Causes and consequences of ecological gradients in southwestern Appalachian streams of Alabama: insights from fish, fish parasite, benthic macroinvertebrate, and crayfish assemblages

by

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Abstract

Biodiversity in freshwaters is highly threatened by short-term and long-term anthropogenic impacts such as landuse-landcover change (e.g., resource extraction, urbanization) and climate change, respectively. One complication for biodiversity conservation is that biodiversity is patchily distributed among freshwater habitats, and thus documenting spatial distribution of biodiversity is important for prioritizing conservation efforts. On a smaller scale, biodiversity often corresponds to various environmental gradients and tends not to be evenly distributed among streams within a network. At both the community and population levels, biodiversity can be influenced by habitat characteristics and connectivity among locations. The southeastern US, including Alabama, is an aquatic biodiversity hotspot where most of this diversity occurs in streams and rivers. I studied streams in the Southwestern Appalachians ecoregion of Alabama to investigate the spatial distribution of 1) taxonomic diversity among assemblages of fishes, macroinvertebrates, and fish parasites and 2) genetic diversity in 2 species of crayfishes. In Chapter 2, I developed tools (Ecological Endpoint Curves; EEC) for resource managers to assess the biotic integrity of fish assemblages in small streams of this ecoregion and compared these to a previously developed index of biotic integrity (IBI) for stream fishes. I found that EECs may be more appropriate assessment tools than an IBI for small stream fishes because several IBI sub-metrics are strongly influenced by the low species richness of fish assemblages naturally occurring in these streams. In Chapter 3, I described the spatial distributions of fish and macroinvertebrate diversity as they related to stream size within the stream networks of this ecoregion. Results suggested that large streams, where fish richness was highest, are essential for conservation efforts, whereas headwater streams should be prioritized for conservation efforts targeting macroinvertebrates because taxa richness was evenly distributed among streams of different sizes and headwaters necessarily outnumber larger streams. In Chapter 4, I investigated stream size–diversity relationships in fish parasites communities and report strong stream size–parasite richness relationships, which may have important ecological and evolutionary implications for their host (*Etheostoma artesiae*). In Chapter 5, I investigated the association between the spatial distribution of genetic diversity and crayfish burrowing and habitat use traits using 2 sympatric crayfishes (*Cambarus striatus* Hay and *Faxonius validus* Faxon). Results suggested that crayfish burrowing behaviors and habitat use traits are useful in predicting population genetic structure, and also may reflect cryptic diversity in *F. validus*, which may require conservation attention.

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Chapter 1: Introduction to the dissertation

INTRODUCTION

Rivers and streams are some of the most imperiled ecosystems on the planet, with threats coming from anthropogenic impacts such as landuse–landcover change (e.g., resource extraction, urbanization) and climate change among others (Dudgeon et al. 2006). One complication for biodiversity conservation is that biodiversity is not evenly distributed in space and thus documenting the spatial distribution of biodiversity is a prerequisite for conservation efforts (Gering et al. 2003; Clarke et al. 2010; Kanno et al. 2012). Within stream networks, several environmental gradients associated with stream size are predicted to influence biodiversity, resulting in unevenly distributed biodiversity among streams within a network (Vannote et al. 1980; Finn et al. 2011). The spatial distribution of biodiversity of aquatic communities (as species richness and composition) and populations (as genetic diversity) also may be influenced by habitat characteristics and connectivity as well as organismal traits (Hughes et al. 2009, 2013; Brown and Swan 2010).

Understanding stream biodiversity patterns and their causes is particularly important for conservation efforts in the southeastern US, including Alabama, which is a lotic biodiversity hotspot for streams and rivers (Lydeard and Mayden 1995). In this dissertation I investigated relationships between biodiversity and environmental correlates or organism traits in the Southwestern Appalachians ecoregion of Alabama; specifically, I sampled streams with minimal anthropogenic impacts within the Bankhead National Forest to quantify these relationships. I addressed 4 questions: (1) Are stream size–biotic metric relationships predictive enough to be used to assess ecological integrity of fish assemblages of the Southwestern Appalachians ecoregion of Alabama (Chapter 2)? (2) Are there relationships between stream size and biodiversity richness, dissimilarity, or nestedness in fish and benthic macroinvertebrate assemblages (Chapter 3)? (3) Are there relationships between stream size and richness, dissimilarity, and nestedness of parasite communities within host fish (Chapter 4)? (4) To what extent does differential habitat use and burrowing traits of 2 sympatric crayfishes relate to patterns of population genetic structure (Chapter 5)?

Tools for assessing ecological integrity in streams

A common tool used by most state and federal agencies to assess ecological condition of streams is the multi-metric index of biotic integrity (IBI). IBIs typically use up to 12 sub-metrics of assemblage structure (e.g. richness, composition, trophic structure, and reproductive or habitat use traits) to score assemblage quality relative to values obtained from minimally disturbed reference sites (ecological endpoints) and sum these values for an overall ecological integrity score (Karr, 1981, 1991; Karr et al., 1986). Richness-based fish IBI sub-metric thresholds are often adjusted for stream size because of the well-known influence of size on richness of aquatic assemblages along the stream continuum (e.g. Vannote et al., 1980; Grenouillet et al., 2004). In contrast, fish assemblage composition and trait-based sub-metric thresholds often are not adjusted for stream size and thus are constant longitudinally (Karr, 1981; O'Neil and Shepard, 2011a and 2011b).

Fish IBIs have several advantages in bioassessment, including an ability to be regionally calibrated (Karr 1981), but a major limitation is that IBIs require at least moderate species richness (~5 in minimally disturbed sites, Fausch et al. 1990). Species-poor systems lack sufficient among-site variation in richness and composition, resulting in redundant IBI sub-metrics (Langdon 2001); in such cases IBI scores reflect stream size stream size rather than assemblage quality reducing IBI performance (Van Sickle 2010). Thus, fish-based IBIs often perform poorly in headwater streams with limited richness (Fausch, 1990; Langdon, 2001). An additional problem is that headwater streams often are excluded from datasets when developing IBIs. For example, the datasets used by the Geological Survey of Alabama in their fish-based IBIs for the Appalachian Plateau reported a minimum stream size of 10.1 km^2 (O'Neil and Shepard 2011a and 2011b). Therefore, alternative assessment tools for fish assemblages in naturally depauperate headwater streams, where fish-based IBIs may be inappropriate, are sorely needed. In lieu of IBIs, researchers have used relationships between biotic metrics and stream size, known as ecological endpoint curves (EECs), as assemblage assessment tools in minimally disturbed headwater streams (Helms et al. 2016). However, EECs have only been developed for the Piedmont ecoregion of Alabama. In Chapter 2, I developed EECs for fish assemblages in the Southwestern Appalachians ecoregion on the Appalachian Plateau, which confirmed that the fish-based IBI for the Appalachian Plateau performs poorly in these small headwater streams. To determine if fish IBIs developed for other regions of Alabama also underperform when assessing headwater assemblages, I compared the performance of EECs and the fish-based IBI developed for the Alabama Piedmont. I show that IBI sub-metric redundancy in headwater streams is especially problematic in the Alabama Piedmont resulting in IBI scores correlating with stream size and not ecological integrity.

Taxonomic and trait diversity in streams

Stream ecosystems exhibit unidirectional flow and inherent dendritic spatial structure, and thus are substantially different environments from terrestrial and lentic ecosystems; this difference places unique limitations on associated organisms (Power and Dietrich 2002). For almost 40 years stream ecology has operated within the predictive framework of the River Continuum Concept (RCC; Vannote et al. 1980), which predicts biological diversity (α-diversity) of stream communities function as a result of multiple environmental gradients (e.g. geomorphology, hydrology, temperature) from headwaters to river mouth (Leopold et al. 1964, Vannote et al. 1980). Specifically, the RCC predicts that species richness increases with stream size (Vannote et al. 1980). More recently, Finn et al. (2011) provided a complementary model to the RCC, the Mighty Headwater Hypothesis (MHH), by focusing on β-diversity (community dissimilarity) among streams of dissimilar size. MHH predicts that β-diversity declines with stream size (Finn et al. 2011). In Chapter 3, I investigated the predicted patterns of species richness and β-diversity from the RCC and MHH using fish and benthic macroinvertebrate assemblages, and also in Chapter 4 using parasite communities of the Redspot Darter, *Etheostoma artesiae*. In Chapter 4, I also investigated the relationship between stream size and diversity of parasites within trait groups to explore potential mechanisms driving diversity relationships.

Crayfish genetic diversity in streams

Assessment of genetic diversity among populations of organisms often is a prerequisite for conservation efforts (Hughes et al. 2009) because diversity often is correlated with population fitness (Reed and Frankham 2003). Predictive frameworks using organismal traits, specifically reproductive (e.g., generation time), habitat (e.g. generalists or specialists), and dispersal (e.g., swimming, crawling, flying, aquatic or terrestrial) traits are increasingly used to predict spatial patterns of genetic diversity (Hughes et al. 2013, Paz et al. 2015, Mims et al. 2018). When validated, such approaches can be invaluable in conservation because they can accurately predict population connectivity and genetic diversity of species for which no published genetic data exists (Mims et al. 2018).

Freshwater crayfishes are among the most-imperiled groups in the world (Lodge et al. 2000; Capinha et al. 2013; Richman et al. 2015) and, as of 2016, less than <44% of North

American crayfish species have published data describing population connectivity and genetic diversity (Figiel 2016). Therefore, a trait-based phylogeographic approach may be especially useful in crayfish conservation because it could prioritize particular species for conservation despite limited genetic diversity data. For example, species anticipated to have lower population connectivity could be prioritized relative to those with higher population connectivity.

A previously developed trait-based approach developed for Australian stream invertebrates assumed that all crayfish are equally capable of terrestrial dispersal (Hughes et al. 2013). This framework overestimated population connectivity in several crayfish species (12 of 34 studies), suggesting that some have more limited terrestrial dispersal than previously thought and dispersal abilities are not equal among species (Hughes et al. 2013). The limitation of this simple approach to accurately predict population connectivity suggests that additional crayfish traits need to be explored in such approaches to increase prediction accuracy across a range of species.

One of the most variable traits among crayfish species is burrowing behavior, which differs widely among species (Hobbs 1981). Burrowing ability is a trait that could be useful in predicting population connectivity because burrowing classifications often relate to habitat requirements. For example, in Tasmanian crayfishes, burrowers had more restricted range sizes than stream-dwellers which may be associated with dispersal (Richardson et al. 2006). In Chapter 5, I proposed that crayfish burrowing classification, and associated habitat requirements, relate to terrestrial dispersal opportunities and thus population genetic structure in 2 crayfish species with strongly contrasting burrowing behaviors and habitat use patterns. Specifically, I studied *Faxonius validus* (Faxon) and *Cambarus striatus* (Hay), to determine if these traits corresponded with their respective population genetic structure. In Chapter 6, I summarize my findings of biodiversity patterns at the community and population genetic levels in the streams of the Southwestern Appalachians ecoregion.

LITERATURE CITED

Brown, B. L., and C. M. Swan. 2010. Dendritic network structure constrains metacommunity properties in riverine ecosystems. Journal of Animal Ecology 79: 571–580.

Capinha, C., E. R. Larson, E. Tricarico, J. D. Olden, and F. Gherardi. 2013. Effects of climate change, invasive species, and disease on the distribution of native european crayfishes. Conservation Biology 27: 731–740.

Clarke, A., R. Mac Nally, N. R. Bond, and P. S. Lake. 2010. Conserving macroinvertebrate diversity in headwater streams: the importance of knowing the relative contributions of α and β diversity. Diversity and Distributions 16: 725–736.

Dudgeon, D., A. H. Arthington, M. O. Gessner, Z.-I. Kawabata, D. J. Knowler, C. Lévêque, R. J. Naiman, A.-H. Prieur-Richard, D. Soto, M. L. J. Stiassny, and C. A. Sullivan. 2006. Freshwater biodiversity: importance, threats, status and conservation challenges. Biological reviews of the Cambridge Philosophical Society 81: 163–182.

Figiel Jr, C. R. 2016. Crayfish population genetic studies:assessment and trends. Freshwater Crayfish 22: 19–42.

Finn, D. S., N. Bonada, C. Múrria, and J. M. Hughes. 2011. Small but mighty: headwaters are vital to stream network biodiversity at two levels of organization. Journal of the North American Benthological Society 30: 963–980.

Gering, J. C., T. O. Crist, and J. A. Veech. 2003. Regional conservation of biodiversity additive partitioning of species diversity across multiple spatial scales: implications for regional conservation of biodiversity. Conservation Biology 17: 488–499.

Helms, B., J. Zink, D. Werneke, T. Hess, Z. Price, G. Jennings, and E. Brantley. 2016. Development of ecogeomorphological (EGM) stream design and assessment tools for the Piedmont of Alabama, USA. Water 8: 161.

Hobbs, H. H. 1981. Crayfishes of Georgia. Smithsonian Contributions to Zoology 318: 549p.

Hughes, J. M., J. A. Huey, and D. J. Schmidt. 2013. Is realised connectivity among populations of aquatic fauna predictable from potential connectivity? Freshwater Biology 58: 951–966.

Hughes, J. M., D. J. Schmidt, and D. S. Finn. 2009. Genes in streams: using DNA to understand the movement of freshwater fauna and their riverine habitat. BioScience 59: 573–583.

Kanno, Y., W. T. Russ, C. J. Sutherland, and S. B. Cook. 2012. Prioritizing aquatic conservation areas using spatial patterns and partitioning of fish community diversity in a near-natural temperate basin. Aquatic Conservation: Marine and Freshwater Ecosystems 22: 799–812.

Karr, J. R. 1981. Assessment of biotic integrity using fish communities. Fisheries 6: 21–27.

Lodge, D. M., C. A. Taylor, D. M. Holdich, and J. Skurdal. 2000. Nonindigenous crayfishes threaten North American freshwater biodiversity: lessons from Europe. Fisheries 25: 7–20.

Lydeard, C., and R. L. Mayden. 1995. A diverse and endangered aquatic ecosystem of the southeast United States. Conservation Biology 9: 800–805.

Mims, M. C., E. E. Hartfield Kirk, D. A. Lytle, and J. D. Olden. 2018. Traits-based approaches support the conservation relevance of landscape genetics. Conservation Genetics 19: 17–26. Springer Netherlands.

Paz, A., R. Ibáñez, K. R. Lips, and A. J. Crawford. 2015. Testing the role of ecology and life history in structuring genetic variation across a landscape: a trait-based phylogeographic approach. Molecular Ecology 24: 3723–3737.

Reed, D. H., and R. Frankham. 2003. Correlation between fitness and genetic diversity. Conservation Biology 17: 230–237.

Richardson, A., N. Doran, and B. Hansen. 2006. The geographic ranges of Tasmanian crayfish: extent and pattern. Freshwater Crayfish 15: 347–364.

Richman, N. I., M. Böhm, S. B. Adams, F. Alvarez, E. A. Bergey, J. J. S. Bunn, Q. Burnham, J. Cordeiro, J. Coughran, K. A. Crandall, K. L. Dawkins, R. J. Distefano, N. E. Doran, L. Edsman, A. G. Eversole, L. Füreder, J. M. Furse, F. Gherardi, P. Hamr, D. M. Holdich, P. Horwitz, K. Johnston, C. M. Jones, J. P. G. Jones, R. L. Jones, T. G. Jones, T. Kawai, S. Lawler, M. López-Mejía, R. M. Miller, C. Pedraza-Lara, A. M. M. Richardson, M. B. Schultz, G. A. Schuster, P. J. Sibley, C. Souty-Grosset, C. A. Taylor, R. F. Thoma, J. Walls, T. S. Walsh, and B. Collen. 2015. Multiple drivers of decline in the global status of freshwater crayfish (Decapoda: Astacidea). Philosophical Transactions of the Royal Society B: Biological Sciences 370: 1–11.

Vannote, R. L., W. G. Minshall, K. W. Cummins, J. R. Sedell, and C. E. Cushing. 1980. The River Continuum Concept. Canadian Journal of Fisheries and Aquatic Sciences 37: 130–137.

Chapter 2. Ecological endpoint relationships to hydraulic geometry for streams of the southwestern Appalachians of Alabama, USA

ABSTRACT

Stream restoration design and assessment tools typically include regional geomorphic curves (RC) and often a biotic-based tool like ecological endpoint curves (EEC) or index of biotic integrity (IBI). EECs may be redundant with IBI sub-metrics; however, IBIs often perform poorly in headwater streams. I quantified bankfull channel geomorphology of 34 reference stream reaches in the Southwestern Appalachians (SWA) ecoregion of Alabama (AL) and related them to drainage area (DA) to create RCs. I also quantified fish assemblages in 17 of these sites and correlated them with stream size or geomorphology to create EECs for the SWA. I compared these data, and those from a similar study in the AL Piedmont, to fish IBI sub-metric scoring threshold values. I found predictable relationships between DA and geomorphology of streams in the SWA ecoregion. I also identified 13 fish metrics that were strongly related to stream size or geomorphology, and thus may serve as useful EECs. Bankfull discharge was the environmental variable most strongly related to 7 of 13 fish assemblage metrics. IBIs for both the SWA and Piedmont ecoregions perform poorly in small streams of those regions largely because proportion-based IBI sub-metrics did not sufficiently account for compositional changes in fish assemblages from 1st to 4th order streams. The strong relationships between fish metrics and geomorphology highlight the utility of the EEC approach in small streams and provide useful tools for stream assessments and for setting stream restoration objectives in the SWA ecoregion of AL.

Key words: Fish, Geomorphology, Streams, Hydraulic Geometry, Bankfull Channel Dimensions

INTRODUCTION

A recent national assessment of US rivers and stream conditions using a fish-based multi-metric index found that 32% of the nation's streams were in poor condition (USEPA, 2016). Thus, it is not surprising that more than $1B annually was spent on stream restoration in the US between 1990 and 2003 (Bernhardt et al., 2005), a number that likely has increased since that time. Stream restoration typically involves manipulation of channel form, longitudinal profile, and riparian vegetation structure to improve erosion control, channel stability, physical habitat, and biotic structure and function to predetermined end points (Rosgen, 2004; Roni et al., 2008; Lave, 2009; Bennett et al., 2011). Restoration endpoints are target ranges of hydraulic geometry, physical habitat, biotic parameters, or composite metrics with societal, hydraulic, or ecological value (Karr, 1981; Rosgen, 1998; Barbour et al., 1999; Gentile et al., 2001; Palmer et al., 2005). Thus, endpoints are determined by the natural range of parameter variability in reference or minimally disturbed systems (Karr, 1981; Wohl et al., 2005; Stoddard et al., 2006; Wohl et al. 2015). End points are critical for assessing restoration need, setting goals for individual restoration projects and, ultimately, determining success of interventions (Suter 1990, 2000; Barbour et al. 1999; Rosgen, 1998; Palmer et al., 2005; Wohl et al., 2005; Dey and Schweitzer 2014; Helms et al., 2016).

The primary method for determining stream channel geometry end points for natural channel design restoration is the use of regional curves (RCs; Rosgen 1996; Hey 2006), which are region-specific power relationships between physical channel dimensions (e.g., width, depth, cross-sectional area, bankfull discharge) and drainage area across reference streams of contrasting sizes (Dunne and Leopold, 1978). Regional curves typically encompass a broad range of stream sizes, although they often lack data from headwater streams (Bieger et al. 2015). Failure to include such small streams is problematic because of high variability in hydraulic geometry in these streams (Mulvihill et al. 2009). Additionally, headwater streams are more likely to be impacted by human activity (Creed et al. 2017), in large part because such streams may compose >50% of total stream length, as is the case for the US (Olsen and Peck 2008). There also is increasing recognition that RCs require supplementation with similar biotic tools to set more holistic recovery goals (Sullivan et al., 2004; Walsh et al., 2005; Woolsey et al., 2007; Poole, 2012; Sullivan, 2012) because physical manipulation of stream channels does not always correspond with biotic recovery (Lepori et al., 2005; Louhi et al., 2011; Ernst et al., 2012; Nilsson et al., 2016). The ultimate goal of many restoration efforts is ecological recovery; thus, quantifying ecogeomorphological feedbacks and relationships (Wheaton et al., 2011) is vital for assessing need, design, and efficacy of restoration success (Helms et al., 2016).

One of the primary tools used by most state and federal agencies to assess stream ecological condition is the multi-metric index of biotic integrity (IBI). For fish assemblages, biotic integrity indices typically score assemblage quality relative to expected values from minimally disturbed sites (ecological endpoints) for up to 12 measures of assemblage structure (e.g. richness, composition, trophic structure, and reproductive or habitat use traits) and sum these scores for an overall ecological integrity score (Karr, 1981, 1991; Karr et al., 1986). Sub-metrics of an IBI must meet several criteria including: low redundancy with other sub-metrics (Karr, 1981; Langdon 2001; Stoddard et al., 2008), sufficient responsiveness to environmental stressors or habitat quality (Karr 1981), sufficient metric range (Whittier et al, 2007; Stoddard et al., 2008), and sufficiently high signal-to-noise ratios (Whittier et al, 2007; Stoddard et al., 2008). Many scoring systems have been devised, but the original system of Karr (1981) trisected the datapoints with 2 threshold values or lines for each sub-metric, establishing an upper, middle, and lower range corresponding to scores of 5, 3, and 1 respectively (Karr, 1981). Richness-based fish IBI sub-metric thresholds often are adjusted for stream size because of the well-known influence of size on richness and composition of aquatic assemblages along the continuum (e.g. Vannote et al., 1980; Grenouillet et al., 2004). In contrast, fish assemblage composition and trait-based sub-metric thresholds often are not adjusted for stream size and are longitudinally constant (Karr, 1981; O'Neil and Shepard, 2011a and 2011b).

Use of an IBI has many advantages in bioassessment (including regional calibration, Karr 1981), but a major limitation is that IBIs require at least moderate species richness (i.e., 5 species in minimally disturbed sites, see Fausch et al. 1990). Systems with depauperate assemblages lack sufficient among-site variation in richness and composition, resulting in redundancy among potential IBI sub-metrics and a reduction in the number of IBI sub-metrics (Langdon 2001). Furthermore, redundancy among sub-metrics can decrease overall IBI performance (Van Sickle 2010). These limitations mean that fish-based IBIs often perform poorly in headwater streams with limited richness (Fausch, 1990; Langdon, 2001). Additionally, headwater streams often are excluded from datasets used to develop IBIs. For example, datasets used by the Geological Survey of Alabama (GSA) in their fish-based IBIs for the Appalachian Plateau (AP) and Ridge and Valley/Piedmont regions of Alabama reported a minimum stream size of 10.1 and 4.35 km^2 respectively. Thus, there is need to develop assessment tools for fish assemblages in naturally depauperate headwater streams where fish-based IBIs may be inappropriate.

In lieu of IBIs, researchers investigating minimally disturbed headwater streams have paired RCs with analogous relationships between biotic metrics and stream size, known as ecological endpoint curves (EECs, Helms et al. 2016). EECs can identify the natural range of variation in biotic metrics in minimally disturbed streams and also predict expected assemblage structure (Helms et al. 2016). However, these tools have only been developed for the Piedmont ecoregion of Alabama and thus their development in other regions of the state and beyond is still needed.

Approximately one-third of streams in the Southern Appalachian ecoregion, which includes the Southwestern Appalachians (SWA; Omernik, 1987), are considered in poor biotic condition using a fish-based multi-metric index similar to an IBI (USEPA, 2016). Low stream biotic integrity in this region stems from an amalgamation of agriculture, silviculture, mining, and anthropogenic hydrologic impacts (USEPA, 2006). More specifically, the streams in the SWA Alabama are primarily impacted by agriculture (O'Neil and Shepard 2011b). Biotic impairment in the Southern Appalachian ecoregion is of particular concern because it is a biodiversity hotspot (Lydeard and Mayden, 1995). Thus, it is critical to assess restoration need, create appropriate designs, and set appropriate restoration objectives to mitigate impacts in this ecoregion.

RCs have been developed for the AP of New York (Miller and Davis, 2003; Westergard et al. 2004), Pennsylvania (Chaplin, 2005), Pennsylvania and Maryland (McCandless 2003), West Virginia (Messinger, 2009), and Tennessee (Babbit, 2005). However, no such curves exist for the AP of Alabama. Development of region-specific curves is important because finer stratification of stream geomorphology data sets often improves reliability of predictive equations (Bieger et al., 2015 but see Johnson and Fecko 2008) and because RC relationships can be affected by geology, vegetation, and climate (McCandless and Everett, 2002).

In this study, I developed RCs and associated EECs for the SWA ecoregion of Alabama that incorporated minimally disturbed headwater streams, predicting that EECs will show similar, yet more variable, trends than RCs. Using these relationships, I evaluated the performance of fish-based IBIs designed for the SWA to determine if IBIs systematically under-scored small streams and also tested for redundancies between IBI sub-metrics that might compound under-scoring issues. As a secondary objective, I repeated these analyses for the Piedmont ecoregion of Alabama using data from Helms et al. (2016) and the fish-based IBIs developed for that ecoregion to determine if IBI performance was consistent between the 2 ecoregions. Specifically, I sought to assess if EECs may be used to complement IBIs, especially in small streams where fish-based IBIs often underperform.

METHODS

Study Area and Site Selection

I quantified reference stream reaches within the SWA ecoregion of AL for geomorphology (n=34) and a subset of these reaches in the Bankhead National Forest within the Sipsey Fork - Black Warrior watershed of AL (n=17) (Fig. 2.1) for fish assemblages and habitat during summer base flows from 13 June to 10 July 2014 and 12 May to 5 June. All streams drained heavily forested watersheds dominated by hemlock (*Tsuga canadensis* (L.) Carrière) or mixed-hardwood forests. White oak (*Quercus alba* L.) and American beech (*Fagus grandifolia* Ehrh) were the primary canopy species associated with hemlock-dominated riparian zones, whereas mixed-hardwood sites were a combination of white oak, American beech, chestnut oak (*Quercus prinus* Willd), and red maple (*Acer rubrum* L.) (Brantley et al., 2016). Sites were selected initially based on professional judgement of the condition of geomorphology, riparian vegetation, and instream habitat.

To confirm that that sites had suitable reference stream reaches I calculated Human Disturbance Gradient (HDG) scores for watersheds of each stream (O'Neil and Shepard, 2011a and 2011b). HDG was based on weighted scores of 8 landscape characteristics (P load, human density, % urban, % barren, % pasture, % cropland, road density, and number of upstream road crossings), derived using 2011 landcover data (USGS, 2014) and GRASS GIS (GRASS Development Team, 2017), and with weights derived from the Landscape Development Intensity (LDI) Index (Brown and Vivas, 2003; sensu O'Neil and Shepard, 2011a). However, P loads have little influence on final HDG scores in the Appalachian Plateau (O'Neil and Shepard, 2011b) and I lacked the data to calculate P load; thus, I excluded P load from HDG scores. The GSA considered streams with an HDG <100 as least-disturbed, the best attainable category (O'Neil and Shepard, 2011a and 2011b), so I only included sites in the SWA of AL that were in this category.

I selected reference study reaches based on the following geomorphic criteria: stable channels with minimal bank erosion, free-form meander patterns, unconfined lengths of at least 20 times bankfull width, well-connected and vegetated floodplains, and presence of riffles, runs, and pools (sensu Helms et al., 2016). All reaches had upstream watersheds that were mostly forested with minor contributions by agricultural land uses. Additionally, representative reaches were selected based on the USEPA's Rapid Bioassessment Protocols (sensu Barbour et al., 1999). For each site, I identified a representative stream reach at least 100 m upstream of the nearest road crossing to minimize effects of bridges and culverts. Reach lengths were 20 times wetted width or a minimum of 150 m and maximum of 300 m long with most available instream physical structures and habitat types (boulders, coarse woody debris, riffles, runs, and pools;

Barbour et al., 1999; Zink et al., 2012). Study reaches included at least 3 riffle-run-pool sequences with substrates consisting of shallow gravel and cobbles overlying bedrock.

Stream Geomorphology Measurement

Channel geomorphology was quantified for study reaches to generate the data needed to create RCs. Riffle cross-sections and profiles of each reach were quantified using a Trimble M1 Total Station (Trimble, Sunnyvale, California, USA) and computer-based laser-level survey equipment (Harrelson et al., 1994). Points were recorded along the channel thalweg, water surface, and top of right and left banks for each reach at the beginning and end of geomorphic breaks in channel slope for 3 riffle cross-sections per reach, which were identified using stable riffle features adjacent to active floodplain indicators such as vegetation, active channel, accumulated detritus, etc. (Zimmermann and Church, 2001; Zimmermann et al., 2008). Bankfull width was determined by professional judgement based on elevation of incipient flooding as indicated by sandy deposition, debris from high water, and other indicators of bankfull elevation (USDA 2009). Bankfull channel width (W_{bkf}), bankfull channel mean depth (d_{bkf}), bankfull channel cross-section area (A_{bkf}), bankfull discharge (Q_{bkf}), width-to-depth ratio (W_{bkf}/d_{bkf}), flood-prone area width (W_{fpa}) and entrenchment ratio (W_{fpa}/W_{bkf}) were estimated for each reach. These geomorphologic metrics are indicators of channel condition and potentially directly related to ecological condition (Helms et al., 2016). I then combined the geomorphologic measurements with drainage area (DA) estimated in GRASS GIS (GRASS Development Team, 2017) to derive geometric RCs (Harrelson et al., 1994). Stream roughness (Manning's $\underline{n}$; Cowan, 1956) was estimated to calculate Q_{bkf} (Arcement and Schneider, 1989) and at least 100 randomly selected particles from the streambed were counted to quantify reach-specific median substrate size (D_{50}, Kondolf and Li, 1992). Last entrenchment ratios were calculated, as the width of the flood prone area divided by the bankfull width (W_{fpa}/W_{bkf}), as an estimate of stream incision for each reach.

Stream Habitat and Fish Sampling

I quantified a suite of instream physicochemical habitat parameters at the time of fish collections. I measured conductivity using C66 Sharp meter (Milwaukee Instruments Inc., Rocky Mount, NC, USA), pH using a Sharp pH52 meter (Milwaukee Instruments Inc., Rocky Mount, NC, USA), and dissolved oxygen and water temperature using a YSI 55 meter (Yellow Springs Instruments, Yellow Springs, OH, USA) placed at the downstream end of each study reach. In addition, I measured depth and streamflow velocity at 5 points along 10 equidistant transects in each reach using a Hach-FH950 Portable Flow Meter (Hach, Loveland, CO, USA), where I also measured wetted width (W_{wetted}). Last, I estimated discharge ($Q_{measured}$) using the incremental method (Gore, 1996) and a Hach-FH950 Portable Flow Meter (Hach, Loveland, CO, USA) at the downstream end of each study reach.

I sampled fish by delineating reaches, which were 20 times mean W_{wetted} long, using block seines at the downstream and upstream reach terminus. I quantified fish in a single pass from the entire reach with a backpack electroshocker (Smith-Root LR-24) and used a 3-person team working in an upstream direction, collecting stunned animals with dip nets (sensu Barbour et al., 1999). I estimated catch per unit effort (CPUE) on a per-area basis. I identified all individuals to species and classified them into functional reproductive and functional feeding groups using the FishTrait database (Frimpong and Angermeier, 2009) and as tolerant or intolerant using GSA designations (O'Neil and Shepard, 2011a). I quantified species richness, Shannon's *H'*, CPUE, total number caught, proportion of breeding guilds, proportion of feeding guilds, all 12 fish IBI sub-metrics for the AP (i.e., number of native species, cyprinids, suckers, *Lepomis* spp., darters and madtom species, percentages of dominant, tolerant, *Lepomis* spp., omnivores and herbivores, top carnivores, and simple lithophilic spawners) and proportion of endemics (Boschung and Mayden 2004).

Statistical Analyses

I used power models to describe relationships between cross-section dependent bankfull measures (W_{bkf}, d_{bkf}, A_{bkf}, and Q_{bkf}) and DA using the equation:

$$\text{Bankfull measure} = a * DA^{b} \qquad (1)$$

where a is the coefficient and b is the slope. I then plotted these relationships as RCs on log-log plots.

I then correlated fish response variables with geomorphic variables, physicochemistry, and habitat parameters. I logit transformed proportional biotic response variables (sensu Warton and Hui, 2011) and log-transformed DA and bankfull measures prior to analyses. I then calculated Pearson correlation coefficients between bankfull measures and fish variables and tested for significance using a Holm's correction method (Holm, 1979) to control for family-wise error rates (alpha=0.05). Fish response variables that were significantly correlated with stream size or geomorphic variables were then used to create EECs, using the equation:

$$\text{Fish response variable} = b + a * \ln(x) \qquad (2)$$

where b is the y-intercept, a is the coefficient, and x is stream size or a geomorphic variable.

I used linear models to test for significant relationships between all IBI sub-metrics and DA (sensu O'Neil and Shepard 2011b). I then compared these EECs with IBI sub-metric scoring threshold values extracted from GSA data (O'Neil and Shepard 2011b) and the AL fish IBI worksheet (P.E. O'Neil, GSA, personal communication) by inspecting EEC models and IBI threshold values plotted together on the same graph. I then calculated Holm-corrected Pearson correlation coefficients to test for redundancies among IBI sub-metrics and with the metric % Creek Chub (*Semotilus atromaculatus* Mitchill) a prevalent species that ranged from high to low abundance across study reaches. Last, I applied the same procedures to determine if any observed discrepancies (e.g. redundancies among IBI sub-metrics or relationships with drainage area for proportional sub-metrics) were consistent across the SWA and Piedmont ecoregions of AL (Fig. 2.1). For this comparison, I used data from Helms et al., (2016) for streams in the Piedmont ecoregion to compare EECs with fish IBI scoring thresholds extracted from the AL fish IBI worksheet (P. E. O'Neil, GSA, personal communication) for the Ridge and Valley/Piedmont region of AL (O'Neil and Shepard 2011a).

RESULTS

SW Appalachians Regional Reference Curve Relationships

The 34 AL streams surveyed for geomorphology showed DA ranging from 0.05 to 261.6 km^2. Reach slopes ranged from 0.0007 to 0.0866 m/m, W_{bkf} ranged from 1.2 to 38.3 m, mean d_{bkf} ranged from 0.1 to 3.7 m, A_{bkf} 0.1 to 103.4 m^2, and Q_{bkf} ranged from 0.1 to 89.6 m^3/s (Table 2.1). D_{50} values ranged from 0.5 to 250.0 mm (Table 2.1). W_{bkf}/d_{bkf} ranged from 25.4 in wide, shallow channels to 6.7 in narrow, deep channels. Entrenchment ratios ranged from 1.1 to 6.6 (Table 2.1). Relationships between DA and W_{bkf}, d_{bkf}, A_{bkf}, and Q_{bkf} for SWA ecoregion were significant ($R^2 = 0.91\text{-}0.98$, $p < 0.001$, Figure 2.2). Channel slopes decreased with increasing DA ($R^2 = 0.63$, $p < 0.001$) but there was no relationship between D_{50} and DA ($R^2 = 0.01$, $p = 0.42$).

Fish Collections

All study sites where fish were collected were in the least-disturbed category (HDG < 100) with the highest HDG score being 64.5 at UT North Fork (Elephant) Creek (Table 2.2). Mean W_{wetted} in these sites ranged from 1.1 to 12.0 m across and Q_{measured} ranged from 0.0004 to 0.1416 m^3/s (Table 2.3). A total of 2791 fishes were collected from 12 of the 17 SWA streams in AL streams (Table 2.3) including 30 species in 7 families. No non-native fishes were collected. Fish richness ranged from 0 in headwater streams (sites generally upstream of waterfalls) to 19 in Capsey Creek (Table 2.4), a site with a high DA (52.09 km^2). In general, uppermost reaches of headwater streams were inhabited either only by Creek Chub (*Semotilus atromaculatus*) or by Creek Chub, Redspot Darter (*Etheostoma artesiae* O. P. Hay), and sometimes Western Creek Chubsucker (*Erimyzon claviformis* Girard). More than half of the study sites (10 of 17, 59%) either were fishless or were inhabited by 1 or all 3 species, with Creek Chub always being the most abundant (Table 2.5).

Fish Assemblage-Stream Size Relationships and EECs

Thirteen fish assemblage metrics were significantly correlated with >1 of the stream size metrics, indicating their potential utility as EECs (Table 2.6). Q_{bkf} showed the highest correlation with the most assemblage metrics including: Shannon's *H'* ($r = 0.99$, $p < 0.001$), total richness ($r = 0.92$, $p = 0.003$), cyprinid richness ($r = 0.91$, $p = 0.013$), centrarchid richness ($r = 0.90$, $p = 0.027$), darter richness ($r = 0.95$, $p < 0.001$), darter and madtom richness ($r = 0.95$, $p < 0.001$), and lithophilic species richness ($r = 0.92$, $p = 0.005$, Fig. 2.3). DA showed the highest correlation with % tolerant species ($r = -0.96$, $p = 0.012$) and % Creek Chub ($r = -0.97$, $p = 0.003$), whereas W_{wetted} had the highest correlation with % dominant species ($r = -0.99$, $p < 0.001$) and % simple lithophilic and tolerant species ($r = -0.97$, $p = 0.003$, Fig. 2.4). Q_{measured} had the highest correlation with shiner richness (r = 0.88, p = 0.033) and % simple lithophilic species (r = 0.95, p = 0.027), Fig. 2.4). Correlation analyses suggested that most richness metrics were positively correlated with each other (r ≥ 0.92, p ≤ 0.001). % DELT (deformities, eroded fins, lesions, tumors plus hybrids), a measure included in the AP IBI, was eliminated because no hybrids nor DELT were collected at most sites.

Of the 13 potential EECs identified above, correlation analyses suggested % Creek Chub was negatively correlated with *H'* (r = -0.97, p = 0.002) and % dominant species (r = -0.97, p = 0.003), and was positively correlated with % tolerant species (r = 0.99, p < 0.001) and % simple lithophilic and tolerant species (r = 0.95, p = 0.03). Percent Creek Chub also was negatively correlated with darter richness (r = -0.98, p < 0.001).

EECs and IBI sub-metric comparisons

Linear models showed significant relationships between 11 AP IBI sub-metrics and DA ($R^2 = 0.42 – 0.93$, $p \leq 0.01$; Fig. 2.5). Sucker richness and *Lepomis* spp. richness did not have sufficient metric range (> 4) required for use as IBI sub-metrics (Whittier et al, 2007; Stoddard et al., 2008) in the streams I sampled, whereas those sampled by GSA did (Fig. 2.5C and D). Except for % *Lepomis* sp., most of the proportional-based metric EEC relationships crossed the thresholds for different IBI scores. Streams <1.4 km^2 DA consistently received the same IBI score for each of the proportion-based IBI sub-metrics; scoring as 5 for % *Lepomis* spp. and % omnivores and as 1 for % dominant species, % tolerant species, % top piscivores, and % simple lithophils (Table 2.7). Overall IBI scores for SWA streams sampled for fish were not significantly correlated with DA ($R^2 = -0.08$, p = 0.64; Fig. 2.6)

Most of the 11 IBI sub-metrics for the Piedmont streams from Helms et al. (2016) were significantly related to DA ($R^2 = 0.27$-0.82, $p \leq 0.02$) except for % *Lepomis* spp. ($R^2 = 0.09$, $p = 0.14$) and % omnivores ($R^2 = 0.17$, $p = 0.07$, Fig. 2.7). Intolerant species richness did not have sufficient range for use as an IBI sub-metric because the maximum value was 3 for Piedmont streams sampled by Helms et al. (2016) and the GSA (O'Neil and Shepard, 2011a) (Fig. 2.7). In addition, except for % *Lepomis* spp. and % omnivores, proportion-based sub-metric relationships with DA crossed IBI scoring thresholds. For all 5 of these proportional IBI sub-metrics (i.e., % tolerant species, % insectivorous cyprinids, % top piscivores, % simple miscellaneous spawners) most Piedmont streams with DA <8 km^2 were assigned the lowest possible score by the IBI thresholds. Because of the consistent low scoring of the smallest streams there was a positive relationship between stream size and overall IBI score ($R^2 = 0.49$, $p = 0.002$, Table 2.7, Fig. 2.6)

Creek Chub contribution to the fish assemblages of headwater streams was high in both the SWA and Piedmont regions and declined with increasing stream size (Fig. 2.8). However, DA explained more of the variation in % Creek Chub in the SWA ($R^2 = 0.93$, $p < 0.001$) than in the Piedmont ($R^2 = 0.51$, $p = 0.001$). Percent Creek Chub also explained a large amount of variation in % tolerant species ($R^2 = 0.97$, $p < 0.001$), % dominant species ($R^2 = 0.93$, $p < 0.001$), and % simple lithophilic species ($R^2 = 0.83$, $p < 0.001$) in the SWA as well as % tolerant species ($R^2 = 0.85$, $p < 0.001$), % insectivorous cyprinids ($R^2 = 0.53$, $p < 0.001$), and % simple miscellaneous spawners ($R^2 = 0.60$, $p = 0.001$) in the Piedmont (Fig. 2.9).

DISCUSSION

The primary findings of my research are that: 1) bankfull channel geometry is strongly related to stream size (as drainage area), 2) bankfull channel geometry, specifically bankfull discharge, was a better predictor of fish assemblage metrics than drainage area in many cases, 3) IBIs developed for relatively large streams in the Appalachian Plateau and Piedmont regions of AL may not be suitable for use in small streams of these regions because of high redundancy among several sub-metrics. In addition, I identified 13 predictable EEC relationships that can be used in small streams of the Appalachian Plateau of AL in lieu of the fish-based IBI developed by the GSA. Taken together, these results have documented a diverse set of geomorphological and associated ecological assessment tools identifying the natural range of variation for fish assemblage metrics in reference streams, which can be used to better inform stream restoration efforts in small streams of the Appalachian Plateau of AL. Furthermore, these findings validate the EEC approach and suggest that these small stream assessment tools can be developed for other ecoregions of the US.

Appalachian Plateau Regional Reference Curves

Channel dimensions in the SWA streams of AL were highly related to stream size, as indicated by DA. These strong and highly predictable RC relationships reflect prior research, and thus further support their use in planning and assessment of natural channel design associated with restoration efficacy (Leopold, 1994; Rosgen, 2004; Lave, 2009; Bieger et al., 2015). Specifically, there were highly predictable relationships between stream channel dimensions (W_{bkf}, d_{bkf}, A_{bkf}, and Q_{bkf}) and drainage area in the SWA ecoregion, which is consistent with findings from other studies of stream channel hydraulic geometry from other ecoregions (e.g., Chaplin, 2005; Messinger, 2009; Brockman et al., 2012; Helms et al., 2016). It important to note that my regional curves include streams of much smaller DA than many other RC studies, particularly in the northern portions of the Appalachian Plateau (see Bieger et al. 2015). This lack of data from small streams can limit accurate prediction of geomorphology of these streams because of their higher variability in hydraulic geometry (Mulvihill et al., 2009; Bieger et al., 2015).

IBI performance in small streams of AL ecoregions

Linear models suggested that all IBI sub-metrics had significant relationships with DA in the SWA and thus could be potential EECs. These relationships were not problematic for richness-based IBI sub-metrics because their thresholds are already adjusted for stream size, but these scoring thresholds underestimated overall species richness, cyprinid species richness, and sucker species richness in streams with < 5 km^2 drainage area in the SWA (Fig. 2.4A-C). This underestimation is likely a result of not including small stream assessments in the development of the IBI for Appalachian Plateau, although IBI thresholds for the Piedmont ecoregion were more accurate. Positive stream size-IBI sub-metric relationships were not problematic for richness-based IBI sub-metrics because thresholds for these measures are adjusted for stream size; however, these relationships were problematic for the proportion-based IBI sub-metrics because thresholds for these metrics are constant for all stream sizes. Except for % *Lepomis* species in the SWA and Piedmont ecoregion and % omnivores in the Piedmont ecoregion, all the relationships between proportion-based metrics and DA crossed IBI sub-metric scoring thresholds, suggesting that IBI sub-metric scores are, at least in part, determined by stream size. There were no relationships between these measures and DA in the original GSA assessment of mostly large streams (O'Neil and Shepard 2011a and 2011b), although omission of smaller streams in that sudy may have led to setting unrealistic scoring thresholds particularly at the lower end of the stream size distribution. Such omission could result in under- or over-scoring some small sites despite their metric values falling within the natural variation of a least-disturbed site as shown in my study.

Some caution is warranted in comparing my fish data with those collected by the GSA because of differences in fish collection methods. The GSA protocol includes 30 sampling efforts in channel habitats plus 2 efforts along the stream bank (O'Neil et al., 2006); in contrast, I followed the EPA rapid bioassessment protocols (Barbour et al., 1999) where sampling area is proportional to stream size (20X wetted width). Despite differences in sampling approaches, both approaches appeared comparable because both studies yielded accurate representation of the fish assemblage present. Further, differences in collection methods are more likely to affect samples of larger streams than smaller streams, as single-pass backpack electrofisher capture efficiency for all fishes strongly declines with increasing cross-sectional area (Price and Peterson 2010). Additionally, IBI sub-metric values for larger streams were often in the range of those calculated for least-disturbed and similarly sized streams sampled by the GSA (see Fig. 2.5 and Fig. 2.7) suggesting that these values were not abnormal for larger streams despite differences in sampling approaches.

Assessing small streams in AL using the developed IBIs also may misclassify small streams because of the high degree of correlation and thus redundancy among proportion-based metrics in these systems. In the SWA, 4 of 6 proportion-based metrics scored all streams with $< 5\ \mathrm{km}^2$ DA as 1s but these low scores were partially offset by the other 2 proportion-based metrics scoring these same streams as 5s. This partial offsetting resulted in there being no relationship between overall IBI score and DA. However, in the Piedmont ecoregion, the worst-case scenario emerges where all proportion-based IBI sub-metrics that were related to DA scored most streams with <5 km^2 DA as 1s. The result of this consistent low scoring of small streams resulted in a significant positive relationship between DA and overall IBI score for Piedmont streams. These results are not surprising, as these IBIs were not developed using small streams. Additionally, several studies that have developed IBIs for use in small streams concluded that many potential IBI sub-metrics are redundant in systems with low fish species richness (Fausch, 1990; Langdon, 2001), which can reduce IBI performance (Van Sickle 2010).

Ultimately, redundancy among several proportion-based IBI sub-metrics and relationships between these sub-metrics and stream size led to IBI scores of small streams being strongly influenced by stream size. In some cases, such as the SWA, biases in sub-metric scores for small streams may offset because some sub-metrics are biased to score small streams high whereas others are biased towards low scores for theses streams resulting in an overall IBI score that is not related to stream size. However, in other cases, such as the Piedmont, these biases are additive rather than subtractive resulting in strong correlations between overall IBI score and stream size. These limitations suggest that either 1) future IBI development studies, in Alabama and likely other southeastern streams, need to incorporate data from small streams or 2) other tools, such as EECs, are needed to when using fish assemblages as indicators of stream ecological integrity in small streams.

Ecological Endpoint Curves

Our correlation results suggest that several instream biotic measures were strongly linked to various measures of stream size (DA, Q_{bkf}, $Q_{measured}$, and W_{wetted}) in small streams of the AL portion of the SWA ecoregion; thus, they have potential for use as EECs alongside RCs for planning and assessment of restoration efforts in this ecoregion. Several of these relationships were of similar strength as that of RCs (Fig. 2.3A, E, 2.4A, B, D, and E) but just as many were weaker and more variable (Fig. 2.3B, C, D, F, G, 2.4C, and F). My results also support prior studies reporting strong linkages between biotic response variables and stream size or hydraulic geometry (e.g., Vannote et al., 1980; Gangloff and Feminella, 2007; Helms et al., 2016). Additionally, my results suggest that fish assemblage metrics were not related to habitat quality variables, such as entrenchment ratio, supporting the finding that there was no relationship between habitat quality and IBI scores in this region (O'Neil and Shepard 2011b). Alternatively, this result also may suggest that I consistently chose reference reaches of high habitat quality. Several biotic metrics lacked predictable relationships with stream size (e.g., fish CPUE), but they still provided useful estimates of the natural range of variation for biotic metrics that were unrelated to stream size, which also is useful in restoration assessments and natural channel design in this ecoregion.

Stream size has long been considered a primary driver of biotic community structure and function (Hynes, 1970; Vannote et al., 1980; Allan and Castillo, 2007). However, recent attention has been paid to the complexity and influence of spatial scale on ecogeomorphological connections, specifically how local geomorphic conditions influence ecological processes (Sullivan et al., 2004, 2006; Stallins, 2006; Post et al., 2007; Sullivan, 2012). My results clearly showed that several biotic metrics (e.g. fish species richness, cyprinid richness, centrarchid richness, darter richness, and % tolerant species) were highly correlated with stream size (as Q_{bkf} and DA), and thus appear useful as EECs for assessing the need for, or efficacy of, restorations in small streams of the SWA. In contrast, other biotic metrics (i.e., fish CPUE) were less useful as EECs because they were statistically unrelated to stream size or channel geomorphology. Although these latter biotic metrics did not fit the criteria of EECs, they may still provide useful information as traditional ecological benchmarks for determining restoration need and efficacy

(Storey and Cowley, 1997; Collier et al., 2001). Identification of the range of natural variation for these metrics in reference streams is an important step in incorporating these metrics into biological integrity indices (Karr, 1991) and for selecting appropriate ecological endpoints for stream assessments and restorations (Gentile et al., 2001).

I found that various measures of fish assemblage richness (e.g. species richness, cyprinid richness, centrarchid richness, and darter richness) were highly correlated ($R^2 = 0.79 – 0.90$) with a full suite of stream size indicators (i.e., DA, W_{bkf}, d_{bkf}, A_{bkf}, Q_{bkf}, mean W_{wetted}). The positive relationship between richness and habitat area is a pervasive pattern observed for freshwater fishes (Matthews, 1998). Globally, the amount of surface area drained by the river basin (i.e., DA) was the most important predictor of freshwater fish species richness when compared to measured annual discharge (Oberdorff et al., 1995); relationships between fish richness and DA in the SW Appalachians streams in my study partially supports this prior work because DA was highly predictive of assemblage richness measures. However, estimated bankfull channel discharge (Q_{bkf}) had a stronger relationship with richness measures than DA, although the increase in strength of the relationship was marginal. This result suggests that geomorphological measurements may help improve the predictability of stream size-assemblage structure relationships, although DA, because of the relative ease of its calculation by GIS without the need of equipment for onsite measurement, has its logistical benefits. In my study, the strength of the relationships between stream size and fish richness measures would have been even stronger without one notable outlier, Tedford Creek, which had relatively low fish richness for its relatively large DA. It is possible that low species diversity of Tedford Creek resulted from its unique intermittent hydrology; on subsequent visits I observed this stream to have no running or standing surface water for hundreds of meters (personal observation). However, this contention needs verification and the explanatory power of long-term hydrology patterns in explaining fish richness in the SWA is needed.

I also found that many of the compositional metrics (e.g., % dominant, % tolerant, % simple lithophilic spawners) also were strongly correlated with several measures of stream size in the SWA. However, many of these metrics were intercorrelated and all appear to primarily reflect the influence and dominance of Creek Chub in fish assemblages of small streams. Thus, the use of relationships between stream size and fish community compositional metrics as EECs for the SWA of AL may be less informative, because these metrics primarily reflect redundant information (% Creek Chub), than in geographic regions with more diverse headwater assemblages (e.g., Felley, 1992; Casatti 2005). However, relationships between compositional metrics and stream size are likely to be pervasive in nearly all stream networks because this pattern is a natural outgrowth of the well-documented positive relationship between stream size and fish species richness (e.g. Vannote et al., 1980; Grenouillet et al., 2004). Additionally, the influence of Creek Chub dominance on ecological traits of fish assemblages also supports the hypothesis that trait-based approaches may be more informative at broader spatial scales where species traits are more likely to differ among catchments (Pyron et al., 2011).

In this study I identified 13 EEC relationships between biotic measures and stream size or hydraulic geometry that can be used by resource managers to assess fish communities in small streams of the SWA in Alabama. Rather than subjectively eliminating redundant metrics, I present all 13 for resource managers to use as they see fit. However, I caution managers to be aware of redundancy among proportion-based metrics because deviation from the natural range of variation in one of these measures also will likely result in deviation from expected values for many other measures. It may be prudent to use only one of these metrics in a given stream assessment. My recommendation is to use % tolerant species because this trait-based measure offers a mechanistic link to disturbance and the GSA has found that it is responsive to changes in Human Disturbance Gradient values. Additionally, this measure was more variable in reference streams of the SWA and may be sensitive to disturbances or human influence even in the Bankhead National Forest where human influence was minimal. For example, % tolerant species was high in Tedford Creek, which is subject to more frequent drying, because of higher % Creek Chub (a tolerant species) than expected for its size. For another example, % tolerant species was high in Owl Creek because of the abundance of Striped Shiner (*Luxilus chrysocephalus* Rafinesque), the only other relatively numerous tolerant species that was not Creek Chub, possibly as a result of these fish moving upstream from Brushy Lake. However, depending upon the expected stressor, other metrics may be more suitable. For instance, if the primary stressor is sedimentation, then using a metric such as % simple lithophilic spawners may be more appropriate than % tolerant species.

In summary, the empirical tools designed in my study provide rigorous geomorphological and ecological criteria useful for site assessments, restoration design, and evaluating restoration success in streams of the SWA ecoregion of Alabama. Geomorphological stream channel measurements were highly predictable with stream size, as indicated by drainage area, thus confirming the validity of regional curves as restoration design tools. As a caveat, however, I caution practitioners to consider the amount of natural variability shown in these data and encourage inclusion of data from additional reference streams, whenever possible, for management applications. Additionally, I have shown that previously developed fish-based IBIs for the SWA and Piedmont ecoregions of AL underperform in small streams in these ecoregions because IBI sub-metric thresholds developed using larger streams do not account for the relationships between these proportion-based assemblage metrics and stream size, and that high degree of redundancy among these metrics can bias IBI scores for small streams. Development of these EECs for other ecoregions could, in addition to developed IBIs, improve assessments, restoration designs, effectiveness of restoration projects, and thus ecological function of restored streams in the 0.1-100 km^2 size range where fish assemblages are simplified.

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LITERATURE CITED

Allan, J. D., and M. M. Castillo. 2007. Stream ecology: structure and function of running waters. Springer, Dordrecht, The Netherlands.

Arcement, G. J. J., and V. R. Schneider. 1989. Guide for selecting Manning's roughness coefficients for natural channels and flood plains. Federal Highways Administration, US Department of Transportation, Report No. FHWA-TS-84-204, Washington, 62 pp.

Babbit, G. S. 2005. Bankfull hydraulic geometry of streams draining the Southwestern Appalachians of Tennessee. Master's thesis, The University of Tennessee – Knoxville, 130 pp.

Barbour, M.T., J. Gerritsen, B.D. Snyder, and J.B. Stribling. 1999. Rapid bioassessment protocols for use in streams and wade-able rivers: periphyton, benthic macroinvertebrates and fish. U.S. Environmental Protection Agency, Office of Water, Washington DC.

Bennett, S. J., A. Simon, J. M. Castro, J. F. Atkinson, C. E. Bronner, S. S. Blersch, and A. J. Rabideau. 2011. The evolving science of stream restoration. Geophysical Monograph Series 194: 1–8.

Bernhardt, E., M. Palmer, J. Allan, G. Alexander, K. Barnas, S. Brooks, J. Carr, S. Clayton, C. Dahm, J. Follstad-Shah, D. Galat, S. Gloss, P. Goodwin, D. Hart, B. Hasset, R. Jenkinson, S. Katz, G. M. Kondolf, P. S. Lake, R. Lave, J. L. Meyer, T. K. O'Donnell, L. Pagano, B. Powell, and E. Sudduth. 2005. Synthesizing US river restoration efforts. Science 308: 636–637.

Bieger, K., H. Rathjens, P. M. Allen, and J. G. Arnold. 2015. Development and evaluation of bankfull hydraulic geometry relationships for the physiographic regions of the United States. Journal of the American Water Resources Association (JAWRA) 51: 842-858.

Boschung, H., and R. L. Mayden. 2004. Fishes of Alabama. Smithsonian Books, Washington D.C.

Brantley, E., B. Helms, A. Ludwig, J. Shaw, G. Jennings, C. Anderson, D. Werneke, and J. Feminella. 2016. Eco-morphological mitigation design and assessment tools for the Alabama and Tennessee Appalachian Plateau. US Environmental Protection Agency, Region IV Grant No. CD 00D01412, Final Report, Atlanta, GA. http://cses.auburn.edu/eve-brantley/wp-content/uploads/sites/114/2016/10/Final-report_CD00D01412_-093016.pdf

Brockman, R. R., C. T. Agouridis, S. R. Workman, L. E. Ormsbee, and A. W. Fogle. 2012. Bankfull regional curves for the inner and outer bluegrass regions of Kentucky. Journal of the American Water Resources Association (JAWRA) 48: 391–406.

Brown, M.T., and M.B. Vivas. 2003, A landscape development intensity index: Tallahassee, Florida, Florida Department of Environmental Protection, 21 p.

Casatti, L. 2005. Fish assemblage structure in a first order stream, southeastern Brazil: longitudinal distribution, seasonality, and microhabitat diversity. Biota Neotropica 5:75–83.

Chaplin, J. J. 2005. Development of regional curves relating bankfull-channel geometry and discharge to drainage area for streams in Pennsylvania and selected areas of Maryland. U.S. Geological Survey, Scientific Investigations Report 2005-5147. Reston, Virginia. URL: http://pubs.usgs.gov/sir/2005/5147/SIR2005-5147.pdf

Collier, K. J., J. C. Rutherford, J. M. Quinn, and R. J. Davies-Colley. 2001. Forecasting rehabilitation outcomes for degraded New Zealand pastoral streams. Water Science and Technology 43: 175-184.

Cowan, W. L. 1956. Estimating hydraulic roughness coefficients. Agricultural Engineering 37: 473–475.

Creed, I. F., C. R. Lane, J. N. Serran, L. C. Alexander, N. B. Basu, A. J. K. Calhoun, J. R. Christensen, M. J. Cohen, C. Craft, E. D'Amico, E. De Keyser, L. Fowler, H. E. Golden, J. W. Jawitz, P. Kalla, L. Katherine Kirkman, M. Lang, S. G. Leibowitz, D. B. Lewis, J. Marton, D. L. McLaughlin, H. Raanan-Kiperwas, M. C. Rains, K. C. Rains, and L. Smith. 2017. Enhancing protection for vulnerable waters. Nature Geoscience 10:809–815. Nature Publishing Group.

Dey, D. C. and C. J. Schweitzer. 2014. Restoration for the future: endpoints, targets, and indicators of progress and success. Journal of Sustainable Forestry 33: S43–S65.

Dunne, T., and L. B. Leopold. 1978. Water in environmental planning. W.H. Freeman, San Francisco, CA.

Ernst, A. G., D. R. Warren, and B. P. Baldigo. 2012. Natural-channel-design restorations that changed geomorphology have little effect on macroinvertebrate communities in headwater streams. Restoration Ecology 20: 532–540.

Fausch, K. D., Lyons, J., Karr, J. R. and Angermeier, P. L. 1990. Fish communities as indicators of environmental degradation. American Fisheries Society Symposium 8: 123–144.

Felley, J. D. 1992. Medium-low gradient streams of the Gulf Coastal Plain. *In* Biodiversity of the southeastern United States, aquatic communities, C. T. Hackney, S. M. Adams, and W. H. Martin (Editors). John Wiley and Sons, Inc, New York, New York, pp. 271–314.

Frimpong, E. A., and P. L. Angermeier. 2009. Fish traits: a database of ecological and life-history traits of freshwater fishes of the United States. Fisheries 34: 487–495.

Gangloff, M. M., and J. W. Feminella. 2007. Stream channel geomorphology influences mussel abundance in southern Appalachian Streams, U.S.A. Freshwater Biology 52: 64–74.

Gentile, J., M. Harwell, W. Cropper Jr, C. Harwell, D. DeAngelis, S. Davis, J. Ogden, and D. Lirman. 2001. Ecological conceptual models: a framework and case study on ecosystem management for south Florida sustainability. Science of The Total Environment 274: 231–253.

GRASS Development Team, 2017. Geographic Resources Analysis Support System (GRASS) Software, Version 7.2. Open Source Geospatial Foundation.

Grenouillet, G., Pont, D. and Hérissé, C. 2004. Within-basin fish assemblage structure: the relative influence of habitat versus stream spatial position on local species richness. Canadian Journal of Fisheries and Aquatic Sciences 61: 93–102.

Hackney, C. T., S. M. Adams, and W. H. Martin (Editors). 1992. Biodiversity of the southeastern United States, Aquatic Communities. John Wiley and Sons, Inc, New York, New York,

Harrelson, C., C. L. Rawlins, and J. Potyondy. 1994. Stream channel reference sites: an illustrated guide to field technique. General Technical Report RM-245: 61 pp. URL: http://www.treesearch.fs.fed.us/pubs/20753

Helms, B., J. Zink, D. Werneke, T. Hess, Z. Price, G. Jennings, and E. Brantley. 2016.

Development of ecogeomorphological (EGM) stream design and assessment tools for the Piedmont of Alabama, USA. Water 8: 1–25.

Herlihy, A. T., Gerth, W. J., Li, J. and Banks, J. L. 2005. Macroinvertebrate community response to natural and forest harvest gradients in western Oregon headwater streams. Freshwater Biology 50: 905–919.

Hey, R. D. 2006. Fluvial geomorphological methodology for natural stable channel design. Journal of the American Water Resources Association 42: 357–374.

Holm, S. 1979. A simple sequentially rejective multiple test procedure. Scandinavian Journal of Statistics 6: 65–70.

Hugueny, B. 1989. West African rivers as ciogeographic islands: species richness of fish communities. Oecologia 79: 236–243.

Hynes, H. B. N. 1970. The ecology of running waters. University of Toronto Press, Toronto, Canada.

Johnson, P. A., and B. J. Fecko. 2008. Regional channel geometry equations: a statistical comparison for physiographic provinces in the eastern U. S. River Research and Applications 24: 823–834.

Karr, J. 1981. Assessment of biotic integrity using fish communities. Fisheries 6:21–27.

Karr, J. R., 1991. Biological integrity: a long-neglected aspect of water resource management. Ecological Applications 1: 66–84.

Karr, J. R., K D. Fausch, P. L. Angermeier, P. R. Yant, and I. J. Schlosser. 1986. Assessing biological integrity in running waters: a method and its rationale. Illinois Natural History

Survey, Special Publication No. 5. Champaign, Illinois.

Kondolf, G. M., and S. Li. 1992. The pebble count technique for quantifying surface bed material size in instream flow Studies. Rivers 3: 80–87.

Langdon, R. W. 2001. A preliminary index of biological integrity for fish assemblages of small coldwater streams in Vermont. Northeastern Naturalist 8: 219–232.

Larsen, S., L. Mancini, G. Pace, M. Scalici, and L. Tancioni. 2012. Weak concordance between fish and macroinvertebrates in Mediterranean streams. PLoS ONE 7: e51115.

Lave, R., 2009. The controversy over natural channel design: substantive explanations and potential avenues for resolution. Journal of the American Water Resources Association (JAWRA) 45: 1519–1532.

Leopold, L. B., 1994. A view of the river. Harvard University Press, Cambridge, Massachussetts.

Lepori, F., D. Palm, E. Brännäs, and B. Malmqvist. 2005. Does restoration of structural heterogeneity in streams enhance fish and macroinvertebrate diversity? Ecological Applications 15: 2060–2071.

Louhi, P., H. Mykrä, R. Paavola, A. Huusko, T. Vehanen, A. Mäki-Petäys, and T. Muotka. 2011. Twenty years of stream restoration in Finland: little response by benthic macroinvertebrate communities. Ecological Applications 21: 1950–1961.

Lydeard, C., and R. L. Mayden. 1995. A diverse and endangered aquatic ecosystem of the southeast United States. Conservation Biology 9: 800–805.

Matthews, W. J. 1998. Physical factors within drainages as related to fish assemblages. *In* Patterns in Freshwater Fish Ecology. Kluwer Academic Publishers, Norwell, Massachusetts, pp. 264–317.

McCandless, T. 2003. Maryland stream survey: bankfull discharge and channel characteristics of streams in the Allegheny Plateau and the Valley and Ridge hydrologic regions. U . S . Fish & Wildlife Service report: CBFO-S03-01, 33 pp.

McCandless, T. L., and R. A. Everett. 2002. Maryland stream survey: bankfull discharge and channel characteristics of streams in the Piedmont hydrologic region. U.S. Fish & Wildlife, Service Chesapeake Bay Field Office, CBFO-S02-01. URL: http://www.fws.gov/chesapeakebay/pdf/Section1.pdf%5C.

Messinger, T. 2009. Regional curves for bankfull channel characteristics in the Appalachian Plateaus, West Virginia. US Geological Survey, Scientific Investigations Report 2009–5242, Reston, Virginia. URL: http://pubs.usgs.gov/sir/2009/5242/pdf/sir2009-5242.pdf

Miller, S.J. and D. Davis. 2003. Optimizing Catskill Mountain regional bankfull discharge and hydraulic geometry telationships. *In* Watershed Management for Water Supply Systems: Proceedings of the American Water Resources Association 2003 International Congress, New York City, New York, June 29- July 2, 2003, 10 pp. http://www.catskillstreams.org/pdfs/catskillregionalcurves.pdf

Mulvihill, C. I., B. P. Baldigo, S. J. Miller, D. DeKoskie, and J. DuBois. 2009. Bankfull discharge and channel characteristics of streams in New York State. United States Geological Survey, Scientific Investigations Report 2009-5144, Reston, Virginia. URL: http://pubs.usgs.gov/sir/2009/5144/pdf/sir2009-5144_mulvihil_bankfull_2revised508.pdf

Nilsson, C., J. M. Sarneel, D. Palm, J. Gardestrom, F. Pilotto, L. E. Polvi, L. Lind, D. Holmqvist, and H. Lundqvist. 2016. How do biota respond to additional physical restoration of restored streams? Ecosystems 20: 144-162.

Oberdorff, T., J. F. F. Guegan, and B. Hugueny. 1995. Global scale patterns of fish species richness in rivers. Ecography 18: 345–352.

Olsen, A. R. and Peck, D. V. 2008. Survey design and extent estimates for the wadeable streams assessment. Journal of the North American Benthological Society 27: 822–836.

Omernik, J. M. 1987. Ecoregions of the conterminous United States. Annals of the Association of American Geographers 77: 118–125.

O'Neil, P.E., and T. E. Shepard. 2011a. Calibration of the index of biotic integrity for the Ridge and Valley/Piedmont ichthyoregion in Alabama. Open-File Report 1109. Geologic Survey of Alabama, Tuscaloosa, Alabama.

O'Neil, P.E., and T. E. Shepard. 2011b. Calibration of the index of biotic integrity for the plateau ichtyoregion in Alabama. Open-File Report 1111. Geologic Survey of Alabama, Tuscaloosa, Alabama.

O'Neil, P.E., T.E. Shepard, and M.R. Cook, 2006. Habitat and biological assessment of the Terrapin Creek watershed and development of the index of biotic integrity for the Coosa and Tallapoosa River systems: Alabama Geological Survey Open-File Report 0601, 210 p.

Paavola, R., T. Muotka, R. Virtanen, J. Heino, D. Jackson, and A. Mäki-Petäys. 2006. Spatial Scale Affects Community Concordance among Fishes , Benthic Macroinvertebrates , and Bryophytes in Streams. Ecological Applications 16: 368–379.

Palmer, M. A., E. S. Bernhardt, J. D. Allan, P. S. Lake, G. Alexander, S. Brooks, J. Carr, S. Clayton, C. N. Dahm, J. Follstad Shah, D. L. Galat, S. G. Loss, P. Goodwin, D. D. Hart, B.

Hassett, R. Jenkinson, G. M. Kondolf, R. Lave, J. L. Meyer, T. K. O’Donnell, L. Pagano, and E. Sudduth. 2005. Standards for ecologically successful river restoration. Journal of Applied Ecology 42: 208–217.

Poole, G. C. 2012. Stream hydrogeomorphology as a physical science basis for advances in stream ecology. Journal of the North American Benthological Society 29: 12–25.

Post, D. M., M. W. Doyle, J. L. Sabo, and J. C. Finlay. 2007. The problem of boundaries in defining ecosystems: a potential landmine for uniting geomorphology and rcology. Geomorphology 89: 111–126.

Price, A. L., and J. T. Peterson. 2010. Estimation and Modeling of Electrofishing Capture Efficiency for Fishes in Wadeable Warmwater Streams. North American Journal of Fisheries Management 30:481–498.

Pyron, M., L. Williams, J. Beugly, and S. J. Jacquemin. 2011. The role of trait-based approaches in understanding stream fish assemblages. Freshwater Biology 56: 1579–1592.

R Core Team, 2016. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Roni, P., K. Hanson, and T. Beechie. 2008. Global review of the physical and biological rffectiveness of stream habitat tehabilitation techniques. North American Journal of Fisheries Management 28: 856–890.

Rosgen, D.L. 1996. Applied river morphology. Wildland Hydrology, Pagosa Springs, CO.

Rosgen, D.L. 1998. The reference reach—a blueprint for natural channel design. *In* Proceedings Wetlands and River Restoration Conference, Hayes, D.F. (ed), American Society of Civil

Engineers, Reston, VA., Denver, CO.

Rosgen, D. 2004. The reference reach - a blueprint for natural channel design. Proceedings of Wetlands Engineering and River Restoration Conference 1998: 1-8.

Roy, A. H., M. C. Freeman, B. J. Freeman, S. J. Wenger, W. E. Ensign, and J. L. Meyer. 2005. Investigating hydrologic alteration as a mechanism of fish assemblage shifts in urbanizing streams. Journal of the North American Benthological Society 24: 656–678.

Smith, G. R. 1981. Late Cenozoic freshwater fishes of North America. Annual Review of Ecology, Evolution, and Systematics 12: 163–193.

Stallins, J. A. 2006. Geomorphology and ecology: unifying themes for complex systems in biogeomorphology. Geomorphology 77: 207–216.

Stoddard, J. L., A. T. Herlihy, D. V. Peck, R. M. Hughes, T. R. Whittier, and E. Tarquinio. 2008. A process for creating multimetric indices for large-scale aquatic surveys. Journal of the North American Benthological Society 27:878–891.

Storey, R. G., and D. R. Cowley. 1997. Recovery of three New Zealand rural streams as they pass through native forest remnants. Hydrobiologia 353: 63–76.

Sullivan, S. M. P. 2012. Geomorphic-ecological relationships highly variable between headwater and network mountain streams of northern Idaho, United States. Journal of the American Water Resources Association (JAWRA) 48: 1221–1232.

Sullivan, S. M. P., M. C. Watzin, and W. C. Hession. 2004. Understanding stream geomorphic state in relation to ecological integrity: evidence using habitat sssessments and macroinvertebrates. Environmental Management 34: 669–683.

Sullivan, S. M. P., M. C. Watzin, and W. C. Hession. 2006. Influence of stream geomorphic condition on fish communities in Vermont, U.S.A. Freshwater Biology 51(10): 1811–1826.

Suter, G. W. 1990. Endpoints for regional ecological risk assessments. Environmental Management 14: 9-23.

Suter, G. W. 2000. Generic assessment endpoints are needed for ecological risk assessment. Risk analysis, an official publication of the Society for Risk Analysis 20: 173–178.

US Department of Agriculture. 2009. Stream visual assessment protocol version 2, vol. subpart B—conservation planning. USDA Natural Resources Conservation Service, 48p.

U.S. Environmental Protection Agency. 2016. National rivers and streams assessment 2008-2009: a collaborative survey (EPA/841/R-16/007). Office of Water and Office of Research and Development, US Environmental Protection Agency, Washington, D.C., pp. 1-116. URL: http://www.epa.gov/national-aquatic-resource-surveys/nrsa C

U.S. Environmental Protection Agency. 2006. Wadeable streams assessment: a collaborative survey of the nation's streams. Office of Water, US Environmental Protection Agency, Washington DC, pp. 1-113.

U.S. Geological Survey. 2014. NLCD 2011 Land Cover (2011 Edition, amended 2014) - National Geospatial Data Asset (NGDA) Land Use Land Cover. U.S. Geological Survey, Sioux Falls, SD.

Vannote, R. L., G. W. Minshall, K. W. Cummins, J. R. Sedell, and C. E. Cushing. 1980. The River Continuum Concept. Canadian Journal of Fisheries and Aquatic Sciences 37: 130–137.

van Sickle, J. 2010. Correlated metrics yield multimetric indices with inferior performance. Transactions of the American Fisheries Society 139: 1802–1817.

Walsh, C. J., A. H. Roy, J. W. Feminella, P. D. Cottingham, and R. P. Morgan. 2005. The urban stream syndrome : current knowledge and the search for a cure The urban stream syndrome: current knowledge and the search for a cure. Journal of the North American Benthological Society 24:706–723.

Warton, D. I., and F. K. C. Hui. 2011. The arcsine is asinine: the analysis of proportions in ecology. Ecology 92:3–10.

Westergard, B. E., C. I. Mulvihill, A. G. Ernst, and B. P. Baldigo. 2004. Regionalized equations for bankfull-discharge and channel characteristics of streams in New York State: Hydrologic Region 5 in Central New York. U. S. Geological Survey, USGS Water-resources Investigations report 5247, pp. 1-16.

Wheaton, J. M., C. Gibbins, J. Wainwright, L. Larsen, and B. McElroy. 2011. Preface: multiscale feedbacks in ecogeomorphology. Geomorphology 126: 265–268.

Whittier, T. R., R. M. Hughes, J. L. Stoddard, G. A. Lomnicky, D. V. Peck, and A. T. Herlihy. 2007. A structured approach for developing indices of biotic integrity: three examples from streams and rivers in the Western USA. Transactions of the American Fisheries Society 136:718–735.

Wohl, E., P. L. Angermeier, B. Bledsoe, G. M. Kondolf, L. MacDonnell, D. M. Merritt, M. A. Palmer, N. L. Poff, and D. Tarboton. 2005. River restoration. Water Resources Research 41:1–12.

Wohl, E., S. N. Lane, and A. C. Wilcox. 2015. The science and practice of river restoration. Water Resources Research 51: 5974–5997.

Woolsey, S., F. Capelli, T. Gonser, E. Hoehn, M. Hostmann, B. Junker, A. Paetzold, C. Roulier, S. Schweizer, S. D. Tiegs, K. Tockner, C. Weber, and A. Peter. 2007. A strategy to assess river restoration success. Freshwater Biology 52: 752–769.

Zimmermann, A., and M. Church. 2001. Channel morphology, gradient profiles and bed stresses during flood in a step-pool channel. Geomorphology 40: 311–327.

Zimmermann, A. E., M. Church, and M. A. Hassan. 2008. Identification of steps and pools from stream longitudinal profile data. Geomorphology 102: 395–406.

Zink, J. M., G. D. Jennings, and G. A. Price. 2012. Morphology characteristics of southern Appalachian wilderness streams. Journal of the American Water Resources Association (JAWRA) 48: 762–773.

Table 2.1. Summary of reference stream locations and geomorphology measurements for the southwestern Appalachians ecoregion of AL including mean particle size (D_{50}), bankfull width (W_{bkf}), bankfull depth (D_{bkf}), bankfull cross-sectional area (A_{bkf}), bankfull discharge (Q_{bkf}), and bankfull mean velocity (V_{bkf}).

Stream Name	Latitude	Longitude	Drainage Area (km^2)	W_{bkf} (m)	D_{bkf} (m)	A_{bkf} (m^2)	Q_{bkf} (m^3/sec)	Mean V_{bkf} (m/sec)	Entrenchment Ratio	Hydraulic radius (m)	Channel Slope (m/m)	D_{50} (mm)	Manning's n
UT1 Collier Creek	34.2649	-87.3057	0.05	1.2	0.1	0.1	0.1	0.85	4.9	0.08	0.0575	6.5	0.0510
UT1 Crooked Creek	33.7517	-86.8872	0.08	1.2	0.1	0.2	0.2	1.22	3.6	0.12	0.0853	180.0	0.0580
UT2 Crooked Creek	33.7509	-86.8919	0.08	1.2	0.1	0.2	0.2	1.06	6.2	0.11	0.0826	171.0	0.0630
UT North Fork Caney Creek	34.2678	-87.4608	0.08	2	0.1	0.3	0.4	1.32	2.1	0.13	0.0735	10.0	0.0520
UT3 Brushy Creek	34.2549	-87.2563	0.11	1.6	0.1	0.2	0.3	1.25	3.2	0.12	0.0589	5.7	0.0460
UT2 Brushy Creek	34.2529	-87.2544	0.13	1.9	0.2	0.4	0.6	1.58	3.6	0.16	0.0666	41.0	0.0490
UT1 Thompson Creek	34.3441	-87.4707	0.16	1.9	0.2	0.4	0.6	1.48	2.3	0.17	0.0866	105.0	0.0610
UT Brown (Bushwhack) Creek	34.3056	-87.2402	0.16	2.0	0.2	0.4	0.5	1.20	2.4	0.17	0.0525	173.0	0.0580
UT Sipsey Creek	34.2825	-87.3986	0.21	2.7	0.3	0.8	1.1	1.35	2.9	0.24	0.0337	1.6	0.0530
UT Capsey Creek	34.2648	-87.2014	0.21	2.4	0.3	0.6	1.1	1.73	2.8	0.21	0.0421	11.0	0.0420
UT Locust Fork	33.7467	-86.8943	0.23	2.1	0.2	0.4	0.5	1.11	3.6	0.18	0.0461	114.0	0.0610
UT W. Fork Beech (Stillwater) Creek	34.3016	-87.3162	0.24	3.0	0.3	0.8	0.8	1.05	3.8	0.22	0.0198	45.0	0.0490
UT N. Fork Caney (Elephant) Creek	34.2772	-87.4595	0.25	2.8	0.303	0.9	0.9	1.05	-	-	-	-	-
UT Rush (Melody) Creek	34.3303	-87.1970	0.30	2.9	0.2	0.6	0.5	0.97	3.5	0.17	0.0281	1.1	0.0530
UT Brushy Creek	34.2525	-87.2476	0.32	2.9	0.3	0.8	1.1	1.33	2.7	0.24	0.0440	123.0	0.0610
UT Brown Creek	34.3040	-87.2443	0.77	3.5	0.3	1	1.1	1.03	1.8	0.25	0.0164	62.0	0.0500
White Oak Creek	34.3454	-87.4539	0.96	6.8	0.4	2.8	3.2	1.14	-	-	-	-	-
UT2 Thompson Creek	34.3423	-87.4735	0.60	3.5	0.2	0.7	0.7	1.14	1.7	0.17	0.0318	34.0	0.0480
UT2 Collier Creek	34.2630	-87.3007	0.98	4.4	0.4	1.6	1.4	0.88	4.8	0.31	0.0095	50.0	0.0510
Rush Creek (Headwaters)	34.3297	-87.1970	1.14	3.0	0.4	1.3	1.8	1.41	3.3	0.33	0.0214	55.0	0.0500
UT2 Capsey Creek	34.2632	-87.1996	1.36	-	-	-	-	-	-	-	-	-	-
Turkey Creek	33.4139	-87.5139	16.00	9.3	0.6	5.7	10.4	1.83	1.5	0.54	0.0037	100.0	*
North Fork Caney Creek	34.2643	-87.4256	13.58	12.5	1.2	15.0	23.9	1.59	-	-	-	-	-
Tedford Creek	34.3490	-87.4701	14.99	17.0	1.0	16.4	13.1	0.80	-	-	-	-	-
Owl Creek	34.3089	-87.2659	15.69	14.0	1	14.2	24.9	1.75	1.6	0.88	0.0073	180.0	0.0450
Thompson Creek	34.3458	-87.4689	38.94	19.4	1.1	20.8	26.4	1.27	2.6	0.97	0.0042	250.0	0.0500
Hubbard Creek	34.2673	-87.4589	48.54	18.5	1.8	33.4	36.1	1.08	-	-	-	-	-
Capsey Creek	34.2648	-87.2014	52.09	15.3	1.3	19.7	32.0	1.63	1.1	1.10	0.0039	80.0	0.0410
Turkey Creek	33.7106	-86.6962	71.00	17.3	1.5	25.5	70.8	2.78	5.1	1.26	0.0060	22.0	*
Blackburn Fork Little Warrior River	33.8603	-86.4455	93.50	16.5	1.2	19.0	15.6	0.82	3.1	1.01	0.0010	45.0	0.0390
Five Mile Creek	33.5957	-86.8679	134.00	23.3	2.3	54.1	115.3	2.13	3.3	1.93	0.0016	3.1	*
Village Creek	33.5452	-86.9273	135.00	32.0	1.7	53.0	56.7	1.07	2.0	1.50	0.0007	5.7	*
Sipsey Fork	34.2853	-87.3989	239.00	27.9	3.7	103.4	89.0	0.86	1.9	2.93	0.0008	0.5	*
Clear Creek	34.0802	-87.4227	262.00	38.3	1.5	57.8	89.6	1.55	2.0	1.40	0.0010	0.5	*

Bankfull discharge was derived from the long-term flow record at a USGS gage station.

Table 2.2. Human disturbance gradient (HDG) score and values for variables that compose the HDG for streams that were sampled for fish in the William B. Bankhead National Forest in the southwestern Appalachians ecoregion of Alabama in the spring season of 2014 and 2015.

Stream Name	Human Density (#/km^2)	Road Density (people/km^2)	Road Crossings	Crossings (per km stream length)	% Open Water	% Developed	% Barren	% Forest	% Shrub and Grassland	% Pasture	% Crop	% Wetlands	% Impervious Surface	% Canopy	HDG Score
UT3 Brushy Creek	0.0	0.0	0	0.00	0.0	0.0	0.0	100.0	0.0	0.0	0.0	0.0	0.0	98.8	0.0
UT2 Brushy Creek	0.0	0.0	0	0.00	0.0	0.0	0.0	100.0	0.0	0.0	0.0	0.0	0.0	99.2	0.0
UT Brown (Bushwhack) Creek	0.0	0.7	0	0.00	0.0	1.4	0.0	98.6	0.0	0.0	0.0	0.0	0.0	98.2	16.5
UT W. Fork Beech (Stillwater) Creek	0.0	0.0	0	0.00	0.0	0.0	0.0	97.3	2.7	0.0	0.0	0.0	0.0	93.4	0.0
UT N. Fork Caney (Elephant) Creek	0.0	3.8	0	0.00	0.0	4.1	0.0	95.9	0.0	0.0	0.0	0.0	0.2	98.3	64.6
UT Rush (Melody) Creek	0.0	1.1	0	0.00	0.0	2.6	0.0	97.4	0.0	0.0	0.0	0.0	0.1	98.5	30.5
UT Brushy Creek	0.0	0.2	0	0.00	0.0	2.5	0.0	97.5	0.0	0.0	0.0	0.0	0.1	96.9	21.1
UT Brown Creek	0.0	1.3	0	0.00	0.0	2.4	0.0	97.6	0.0	0.0	0.0	0.0	0.0	98.1	30.1
White Oak Creek	0.0	0.3	0	0.00	0.0	1.6	0.0	98.4	0.0	0.0	0.0	0.0	0.0	99.4	15.2
Rush Creek (Headwaters)	0.0	0.9	0	0.00	0.0	1.9	0.0	98.1	0.0	0.0	0.0	0.0	0.1	98.9	22.4
UT2 Capsey Creek	0.0	1.3	0	0.00	0.0	1.4	0.0	98.0	0.0	0.1	0.0	0.0	0.1	96.5	22.1
North Fork Caney Creek	2.0	1.2	0	0.00	0.0	1.9	0.0	91.7	3.9	2.4	0.1	0.0	0.1	93.6	35.5
Tedford Creek	0.0	0.8	0	0.00	0.0	0.8	0.0	93.2	4.8	0.1	0.0	1.1	0.0	95.1	13.4
Owl Creek	0.0	0.0	0	0.00	0.0	0.9	0.0	94.2	2.7	1.2	0.0	1.0	0.0	95.1	10.7
Thompson Creek	0.0	0.7	0	0.00	0.0	0.7	0.0	96.5	2.4	0.0	0.0	0.4	0.0	96.7	11.0
Hubbard Creek	7.2	1.4	3	0.22	0.1	2.7	0.0	87.4	3.4	5.7	0.3	0.3	0.2	89.2	62.3
Capsey Creek	3.9	1.2	2	0.11	0.0	1.5	0.0	86.2	5.6	6.1	0.1	0.5	0.1	88.4	45.8

Table 2.3. Summary data of physicochemical conditions including mean wetted width (W_{wetted}) measured discharge ($Q_{measured}$) for reference streams in the Southwestern Appalachians ecoregion reference streams of Alabama sampled for fish in the spring season of 2014 and 2015.

Stream name	Mean W_{wetted} (m)	Mean Depth (m)	Max Depth (m)	Mean Flow (m/s)	$Q_{measured}$ (m^3/s)	pH	Conductivity (uS/m)	DO (mg/L)	Temperature (°C)
UT3 Brushy Creek	1.2	0.04	0.17	0.040	0.0004	6.9	15.0	7.63	13.5
UT2 Brushy Creek	1.2	0.05	0.16	0.029	0.0001	7.6	14.0	8.90	15.6
UT Brown (Bushwhack) Creek	1.4	0.05	0.15	0.067	0.0002	6.3	13.0	8.92	16.7
UT W. Fork Beech (Stillwater) Creek	1.2	0.06	0.17	0.040	0.0003	6.3	17.0	7.60	16.5
UT N. Fork Caney (Elephant) Creek	1.1	0.04	0.17	0.017	0.0003	6.9	15.8	8.19	17.0
UT Rush (Melody) Creek	1.3	0.06	0.20	0.073	0.0007	7.6	20.0	8.83	15.9
UT Brushy Creek	1.9	0.08	0.32	0.020	0.0002	7.7	40.0	7.58	18.6
UT Brown Creek	2.1	0.07	0.14	0.020	0.0006	6.5	25.0	6.94	22.2
White Oak Creek	4.1	0.06	0.23	0.019	0.0001	6.9	19.9	7.28	17.8
Rush Creek (Headwaters)	2.4	0.08	0.29	0.144	0.0022	7.7	18.0	8.74	17.5
UT2 Capsey Creek	3.1	0.08	0.22	0.033	0.0010	7.7	21.0	8.69	18.9
North Fork Caney Creek	8.0	0.19	0.49	0.054	0.0455	*	16.4	7.98	19.3
Tedford Creek	7.2	0.26	1.53	0.026	0.0006	7.8	80.0	7.40	21.7
Owl Creek	7.9	0.16	0.57	0.042	0.0198	7.8	24.0	8.17	19.4
Thompson Creek	10.4	0.32	1.02	0.102	0.0160	8.4	70.0	8.42	19.4
Hubbard Creek	10.5	0.15	0.27	0.127	0.1416	7.4	23.0	7.47	23.2
Capsey Creek	12.0	0.40	1.12	0.041	0.0175	7.5	63.7	9.00	18.4

* pH meter malfunctioned

Table 2.4. Fish assemblage richness and diversity measures for reference streams in the William B. Bankhead National Forest in the southwestern Appalachians ecoregion of Alabama. Measures include catch per unit effort (CPUE) and Shannon's diversity (H').

Stream name	Individuals	CPUE	H'	Species richness	Cyprinid richness	Shiner richness	Sucker richness	*Lepomis* spp. richness	Centrarchidae richness	Darter richness	Darter + madtom richness	Terete minnow richness	Lithophil richness	Intolerant species richness
UT3 Brushy Creek	0			0	0	0	0	0	0	0	0	0	0	0
UT2 Brushy Creek	0			0	0	0	0	0	0	0	0	0	0	0
UT Brown (Bushwhack) Creek	20	0.10	0.00	1	1	0	0	0	0	0	0	1	1	0
UT W. Fork Beech (Stillwater) Creek	100	0.55	0.00	1	1	0	0	0	0	0	0	1	1	0
UT N. Fork Caney (Elephant) Creek	4	0.02	0.00	1	1	0	0	0	0	0	0	1	1	0
UT Rush (Melody) Creek	0			0	0	0	0	0	0	0	0	0	0	0
UT Brushy Creek	0			0	0	0	0	0	0	0	0	0	0	0
UT Brown Creek	150	0.48	0.23	3	1	0	1	0	0	1	1	1	2	0
White Oak Creek	170	0.28	0.84	6	2	0	2	1	1	1	1	2	5	0
Rush Creek (Headwaters)	0			0	0	0	0	0	0	0	0	0	0	0
UT2 Capsey Creek	256	0.52	0.33	3	1	0	1	0	0	1	1	1	2	0
North Fork Caney Creek	200	0.17	2.03	13	4	3	1	1	2	4	5	1	9	1
Tedford Creek	171	0.13	1.47	5	2	0	0	1	1	2	2	2	4	0
Owl Creek	320	0.25	2.38	17	5	2	1	3	4	4	4	3	13	0
Thompson Creek	257	0.10	2.26	12	4	3	1	1	2	4	4	1	8	1
Hubbard Creek	361	0.17	2.29	19	6	4	2	2	4	4	5	2	12	1
Capsey Creek	782	0.27	2.05	18	7	5	2	1	3	5	5	2	14	1

Table 2.5. Proportion-based compositional metrics for fish assemblages from reference streams in the William B. Bankhead National Forest in the southwestern Appalachians ecoregion of Alabama in the spring season of 2014 and 2015. Metrics include Black Warrior River endemics (BWE) and Mobile River endemics (ME).

Stream name	% Nest guarding species	% Non-guarding species	% Lithophils	% Generalist spawners	% BWE	% ME	% Widespread	% Dominant	% Tolerant	% *Lepomis cyanellus*	% *Campostoma oligolepis*	% *Lepomis cyanellus + Amierus natalis*	% *Lepomis cyanellus + Lepomis macrochirus + Amierus natalis*	% *Lepomis* spp.
UT3 Brushy Creek	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
UT2 Brushy Creek	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
UT Brown (Bushwhack) Creek	0.0	100.0	100.0	0.0	0.0	0.0	100.0	100.0	100.0	0.0	0.0	0.0	0.0	0.0
UT W. Fork Beech (Stillwater) Creek	0.0	100.0	100.0	0.0	0.0	0.0	100.0	100.0	100.0	0.0	0.0	0.0	0.0	0.0
UT N. Fork Caney (Elephant) Creek	0.0	100.0	100.0	0.0	0.0	0.0	100.0	100.0	100.0	0.0	0.0	0.0	0.0	0.0
UT Rush (Melody) Creek	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
UT Brushy Creek	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
UT Brown Creek	0.0	100.0	100.0	0.0	0.0	4.7	95.3	95.7	95.7	0.0	0.0	0.0	0.0	0.0
White Oak Creek	0.6	99.4	99.4	0.6	0.0	22.4	77.6	75.8	75.8	0.0	3.3	0.0	0.0	1.1
Rush Creek (Headwaters)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
UT2 Capsey Creek	0.0	100.0	100.0	0.0	0.0	9.0	91.0	89.7	89.7	0.0	0.0	0.0	0.0	0.0
North Fork Caney Creek	9.0	91.0	49.0	5.0	1.0	66.5	33.5	44.7	11.7	0.0	0.0	1.0	1.0	8.7
Tedford Creek	8.2	91.8	92.4	7.6	0.0	12.9	87.1	56.9	56.9	0.0	25.9	0.0	0.0	2.6
Owl Creek	22.2	77.8	88.4	8.1	0.0	21.6	78.4	28.3	44.9	1.1	7.5	2.7	3.7	4.8
Thompson Creek	17.5	82.5	71.2	14.4	2.3	50.6	49.4	28.2	0.0	0.0	28.2	0.0	0.0	12.1
Hubbard Creek	11.1	88.9	65.4	8.9	1.4	37.4	62.6	33.5	4.4	0.6	1.2	1.2	1.2	13.7
Capsey Creek	5.8	94.2	77.1	3.1	0.6	36.2	63.8	31.6	2.7	0.0	31.6	0.0	0.0	4.6

Table 2.5. Continued

Stream name	% Omnivores	% Insectivorous cyprinid	% Invertivore	% Top piscivores	% Simples lithophils	% Manipulative lithophils	% Lithophils	% Simple miscellaneous	% Manipulative miscellaneous	% Manipulative spawners	% Miscellaneous spawners	% Simple Lithophils + tolerants	% *Semotilus atromaculatus*
UT3 Brushy Creek	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
UT2 Brushy Creek	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
UT Brown (Bushwhack) Creek	0.0	0.0	0.0	0.0	0.0	100.0	100.0	0.0	0.0	100.0	0.0	100.0	100.0
UT W. Fork Beech (Stillwater) Creek	0.0	0.0	0.0	0.0	0.0	100.0	100.0	0.0	0.0	100.0	0.0	100.0	100.0
UT N. Fork Caney (Elephant) Creek	0.0	0.0	0.0	0.0	0.0	100.0	100.0	0.0	0.0	100.0	0.0	100.0	100.0
UT Rush (Melody) Creek	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
UT Brushy Creek	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
UT Brown Creek	0.0	0.0	1.4	0.0	0.0	97.1	97.1	2.9	0.0	97.1	2.9	95.7	95.7
White Oak Creek	3.3	0.0	4.4	0.0	1.1	83.5	84.6	15.4	0.0	83.5	15.4	76.9	75.8
Rush Creek (Headwaters)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
UT2 Capsey Creek	0.0	0.0	0.7	0.0	0.0	90.3	90.3	9.7	0.0	90.3	9.7	89.7	89.7
North Fork Caney Creek	0.0	56.3	54.4	1.9	23.3	21.4	44.7	51.5	3.9	25.2	55.3	35.0	10.7
Tedford Creek	25.9	0.0	2.6	0.0	0.9	85.3	86.2	13.8	0.0	85.3	13.8	57.8	56.9
Owl Creek	9.1	32.6	1.6	1.1	20.3	64.2	84.5	10.2	5.4	69.5	15.5	65.2	12.8
Thompson Creek	28.2	41.6	38.3	2.0	21.5	42.3	63.8	36.2	0.0	42.3	36.2	21.5	0.0
Hubbard Creek	1.2	30.4	33.5	1.9	56.5	19.3	75.8	21.1	3.1	22.4	24.2	60.9	3.1
Capsey Creek	31.6	37.6	25.1	4.6	35.7	43.4	79.1	20.9	0.0	43.4	20.9	38.4	0.8

Table 2.6. Holm-corrected Pearson correlation coefficients (r) for the relationships between fish assemblage metrics and stream size or geomorphology where at least one relationship was significant for reference streams in the William B. Bankhead National Forest in the southwestern Appalachians ecoregion of Alabama and were sampled in the spring season of 2014 and 2015.

Assemblage metric	Drainage Area	W_{bkf}	D_{bkf}	A_{bkf}	Q_{bkf}	W_{wetted}	$Q_{measured}$
Shannon's *H'*	__0.96**__	__0.96*__	__0.96*__	__0.97*__	__0.99***__	__0.96*__	0.87
Species richness	__0.90**__	0.88	__0.88*__	__0.90*__	__0.92**__	__0.90**__	__0.88*__
Cyprinid richness	__0.89*__	0.87	__0.89*__	0.88	__0.91*__	__0.88*__	0.84
Centrarchid richness	0.86	0.85	0.85	0.87	__0.90*__	0.86	0.85
Shiner richness	0.84	0.77	0.77	0.80	0.84	0.81	__0.88*__
Darter richness	__0.95***__	__0.92**__	__0.90*__	__0.92**__	__0.95***__	__0.94***__	__0.88**__
Darter and Madtom richness	__0.94***__	__0.90*__	__0.91*__	__0.92**__	__0.95***__	__0.93***__	__0.91**__
Lithophil richness	__0.89*__	0.87	0.87	__0.89*__	__0.92**__	__0.90**__	__0.84*__
% Dominant species	__-0.97**__	__-0.97**__	-0.94	__-0.97**__	__-0.98***__	__-0.99***__	-0.81
% Tolerant species	__-0.96*__	-0.92	-0.93	-0.94	-0.95	__-0.95*__	-0.85
% Simple lithophils	0.87	0.79	0.91	0.79	0.88	0.84	__0.95*__
% Simple lithophils and tolerant species	-0.93	-0.95	-0.89	-0.93	-0.94	__-0.97**__	-0.74
% *Semotilus atromaculatus*	__-0.97**__	-0.93	-0.95	-0.95	__-0.97**__	__-0.97**__	-0.88

*, **, and *** indicate p-values < 0.05, < 0.01, and < 0.001 respectively

Table 2.7. Index of Biotic Integrity sub-metric and total scores for reference streams in the William B. Bankhead National Forest in the southwestern Appalachians ecoregion of Alabama sampled in the spring season of 2014 and 2015.

Stream name	Species Richness	Cyprinid Richness	Sucker Richness	*Lepomis* spp. Richness	Darter + Madtom Richness	% Dominant	% Tolerant	% *Lepomis* spp.	% Omnivores	% Top Piscivores	% DELT	% Simple Lithophils	Total IBI Score
UT3 Brushy Creek	-	-	-	-	-	-	-	-	-	-	-	-	-
UT2 Brushy Creek	-	-	-	-	-	-	-	-	-	-	-	-	-
UT Brown (Bushwhack) Creek	5	5	5	3	5	1	1	5	5	1	5	1	42
UT W. Fork Beech (Stillwater) Creek	5	5	5	3	5	1	1	5	5	1	5	1	42
UT N. Fork Caney (Elephant) Creek	5	5	5	3	5	1	1	5	5	1	5	1	42
UT Rush (Melody) Creek	-	-	-	-	-	-	-	-	-	-	-	-	-
UT Brushy Creek	-	-	-	-	-	-	-	-	-	-	-	-	-
UT Brown Creek	5	5	5	1	5	1	1	5	5	1	5	1	40
White Oak Creek	5	5	5	3	3	1	1	5	5	1	5	1	40
Rush Creek (Headwaters)	-	-	-	-	-	-	-	-	-	-	-	-	-
UT2 Capsey Creek	5	5	5	1	3	1	1	5	5	1	5	1	38
North Fork Caney Creek	3	3	3	1	5	3	5	5	5	3	5	3	44
Tedford Creek	1	1	1	1	3	1	1	5	3	1	5	1	24
Owl Creek	5	3	3	3	5	3	1	5	5	3	5	3	44
Thompson Creek	3	3	1	1	3	3	5	3	3	3	5	3	36
Hubbard Creek	3	3	3	1	5	3	5	3	5	3	5	5	44
Capsey Creek	3	3	3	1	5	3	5	5	1	5	5	3	42

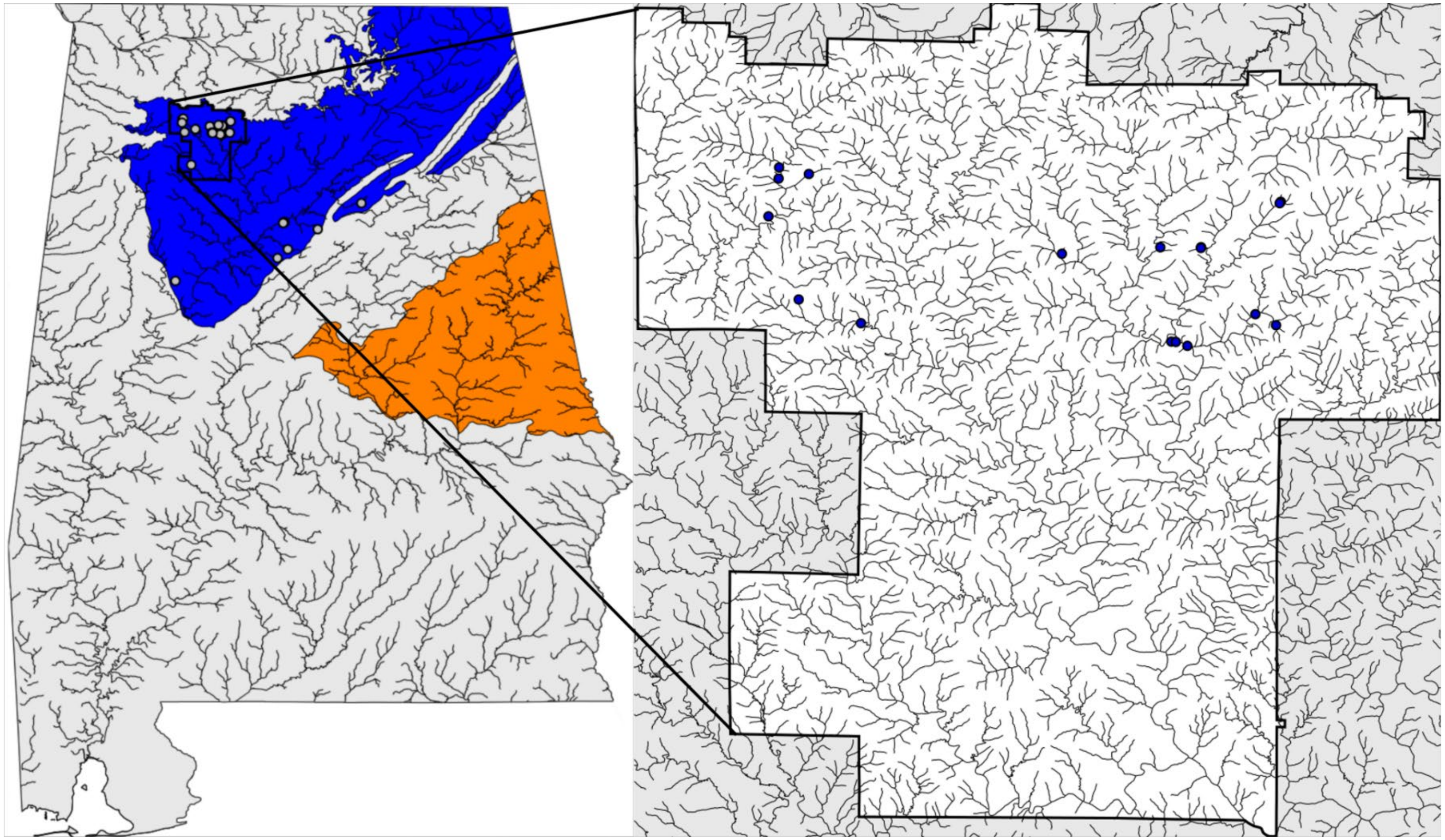


Figure 2.1. Map of Alabama (left) with Piedmont (orange) and southwestern Appalachians (blue) ecoregions highlighted and showing the locations of the William B. Bankhead national forest (black outline) and streams sampled for geomorphology (gray circles), and a map of the William B. Bankhead National Forest (right) and showing the location of streams sampled for fish (blue circles).

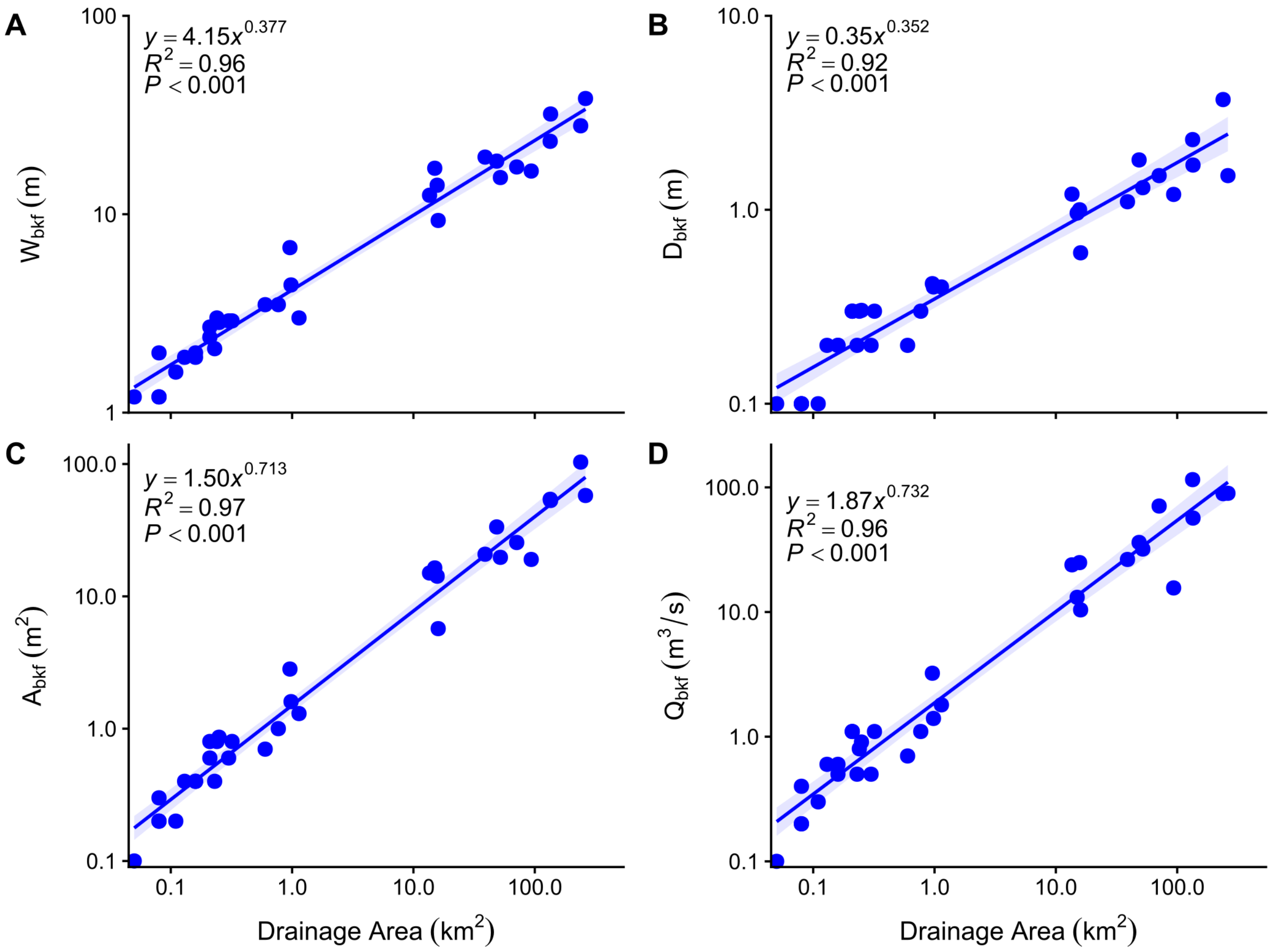


Figure 2.2. Regional curves of the relationships between drainage area and bankfull width ($\text{W}_{\text{bkf;}}$; A), bankfull depth (D_{bkf}; B), bankfull cross-sectional area (A_{bkf}; C), and estimate bankfull discharge (Q_{bkf}; D) for reference streams of the southwestern Appalachians ecoregion of Alabama (blue circles).

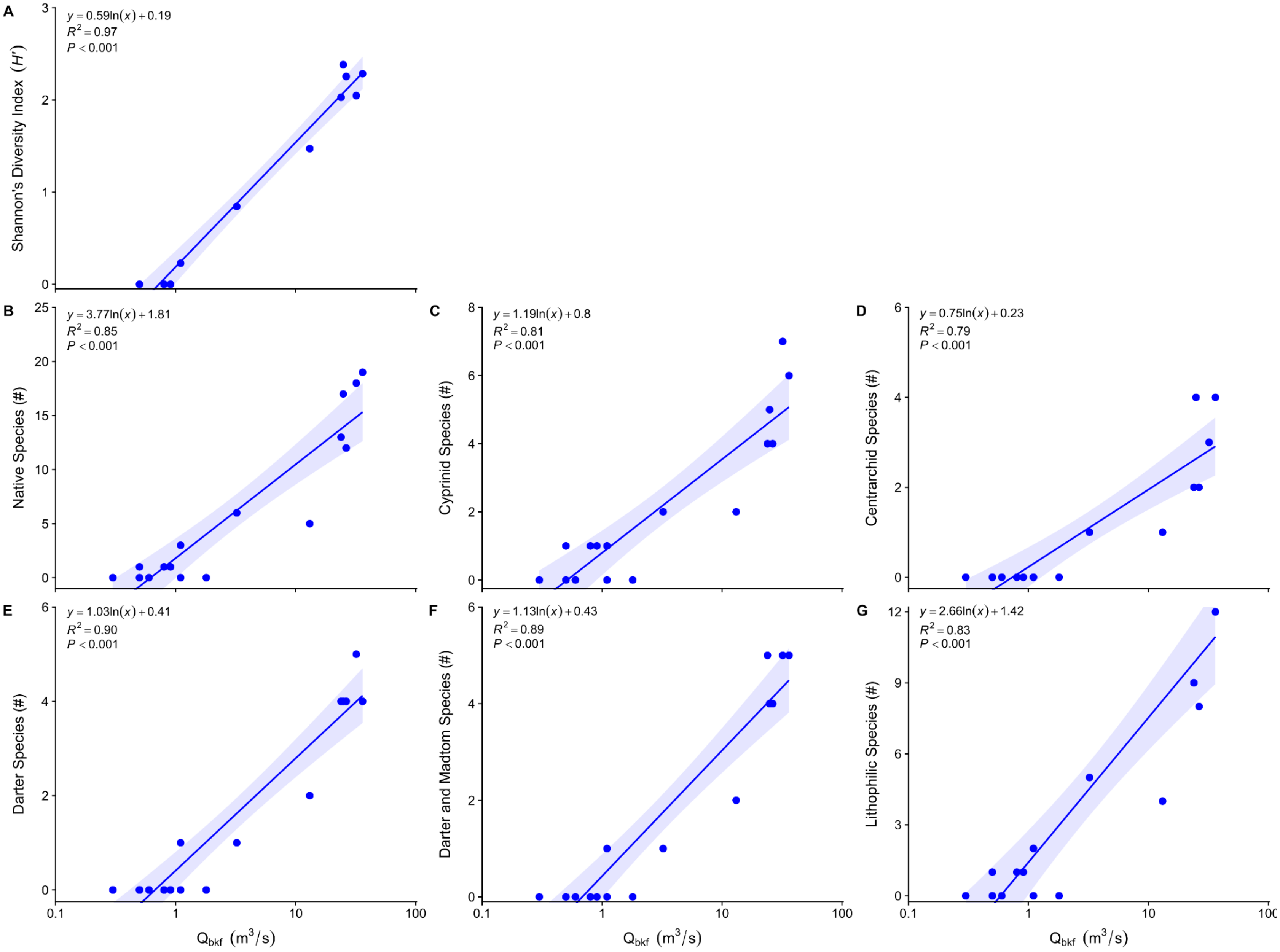
A
$y = 0.59\ln(x) + 0.19$
$R^2 = 0.97$
$P < 0.001$
Shannon's Diversity Index (H′)
0
1
2
3
B
$y = 3.77\ln(x) + 1.81$
$R^2 = 0.85$
$P < 0.001$
Native Species (#)
0
5
10
15
20
25
C
$y = 1.19\ln(x) + 0.8$
$R^2 = 0.81$
$P < 0.001$
Cyprinid Species (#)
0
2
4
6
8
D
$y = 0.75\ln(x) + 0.23$
$R^2 = 0.79$
$P < 0.001$
Centrarchid Species (#)
0
2
4
6
E
$y = 1.03\ln(x) + 0.41$
$R^2 = 0.90$
$P < 0.001$
Darter Species (#)
0
2
4
6
F
$y = 1.13\ln(x) + 0.43$
$R^2 = 0.89$
$P < 0.001$
Darter and Madtom Species (#)
0
2
4
6
G
$y = 2.66\ln(x) + 1.42$
$R^2 = 0.83$
$P < 0.001$
Lithophilic Species (#)
0
3
6
9
12
0.1
1
10
100
Q_{bkf} (m^3/s)

Figure 2.3. Ecological endpoint curves of the relationships between estimated bankfull discharge (Q_{bkf}) and Shannon's diversity index (*H'*; A), native species richness (B), cyprinid species richness (C), centrarchid species richness (D), darter species richness (E), darter and madtom species richness (F), and lithophilic spawner species richness (G) for reference streams of the southwestern Appalachians ecoregion of Alabama (blue circles).

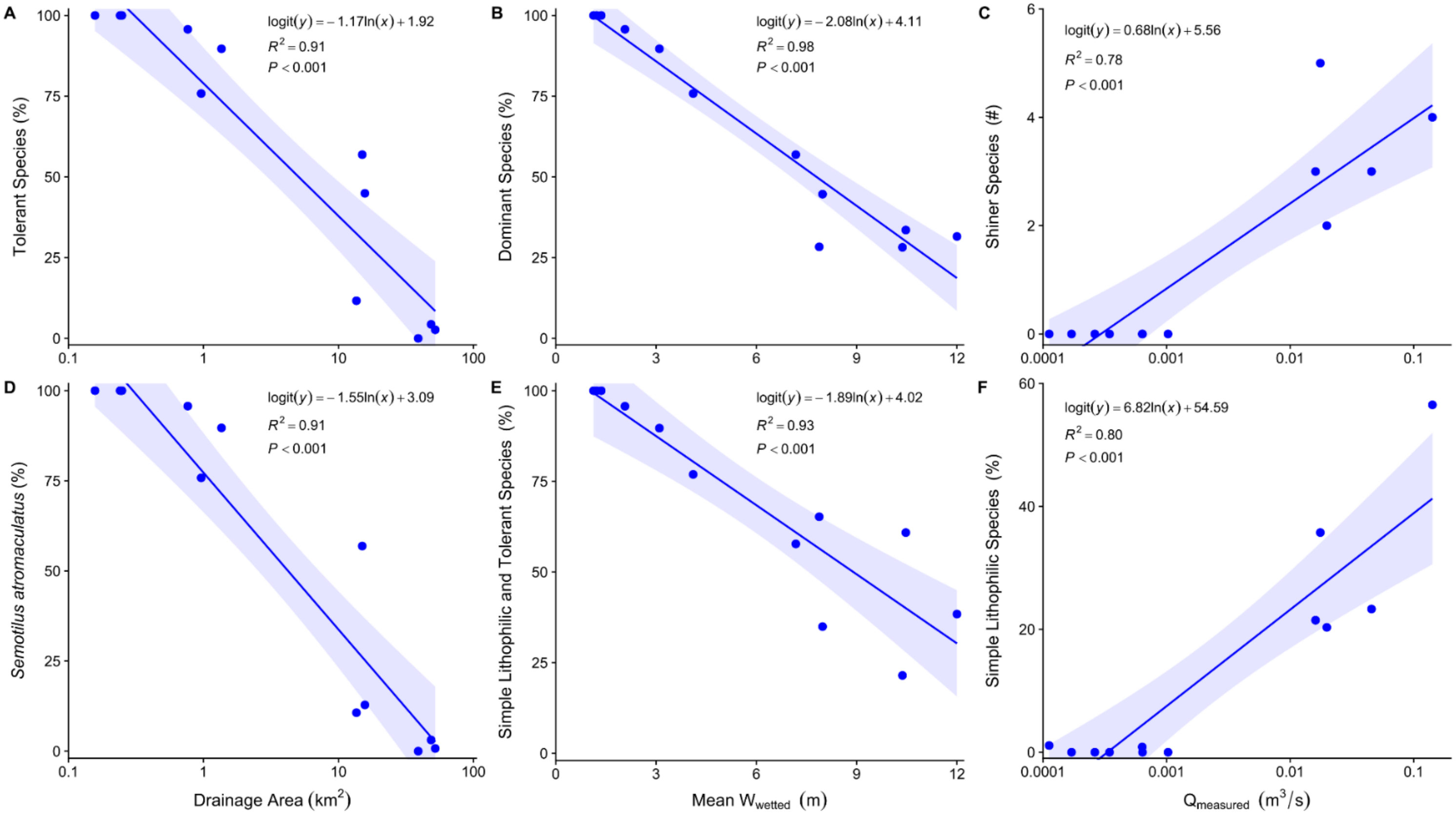


Figure 2.4. Ecological endpoint curves of the relationships between % tolerant species and drainage area (A), % dominant species and mean wetted width (mean W_{wetted}; B), shiner species richness and measured discharge (Q_{measured}; C), % Creek Chub (*Semotilus atromaculatus*) and drainage area (D), % simple lithophilic spawners and tolerant species and mean wetted width (E), and % simple lithophilic spawners and measured discharge (F) for reference streams sampled in the William B. Bankhead National Forest within the southwestern Appalachians ecoregion of Alabama (blue circles).

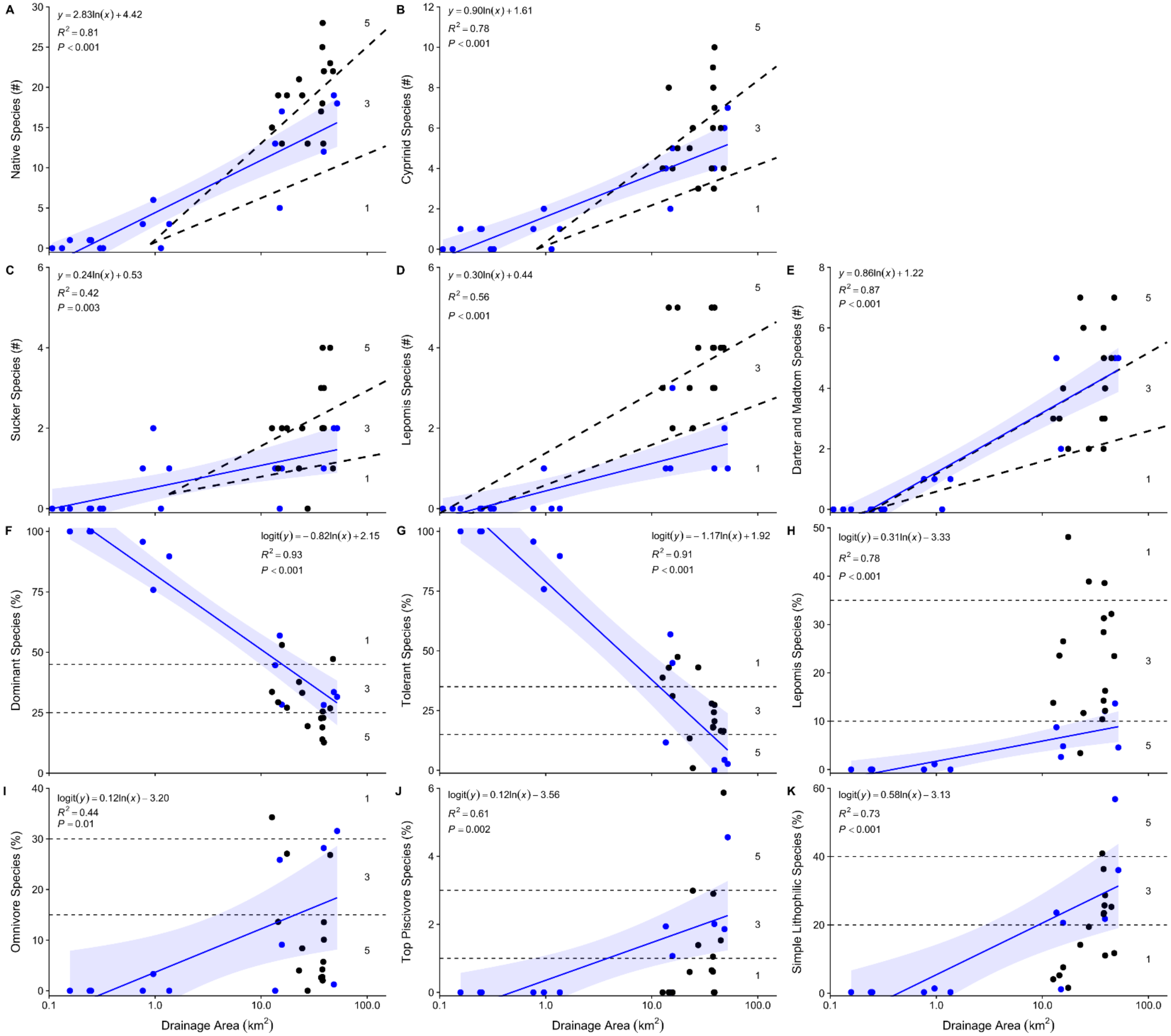

A
$y = 2.83\ln(x) + 4.42$
$R^2 = 0.81$
$P < 0.001$
Native Species (#)
B
$y = 0.90\ln(x) + 1.61$
$R^2 = 0.78$
$P < 0.001$
Cyprinid Species (#)
C
$y = 0.24\ln(x) + 0.53$
$R^2 = 0.42$
$P = 0.003$
Sucker Species (#)
D
$y = 0.30\ln(x) + 0.44$
$R^2 = 0.56$
$P < 0.001$
Lepomis Species (#)
E
$y = 0.86\ln(x) + 1.22$
$R^2 = 0.87$
$P < 0.001$
Darter and Madtom Species (#)
F
$\text{logit}(y) = -0.82\ln(x) + 2.15$
$R^2 = 0.93$
$P < 0.001$
Dominant Species (%)
G
$\text{logit}(y) = -1.17\ln(x) + 1.92$
$R^2 = 0.91$
$P < 0.001$
Tolerant Species (%)
H
$\text{logit}(y) = 0.31\ln(x) - 3.33$
$R^2 = 0.78$
$P < 0.001$
Lepomis Species (%)
I
$\text{logit}(y) = 0.12\ln(x) - 3.20$
$R^2 = 0.44$
$P = 0.01$
Omnivore Species (%)
J
$\text{logit}(y) = 0.12\ln(x) - 3.56$
$R^2 = 0.61$
$P = 0.002$
Top Piscivore Species (%)
K
$\text{logit}(y) = 0.58\ln(x) - 3.13$
$R^2 = 0.73$
$P < 0.001$
Simple Lithophilic Species (%)
5
3
1
0.1
1.0
10.0
100.0
Drainage Area (km^2)

Figure 2.5. Relationships between 11 Appalachian Plateau (AP) fish IBI sub-metrics and drainage area (blue lines), with confidence intervals (blue shading), for reference streams in the William B. Bankhead National Forest and southwestern Appalachians ecoregion of Alabama (blue circles) with AP fish IBI scoring thresholds (black dashed lines) and least-disturbed streams of the southwestern Appalachians ecoregion of Alabama sampled by the Geological Survey of Alabama (black circles) plotted for comparison. Fish IBI submetrics include native species richness (A), cyprinid species richness (B), sucker species richness (C), *Lepomis* spp. richness (D), darter and madtom richness (E), % dominant species (F), % tolerant species (G), % *Lepomis* spp. (H), % omnivores (I), % top piscivores (J), and % simple lithophilic spawning species (K).

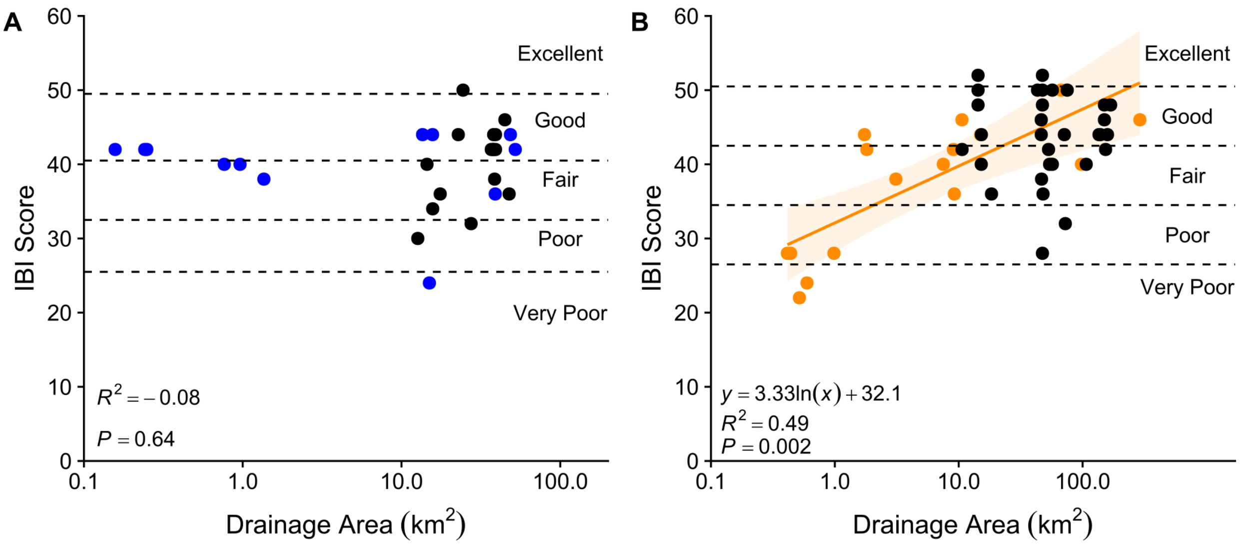
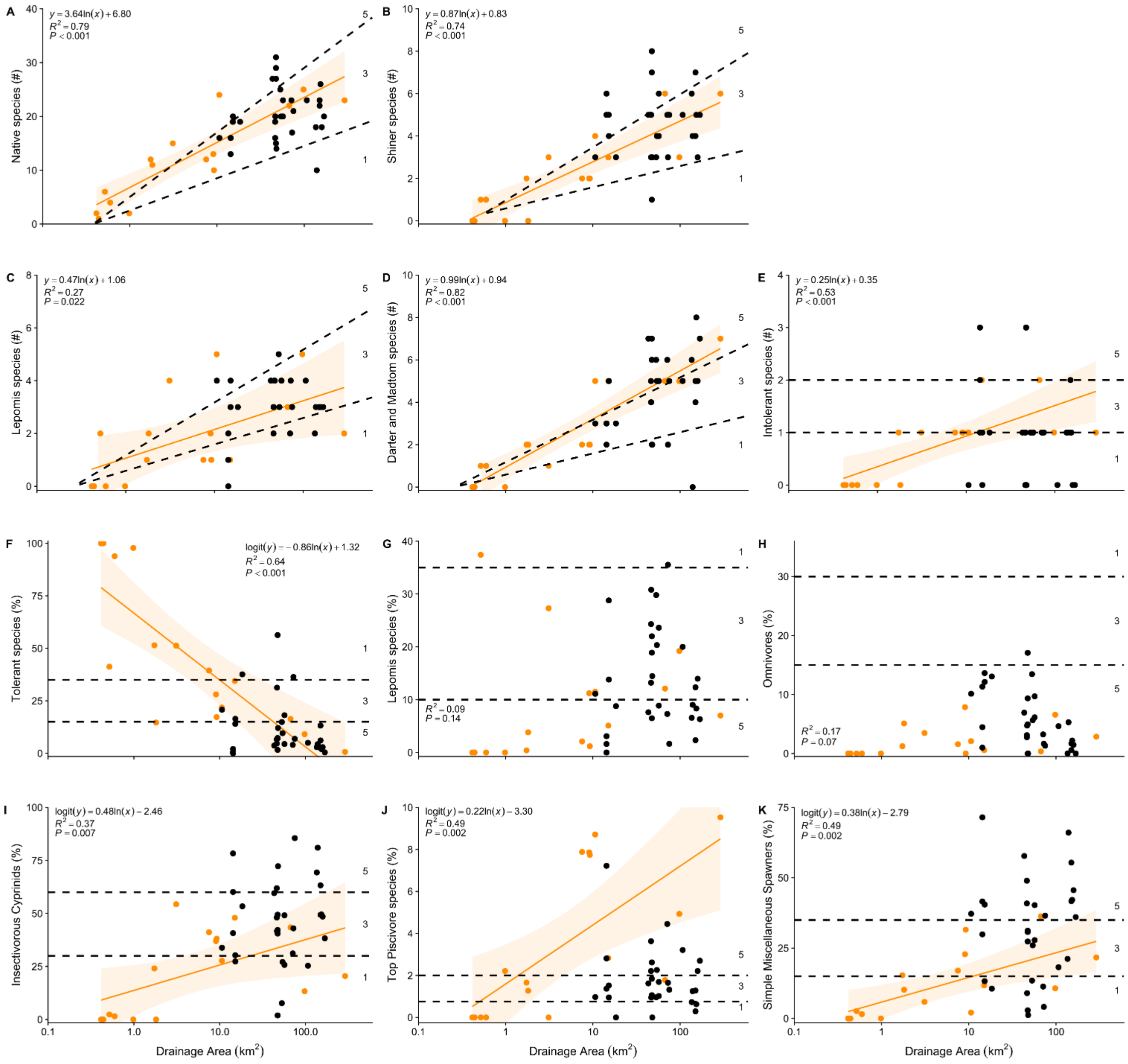


Figure 2.6. Relationships between drainage area and overall IBI scores for reference streams in A) the William B. Bankhead National Forest within the southwestern Appalachians ecoregion of Alabama (blue circles) with Appalachian Plateau fish IBI thresholds (black dashed lines) and least-disturbed streams in the southwestern Appalachians ecoregion of Alabama sampled by the Geological Survey of Alabama (black circles) plotted for comparison and B) the Piedmont ecoregion of Alabama (orange circles) with Piedmont fish IBI scoring thresholds (black dashed lines) and least-disturbed streams in the Piedmont ecoregion sampled by the Geological Survey of Alabama (black circles) for comparison.



A
y = 3.64ln(x) + 6.80
R² = 0.79
P < 0.001
Native species (#)
0
10
20
30
40
5
3
1
B
y = 0.87ln(x) + 0.83
R² = 0.74
P < 0.001
Shiner species (#)
0
2
4
6
8
10
5
3
1
C
y = 0.47ln(x) + 1.06
R² = 0.27
P = 0.022
Lepomis species (#)
0
2
4
6
8
5
3
1
D
y = 0.99ln(x) + 0.94
R² = 0.82
P < 0.001
Darter and Madtom species (#)
0
2
4
6
8
10
5
3
1
E
y = 0.25ln(x) + 0.35
R² = 0.53
P < 0.001
Intolerant species (#)
0
1
2
3
4
5
3
1
F
logit(y) = − 0.86ln(x) + 1.32
R² = 0.64
P < 0.001
Tolerant species (%)
0
25
50
75
100
1
3
5
G
R² = 0.09
P = 0.14
Lepomis species (%)
0
10
20
30
40
1
3
5
H
R² = 0.17
P = 0.07
Omnivores (%)
0
10
20
30
1
3
5
I
logit(y) = 0.48ln(x) − 2.46
R² = 0.37
P = 0.007
Insectivorous Cyprinids (%)
0
25
50
75
100
5
3
1
0.1
1.0
10.0
100.0
Drainage Area (km²)
J
logit(y) = 0.22ln(x) − 3.30
R² = 0.49
P = 0.002
Top Piscivore species (%)
0
2
4
6
8
10
5
3
1
0.1
1
10
100
Drainage Area (km²)
K
logit(y) = 0.38ln(x) − 2.79
R² = 0.49
P = 0.002
Simple Miscellaneous Spawners (%)
0
25
50
75
5
3
1
0.1
1
10
100
Drainage Area (km²)

Figure 2.7. Relationships between 11 Ridge and Valley/Piedmont region fish IBI sub-metrics and drainage area (orange lines), with confidence intervals (orange shading), for reference streams in the Piedmont ecoregion of Alabama (orange circles) with Piedmont fish IBI scoring thresholds (black dashed lines) and least-disturbed streams of the Piedmont ecoregion of Alabama sampled by the Geological Survey of Alabama (black circles) plotted for comparison. Fish IBI submetrics include native species richness (A), shiner species richness (B), *Lepomis* spp. richness (C), darter and madtom richness (D), intolerant species richness (E), % tolerant species (F), % *Lepomis* spp. (G), % omnivores (H), % insectivorous cyprinids (I), % top piscivores (J), and % simple miscellaneous substrate spawning species (K).

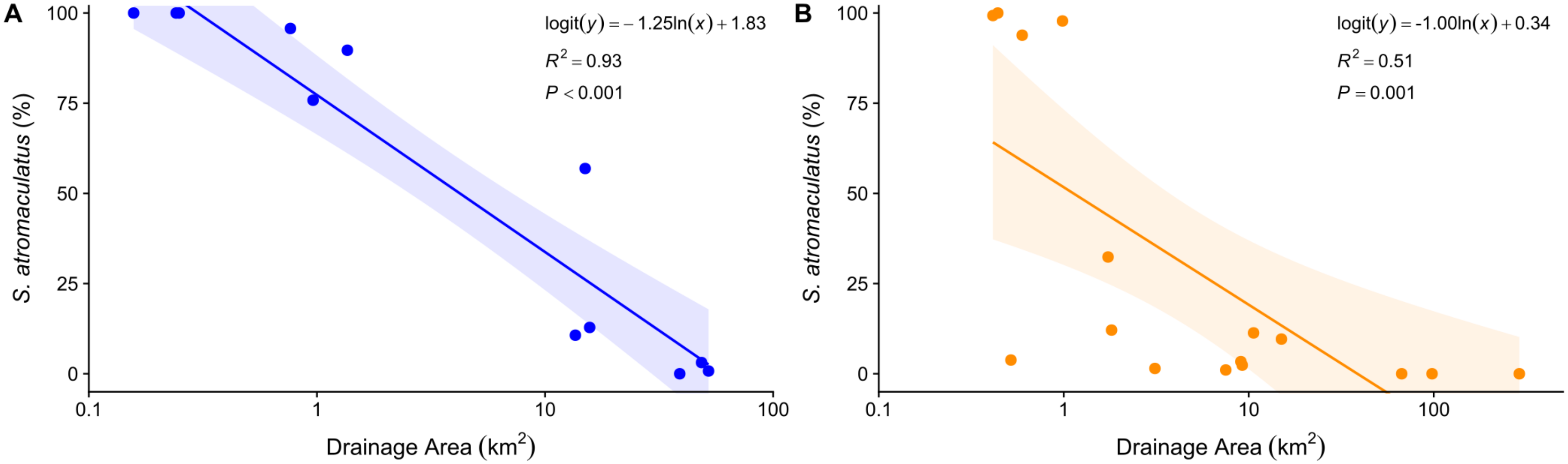


Figure 2.8. Relationships between drainage area and % Creek Chub (*Semotilus atromaculatus*) for A) the southwestern Appalachian (blue circles, line, and shading) and B) the Piedmont ecoregion of Alabama (orange circles, line, and shading).

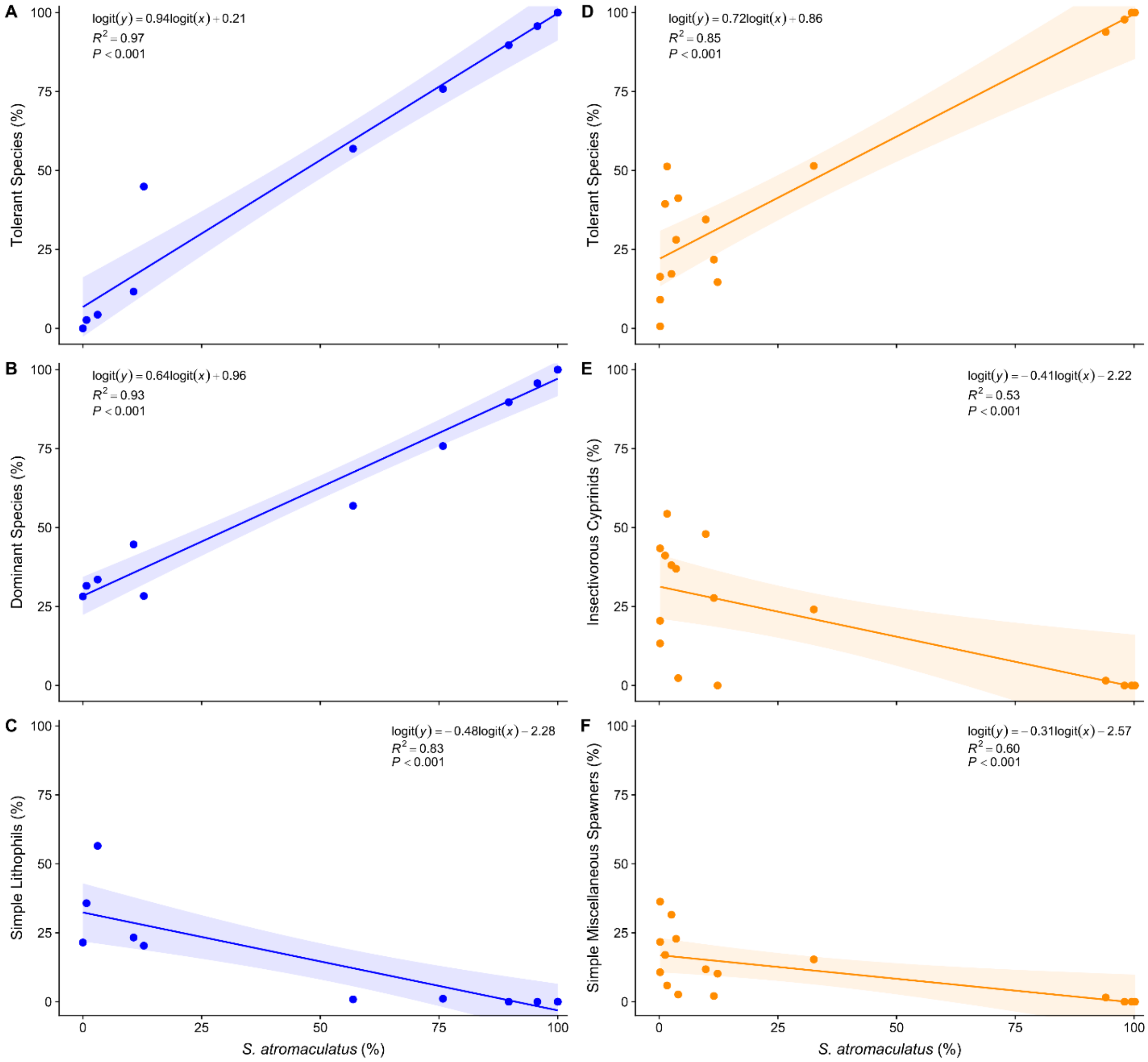


Figure 2.9. Relationships between % Creek Chub (*Semotilus atromaculatus*) and fish IBI submetrics for the southwestern Appalachians (A-C) and Piedmont ecoregions (D-F). Fish IBI sub-metrics for the southwestern Appalachian ecoregion of Alabama includes % tolerant species (A), % dominant species (B), and % simple lithophilic spawning species (C), and the fish IBI submetrics for the Piedmont ecoregion of Alabama includes % tolerant species (D), % insectivorous cyprinids (E), and % simple miscellaneous substrate spawning species (F).

Chapter 3. Spatial patterns of fish and macroinvertebrate assemblages in a biodiversity hotspot: an intraregional test of the river continuum concept and mighty headwater hypothesis

ABSTRACT

The River Continuum Concept (RCC) proposes that physical environmental gradients from headwaters to rivers drive a series of predictable changes in aquatic communities, including an increase in richness (α-diversity) with increasing stream size. In contrast, Mighty Headwater Hypothesis (MHH) predicts community dissimilarity (β-diversity) decreases with stream size because of the spatial isolation and greater habitat heterogeneity among headwaters relative to mid-order streams. I investigated potential stream size – biodiversity relationships of fish and benthic macroinvertebrate assemblages in streams of the Southwestern Appalachians ecoregion of Alabama. Specifically, I predicted that α-diversity would be positively related to stream size, upstream communities would be nested subsets of richer downstream communities, nestedness would be related to stream size, and β-diversity would be highest among headwater streams. I sampled streams in the Bankhead National Forest of Alabama for fish (n = 12) and macroinvertebrates (n = 17) and collected data on a suite of habitat variables. Stream size was positively related to richness of fish assemblages and Ephemeroptera but was unrelated to overall richness and most subsets of macroinvertebrates assemblages. Fish and macroinvertebrate assemblages exhibited nestedness; fish assemblage nestedness generally was stronger than macroinvertebrate assemblage nestedness, except for Ephemeroptera and Trichoptera; nestedness also was associated with stream size in most fishes, Ephemeroptera, and Plecoptera. Mean β-diversity within stream size categories either did not differ among stream size categories or β-diversity was higher in larger streams for subsets of fishes and macroinvertebrates. These results

support the predictions of the RCC in fishes and Ephemeroptera but not for other macroinvertebrate groups. In contrast, there was no support for the predictions of the MHH in any group. Fish conservation efforts should focus on species-rich larger streams rather than headwaters, whereas conservation of headwaters may be important for macroinvertebrates because macroinvertebrate richness was independent of stream size and headwater streams outnumber larger streams in a network.

INTRODUCTION

Conservation priorities and effectiveness of different management strategies can be influenced by spatial patterns of local taxa richness (α-diversity; Allan and Flecker 1993), nestedness (Malmqvist et al. 1999) and compositional dissimilarity among locations (β-diversity). For example, if communities in a region or stream network exhibit a high degree of nestedness where assemblages in species-poor sites are predictable subsets of those in species-rich sites, then most species can be conserved by focusing on managing rich sites (Malmqvist et al. 1999). However, if among-site differences manifest as some species being replaced by others (i.e., high β-diversity), then conservation plans should encompass multiple sites and unique contributions of each site to maintain high regional richness (Clarke et al. 2008). These two patterns are considered to be the two opposing factors determining community composition; when nestedness is high then replacement is low and vice-versa (Leibold and Mikkelson 2002).

In stream networks, several conceptual models of biodiversity have been proposed, beginning with the River Continuum Concept (RCC; Vannote et al. 1980). The RCC suggested that the humped shape pattern of α-diversity along the river course, where headwaters show low richness and richness peaks in 5th and 6th order streams before declining in larger rivers, as a result of environmental heterogeneity and energy sources increasing available niches and promoting greater coexistence (Vannote et al. 1980). However, this linear perspective of a stream or river potentially obfuscates the contribution of headwaters to regional diversity (Clarke et al. 2008). More recently the Mighty Headwater Hypothesis (MHH; Finn et al. 2011), which accounts for the branching nature of stream networks, has been proposed as a complement to the RCC (Finn et al. 2011). The MHH posits that spatial isolation and habitat dissimilarity are higher among headwaters (1st and 2nd order streams) than in larger streams, resulting in β-diversity peaking in headwaters and declining with increasing stream size (Finn et al. 2011). Headwaters are considered to have the highest among-stream habitat dissimilarity of any stream type because local factors (e.g., bedrock type, groundwater inputs, etc.) can more easily influence instream conditions as a result of their small watersheds (Lowe and Likens 2005; Meyer et al. 2007). This habitat dissimilarity among streams is expected to result in parallel dissimilarity among ecological communities because of the evolution of species traits to match their habitat templet (sensu Southwood 1977, Townsend and Hildrew 1994), and some habitat characteristics acting to prevent the establishment of (i.e., filter) some species (Poff 1997). Stream habitat and associated macroinvertebrate assemblages can be spatially autocorrelated, where nearby sites share more similar habitats than distant sites, as close proximity increases chances of shared conditions (Lloyd et al. 2005).

In an extensive review of stream size – biodiversity relationships, Vorste et al. (2017) reported that fish richness increased with stream size whereas macroinvertebrate richness was rarely related to stream size, suggesting support for the RCC in fishes but not macroinvertebrates. Vorste et al. (2017) also found mean macroinvertebrate β-diversity to increase with stream size, contradicting the predictions the MHH and the observation by Finn et al., (2011) that mean β-diversity among headwater streams (1st and 2nd order) was greater than those among mid-order streams (3rd and 4th order) across several regions in the US and Europe. However, Finn et al. (2011) explicitly called for more intraregional comparisons to confirm patterns predicted by the MHH because comparisons of β-diversity among headwaters and mid-order streams within ecoregions were not significant and sample sizes were small. There have been many studies testing the predictions of the RCC using fish and macroinvertebrate assemblages and a few that tested the predictions of the MHH (Finn et al. 2011; Vorste et al. 2017), but my study was designed to explicitly test both of these predictions in streams of the Southwestern Appalachians ecoregion, an aquatic biodiversity hotspot (Lydeard and Mayden 1995).

My primary objective was to test for 1) positive stream size – α-diversity relationships and nestedness and 2) negative relationships between stream size and β-diversity as predicted by the RCC and MHH, respectively. Related to 2, I also tested the MHH assumption that habitat dissimilarity would be higher among low-order streams than among high-order. As a secondary objective, I quantified habitat heterogeneity – biodiversity relationships to explore potential mechanisms driving biodiversity patterns in this ecoregion.

METHODS

Stream reach selection and quantifying stream size

Study streams were in the Southwestern Appalachians ecoregion within the William B. Bankhead National Forest (Fig. 3.1). Streams drained heavily forested watersheds containing either hemlock (*Tsuga canadensis*) or mixed hardwood forests with well-vegetated flood plains. White oak (*Quercus alba*) and beech (*Fagus grandifolia*) were the primary canopy species associated with hemlock dominated riparian zones, whereas mixed hardwood stands were primarily white oak, beech, chestnut oak (*Quercus prinus*), and red maple (*Acer rubrum,* Brantley et al. 2016). Seventeen streams were selected to be quantified for stream biota based on stream size. Drainage area (DA) was used as a continuous measure of stream size, derived watershed delineation in GRASS GIS (Neteler and Mitasova 2008). For equal representation of stream sizes, at least 3 streams were selected from each of the following size categories; < 0.26, 0.26 - 2.59, 2.60 - 25.9, > 25.9 km^2. Small streams were generally well-shaded whereas larger streams often had reduced canopy cover. Representative stream reaches were selected by consensus based on the following criteria: minimal bank erosion, free-form meander patterns, unconfined lengths of at least 20 times bankfull width, stable channels, and well-connected alluvial flood plains (sensu Helms et al. 2016). Sampled reach lengths were 20 times wetted width so that sampling effort was proportional to stream size and reaches were at least 100 m upstream of the nearest road crossing, to minimize effects of bridges and culverts. Reach lengths ranged from 150 m to 300 m long, and contained at least 3 riffles-runs-pools sequences (Barbour et al. 1999; Zink et al. 2012). Bed substrates of most reaches were mostly shallow gravel and cobble overlying bedrock.

Physicochemical measurements

Instream physicochemical habitat parameters were quantified at the reach scale to correspond with habitat sampled for fish and conditions at the time of animal collections. Water conductivity and pH were measured with a C66 Sharp meter (Milwaukee Instruments Inc., Rocky Mount, NC, USA), and dissolved oxygen and water temperature with a YSI 55 meter

(Yellow Springs Instruments, Yellow Springs, OH, USA) placed at the downstream end of each study reach. Hydrological conditions were measured using a Hach-FH950 Portable Flow Meter (Hach, Loveland, CO, USA) by averaging depth, wetted width, and flow at 5 points along 10 equidistant transects along each stream reach. Stream discharge was estimated using the incremental method (Gore 1996) with current measured by a Hach-FH950 Portable Flow Meter (Hach, Loveland, CO, USA) at the downstream end of each study reach. Mean, maximum, and coefficient of variation of depth and flow were calculated for transects and sampling points where benthic macroinvertebrate samples were collected (below). In addition, depth and current velocity were measured at each macroinvertebrate sampling point, and percent inorganic (e.g., % boulder, % cobble etc.) and organic substrate (e.g., % fine particulate organic matter, % course particulate organic matter) of each sampled riffle from each reach were visually estimated to give an assessment of micro-scale habitat conditions (modified from Barbour et al. 1999). Shannon diversity (*H'*) was used to estimate diversity of both organic and inorganic substrates at the reach scale (sensu Boyero 2003) as habitat diversity, including substrate diversity, often is positively related to macroinvertebrate richness (Vinson and Hawkins 1998; Boyero 2003). Total substrate diversity was estimated by summing both inorganic and organic substrate diversity.

Biotic sampling

Biota were sampled from stream reaches from June 13 to July 10 in 2014 and May 12 to June 5 in 2015, during the summer baseflow period. Stream reaches were delineated with small-mesh block seines at the downstream terminus, 1/3 reach length, and the upstream terminus. Habitats (riffles, runs, and pools) in each reach were sampled for fishes with a backpack electroshocker (Barbour et al. 1999). A 3-person team electroshocked reaches, working in an upstream direction, collecting stunned animals with dip nets (sensu Barbour et al. 1999); all fishes were collected in a single pass from the entire reach, with 2 additional passes made in the downstream 1/3 of the reach to increase probability of capturing rare or elusive fish species. All, individuals were collected, preserved, identified to species, and deposited in the Auburn University Museum of Natural History.

Benthic macroinvertebrates were sampled from 3 representative riffles per reach using a Surber sampler (0.09 m^2, 250 µm mesh); riffles were chosen because macroinvertebrate abundance and diversity is typically higher in riffles than other habitat types (Plafkin et al. 1989; Barbour et al. 1999; Feminella 2000). Three Surber samples per riffle were combined resulting in a 0.27 m^2 sample per riffle (0.84 m^2 sample per stream). Excess inorganic substrate was elutriated from macroinvertebrate samples in the field with a 500-µm mesh sieve, and remaining samples were preserved in 95% ethanol and transported to the laboratory and stored at 4 C. In the laboratory, macroinvertebrates were subsampled randomly from a gridded sorting tray and picked under a Nikon SMZ-1 dissecting microscope (30X zoom) until >300 individuals or the entire sample was sorted. After removing subsampled organisms, the remainder of the sample was picked for 30 min removing large or rare organisms not represented in subsamples (Feminella 1996; Vinson and Hawkins 1996; Helms et al. 2016). Most macroinvertebrates were identified to genus using Merritt et al. (2008), and then assigned to functional feeding groups according to classifications in Merritt et al. (2008).

Analyses

Prior to statistical analyses, I reduced collinearity among habitat variables by calculating pairwise Pearson correlation coefficients among habitat variables and then eliminating significantly correlated and redundant variables ($r > 0.5$) by retaining only 1 of the correlated variables; in such a case the variable with the stronger relationship with biodiversity measures was retained. For example, only DA was retained for further analyses because it was correlated with most other stream size measures (maximum depth, mean depth, mean wetted width, max wetted width) and had the strongest correlations with richness variables.

Model-averaging and multi-model inference (sensu Burnham and Anderson 2002) were used to test for relationships between environmental variables (stream size and other habitat parameters) and richness of fishes and macroinvertebrates. Fish and macroinvertebrate subsets analyzed using this method were Cyprinidae, Centrarchidae, Percidae, all macroinvertebrates, Insecta, Coleoptera, Diptera, non-Chironomidae Diptera, Chironomidae, Ephemeroptera, Odonata, Plecoptera, and Trichoptera. Prior to other model fitting procedures, null models were fitted with either Gaussian or Poisson distributions and compared using corrected Akaike Information Criterion (AICc; Hurvich and Tsai 1993) values. AICc values for Poisson null models were always the top model (< 2 ΔAICc) so all further model selection and model averaging were conducted on models with a Poisson distribution. For each response variable (richness of taxonomic groups), top models (< 2 ΔAICc) were selected and models were averaged when there was > 1 top model (Burnham and Anderson 2002).

Nestedness of fish and benthic macroinvertebrate taxonomic groups was tested by calculating nestedness temperature (*T*) of a matrix where rows and columns are ordered by marginal sums and comparing the calculated *T* with the distribution of *T* values, calculated on 999 matrices created using 3 null models used by the BINMATNEST software (Rodríguez-Gironés and Santamaría 2006). Additionally, a Kendall's tau (τ) rank correlation test was used to test for relationships between nestedness packing order (i.e., where packing order values increase with decreasing species richness) and stream size. Kendall's tau (τ) was used because it is more robust than Spearman's rho (Croux and Dehon 2010). Tests for nestedness of Centrarchidae were conducted with and without lentic species (sensu Frimpong and Angermeier 2010) because nestedness among stream fish communities may depend on fish traits (e.g., native vs. non-native; Novak et al. 2011) and several lentic centrarchids were collected from a single site but may reflect stocking efforts in a nearby lake.

To determine if β-diversity decreases with stream size (MHH prediction), null model-derived pairwise Raup-Crick dissimilarity values were calculated to estimate fish and benthic macroinvertebrate β-diversity, this measure isolates β-diversity from differences in α-diversity among sites and is based on presence-absence data (Chase et al. 2011). Homogeneity of variances in β-diversity was then tested by comparing mean distance to centroids among stream size categories (Oksanen et al. 2018). Results were then visualized as boxplots to show significant differences in mean β-diversity among stream size categories. Similarly, to test the underlying assumption of MHH that headwater stream reaches have greater habitat dissimilarity than higher-order streams, Euclidean distances of normalized non-redundant habitat variables were calculated. This method was used because it is appropriate for continuous abiotic variables (Clarke and Warwick 2001). Then a test for homogeneity of variance in habitat dissimilarity among stream size categories was conducted. Last, associations between habitat dissimilarity and overland (i.e., Euclidean distance) or river network distance were quantified using a Mantel test to determine if habitat dissimilarity increased with geographic distance. Statistical tests were conducted using an $\alpha = 0.05$ and all analyses were conducted in R v1.1.442 (R Core Team 2018) using the psych (Revelle 2018), MuMIn (Barton 2018), and riverdist (Tyers 2017) packages.

RESULTS

DA of study streams ranged from 0.11 to 52.09 km^2 (Table 3.1). Of the 17 streams sampled only 12 had fishes present, largely because of downstream waterfalls that blocked upstream passage of fishes. For macroinvertebrates, 188 taxa were identified from 48,800 individuals, whereas 30 species of fish from 4073 individuals were collected. Fish richness ranged from 0 to 24 species/stream and macroinvertebrate richness ranged from 54 to 83 taxa in study streams (Table 3.1). The number of macroinvertebrate taxa only occurring in a single stream (i.e., regionally rare, idiosyncratic taxa, sensu Atmar and Patterson 1993) was 48, and ranged from 0 to 10 per stream (Table 3.1). On average there were 2, 2.5, 3.7, and 4 regionally rare taxa in < 0.26, 0.26 – 2.59, 2.59 – 25.9, and > 25.9 km^2 streams respectively. There were 3, 2, 1, and 3 non-singleton taxa that occurred only in < 0.26, 0.26 – 2.59, 2.59 – 25.9, and > 25.9 km^2 streams respectively.

Environment and α-diversity relationships

Top models were identified for fish, Cyprinidae, Centrarchidae, lotic Centrarchidae, and Percidae richness and the sole predictor variable included in those top models was DA which explained 69 to 92% of the variation in taxa richness (Table 3.2, Fig. 3.2). Top models also were identified for Coleoptera and Ephemeroptera richness, where the sole significant predictor variable was either % gravel or DA, respectively. Percent gravel explained 63.0% of the variance in Coleoptera richness and DA explained 61.0% of the variance in Ephemeroptera richness (Table 3.2, Fig 3.2). No top model was identified for total macroinvertebrate richness, but model averaging identified % gravel as the only significant predictor variable ($z = 2.16$, $p = 0.031$). No top models were identified, nor did model averaging identify variables that explained significant variation in richness of aquatic insects, Diptera, non-Chironomidae Diptera, Chironomidae, Odonata, Plecoptera, or Trichoptera ($z < 1.57$, $p > 0.12$). Rare macroinvertebrate taxa richness also was uncorrelated with DA ($r = 0.32$, d. f. = 15, $p > 0.05$).

Nestedness

All taxonomic groups were more nested among sites than expected by chance using null models 1 and 3 (Table 3.3). Fish, cyprinid, lotic centrarchid, macroinvertebrate, aquatic insect, ephemeropteran, and trichopteran assemblages also were identified as significantly nested using the most conservative null model (null model 2; Table 3.3). Therefore, groups identified as significantly nested under all 3 null models were considered to be strongly nested and others moderately nested. In addition, nestedness of the strongly nested assemblages of fishes, cyprinid, and ephemeropteran was negatively correlated with DA (Table 3.3). Moderately nested assemblages of darters and plecopterans had nestedness that was negatively associated with DA (Table 3.3). No other taxonomic groups showed nestedness that was associated with the stream size gradient (Table 3.3).

Stream size and β-diversity relationships

There was significant heterogeneity in variance of β-diversity among stream size categories for fishes, cyprinids, darters, macroinvertebrates, aquatic insects, chironomids, dipterans, and ephemeropterans (Table 3.4, Fig. 3.3). Among these taxonomic groups, β-diversity was higher in larger streams (> 2.6 km^2) than smaller streams (< 2.6 km^2), except for darters where the smallest (< 0.26 km^2) and largest (> 25.9 km^2) stream size categories showed similar variance in β-diversity (Table 3.4, Fig. 3.3). There were no significant differences in variance of β-diversity among stream size categories for Centrarchidae, Coleoptera, non-chironomid Diptera, Odonata, Plecoptera, or Trichoptera ($F < 1.83$, $p > 0.12$).

Habitat dissimilarity among streams

There was no significant relationship between micro- or macro-habitat dissimilarity (normalized Euclidean distance on habitat variables) among streams and overland or river distance ($R^2 = 0.10$-0.11, $p > 0.05$). Variance in habitat dissimilarity did not differ among stream size categories at either the reach ($F = 1.74$, $p = 0.24$) or riffle levels ($F = 0.42$, $p = 0.74$), although variance tended to increase from the smallest to the largest stream size category at both levels (Fig. 3.5).

DISCUSSION

My study described patterns of biodiversity in the streams of the Bankhead National Forest, specifically to test for the predicted stream size – biodiversity relationships made by the River Continuum Concept and Mighty Headwater Hypothesis. The primary findings were that 1) there were positive relationships between stream size and α-diversity for fish, cyprinid, centrarchid, lotic centrarchid, percid, and ephemeropteran assemblages, 2) most assemblages also were moderately to strongly nested with nestedness associated with stream size, and 3) no assemblages showed greater β-diversity among low-order streams than among higher-order streams. My results suggested that the predictions of the RCC were well supported for stream fish assemblages of the Southwestern Appalachians ecoregion of Alabama. However, my results did not support the RCC-predicted pattern of increasing α-diversity with stream size for benthic macroinvertebrates (Vannote et al., 1980). For taxonomic groups that showed positive stream size–α-diversity relationships, headwater stream assemblages tended to be predictable subsets of richer downstream communities. My results also provided no support for the MHH as β-diversity of all taxa either did not differ among stream size categories or showed higher β-diversity among higher-order streams than lower-order streams (Finn et al., 2011).

Fish α-diversity

Richness of total fish assemblage and all taxonomic subsets were positively related to stream size (as drainage area or stream order), which was the only significant predictor of assemblage richness. This finding is consistent with many studies in other regions that found longitudinal changes in fish assemblages (e.g., Kuehne 1959, Schlosser 1982, Naiman et al. 1987, Araújo et al. 2009, see review by Vorste et al. 2017). The longitudinal patterns and composition of headwater fish assemblages in my study were remarkably similar to those reported in Buckhorn Creek, Kentucky (Kuehne 1962). In both systems, Creek Chub (*Semotilus atromaculatus*) was the sole inhabitant of the smallest streams, to which a darter (*Etheostoma artesiae* in Alabama; *Etheostoma saggita* in Kentucky) and a few other species occurred in streams of the second smallest size category; in both studies, centrarchids become more diverse and common in larger size categories (Kuehne 1962). This apparent similarity in fish community composition between my system and Buckhorn Creek likely result from both residing in the Appalachian Plateau physiographic province and Appalachian ecoregions (Omernik 1995).

Stream size is not considered a direct cause of longitudinal variation in fish richness as a multitude of related environmental variables are correlated with stream size (Vorste et al. 2017). In my study, drainage area was positively correlated with available stream habitat (e.g., depth and wetted width) which may, in fact, be a limiting factor preventing deep bodied fishes like centrarchids or water column specialists from establishing populations in smaller streams. Habitat diversity and stream depth are cited as potential drivers structuring fish diversity and often are correlated with stream size (Sheldon 1968; Gorman and Karr 1978). In my study, some habitat variables (e.g., mean width, mean depth, mean flow velocity) were correlated with stream size and thus might influence fish richness; however, habitat diversity measures (e.g., max flow velocity, depth and flow velocity variation) were independent of stream size and thus had no relationships with fish richness.

Stream size may not be the only environmental variable driving fish assemblage richness in my study. Network position also appeared to structure fish assemblages at some sites, where high-order stream species also occurred in some low-order streams. For example, it is likely that Longear Sunfish (*Lepomis megalotis*), Alabama Hogsucker (*Hypentelium etowanum*), and Central Stonerollers (*Campostoma oligolepis*) in White Oak Creek (2nd order stream) originated from the nearby confluence with Thompson Creek (6th-order stream) where those species were abundant. This finding supports the observation that distance to the nearest confluence and the size of that connecting stream is related to fish species richness in some systems (Osborne and Wiley 1992). Fish richness in Owl Creek (19 species) also appeared inflated but for a different reason. Here, richness was relatively high because of 3 lentic species of centrarchids (*Lepomis macrochirus*, *Lepomis gulosus*, and *Micropterus salmoides*) collected from no other site, and occurred from stocking efforts in nearby Brushy Lake (Rickerson 2004). Taken together, fish assemblages in the Bankhead National Forest appear to be influenced by a combination of stream size, network position, and anthropogenic impacts.

Macroinvertebrate α-diversity

Except for Ephemeroptera richness, taxa richness of total macroinvertebrates as well as taxonomic subsets tended to not be related to stream size. These results are consistent with the finding that stream size – macroinvertebrate richness relationships are weak in the rare instances they occur (reviewed by Vorste et al. 2017), although exceptions exist (Bruns et al. 1987; Clarke et al. 2008). In my study, both headwater and higher-order reaches showed high but variable mean generic richness (~71 – 77 taxa per stream), similar to others reporting high richness in headwater streams (reviewed by Clarke et al. 2008). My finding that Ephemeroptera richness was positively related to stream size also has been reported from many streams at more northern latitudes (Deván and Mucinaz 1986; Giberson and Mackay 1991; Svitok 2006; Beketov 2008) and may result from temperature variability as others have suggested (Deván and Mucinaz 1986; Beketov 2008).

Macroinvertebrate richness often is associated with habitat diversity or complexity (reviewed by Vinson and Hawkins 1998), including substrate diversity (Boyero 2003), or median particle size (Boyero and Bosch 2004, Graça et al. 2004, but see Erman and Erman 1984). In my study, macroinvertebrate richness generally was not associated with measures of habitat diversity (e.g., as total substrate diversity) or heterogeneity (e.g., as CV of depth or flow velocity) although it was negatively associated with % gravel in riffles. Total macroinvertebrate and coleopteran richness were negatively associated with % gravel, suggesting that availability of other types of coarse-grained habitat (e.g., bedrock, boulders, cobble) may limit some taxa. The lack of relationships between other taxa richness and habitat variables may be related to the large spatial scale (stream reach) at which habitat variables were measured. For example, benthic macroinvertebrates in streams of Sierra de Guadarrama, Spain corresponded to habitat variables a very localized scales (among individual samples), whereas relationships at larger riffle or reach scales were absent (Boyero 2003). If true in my study, then the lack of reach-scale habitat – richness relationships suggest that microhabitat filters are stronger than those at the reach or basin-scale (Lamouroux et al. 2004); this pattern would cause greater compositional differences among microhabitats than in the same microhabitat at different localities (Costa and Melo 2008).

Nestedness of aquatic assemblages

Results of my nestedness analyses supported a growing body of evidence that nestedness of fish and macroinvertebrate assemblages are common along environmental gradients, including stream size (Taylor and Warren 2001; Cook et al. 2004; Heino et al. 2009, 2010; Miranda et al. 2019). In support of nestedness theory (sensu Atmar and Patterson 1993), nestedness has been linked to colonization-extinction dynamics along the stream continuum, where upstream reaches experience greater extinction rates (potentially because of intermittence) and/or lower colonization rates (Taylor and Warren 2001). Colonization-extinction dynamics related to flow variability also may be responsible for the assemblage nestedness patterns in my system. During subsequent visits to several smaller streams at summer low flow, I found that some streams become a series of isolated pools, which may cause localized extinctions and impede colonization.

Nestedness was common among all fish and macroinvertebrate assemblages but fish assemblage subsets tended to be more strongly nested (i.e., significantly nested using all 3 null models) than macroinvertebrate assemblage subsets. Additionally, nestedness of fish assemblages was negatively related to DA (i.e., headwater assemblages being non-random subsets richer higher-order stream assemblages), indicating that compositional differences along the continuum may result from species additions rather than replacement; this pattern would be the case because nestedness and species turnover (replacement) are different patterns that contribute to compositional dissimilarity between communities (Leibold and Mikkelson 2002).

In contrast to fish assemblages where nestedness was high and strongly related to stream size, macroinvertebrate assemblages were mostly moderately nested (i.e., significantly nested using null models 1 and 3 only), and generally unrelated to stream size; the two exceptions being

Ephemeroptera assemblages which were strongly nested and Plecoptera assemblages which were moderately nested, but both had nestedness that was related to stream size. Relatively weak nestedness in macroinvertebrate assemblages also was observed in north-eastern Finnish streams (Heino et al. 2009). However, the lack of a relationship between stream size and nestedness of macroinvertebrate assemblages in my study contrasts with finding that stream size the was strongest environmental correlate with nestedness macroinvertebrate assemblages in those Finnish streams (Heino et al. 2009). Subsequent work in 8 river basins in Finland also found that nestedness of combined assemblages of Ephemeroptera, Plecoptera, and Trichoptera was commonly associated with stream size (Heino et al. 2010), and my results are another exception to this pattern. The reason for the lack of a stream size–nestedness relationship is not immediately clear, but there are potential explanations. First, there was a large proportion of rare and idiosyncratic taxa in my study (~26%), which also may explain why nestedness of macroinvertebrates was much weaker than fish assemblages; idiosyncratic taxa increase the randomness or disorder of the site by taxa matrix and thus decreases nestedness (Atmar and Patterson 1993). Second, I included all macroinvertebrates and identified them to genus whereas the Heino et al. (2009, 2010) studies used only a subset of macroinvertebrates (i.e., Ephemeroptera, Plecoptera, Trichoptera, and Coleoptera). In my study, Ephemeroptera and Trichoptera assemblages were both strongly nested, and Ephemeroptera and Plecoptera assemblages had nestedness associated with stream size, which reflected results of (Heino et al. 2009, 2010). Alternatively, nestedness of macroinvertebrate assemblages in my study may be related to environmental gradients (i.e. diversity of basal resources, long-term temperature variability, etc.) other than stream size that were not measured in this study.

Like fishes, strong nestedness of Ephemeroptera assemblages in combination with its positive relationship with stream size, suggest that headwater taxa also are a subset of mayfly assemblages in larger streams, and that longitudinal species additions describes compositional differences along the stream continuum. Ephemeroptera therefore are representative of an assemblage where stream size is strongly related to community composition. In contrast, most macroinvertebrate assemblages I examined represent the other end of the spectrum where stream size has no relationship with assemblage composition. These results are similar to those reported for macroinvertebrates in headwater streams of the Talladega National Forest, where most taxa (75%) showed no relationship stream size (as flow permanence, Feminella 1996).

General dissimilarity of stream fish and macroinvertebrate assemblage richness and nestedness suggest that these groups respond primarily to different environmental factors and/or at different scales (Paavola et al. 2003; Carlisle et al. 2008), although in some cases concordance is stronger than others (Johnson and Hering 2010). Discrepancies in biodiversity patterns of fish and macroinvertebrate assemblages may result from the interaction between their contrasting traits and relevant macro- or micro-habitat characteristics. For example, body size differs drastically between even the largest macroinvertebrates and smallest fishes, thus macro-habitat characteristics such as stream depth (and volume) can limit some large-bodied fishes (e.g., centrarchids) but are less relevant to micro-habitats used by benthic macroinvertebrates (Poff 1997). However, it is notable that Ephemeroptera assemblage patterns were similar to that of fishes despite that they are unlikely to be limited by stream depth or volume. It has been suggested that many Ephemeroptera are warm-adapted, unlike many other aquatic insect taxa such as Plecoptera (Pritchard et al. 1996) and thus temperature relations may structure Ephemeroptera assemblages. For example, population sizes and species richness of mayfly species are commonly positively related to mean summer stream temperatures (Haidekker and Hering 2008; Brittain et al. 2003; Svitok 2006; Beketov 2008). I did not collect long-term stream temperature data, although larger streams were usually warmer than smaller streams as a result of having more open canopy and thus greater insolation. Thus, the stream size–richness and stream size–nestedness relationships in mayflies may be predominantly driven by stream temperature. In contrast, the lack of relationships between mayfly assemblages and microhabitat variables suggest that these factors are less important in structuring mayfly assemblage composition.

β-diversity

In contrast to the support found for RCC predictions in fish and Ephemeroptera assemblages, I found no support for the predictions of the MHH for any taxonomic group. Mean Raup-Crick β-diversity values either were not different among stream size categories or, where differences occurred, β-diversity tended to be higher among large (vs. small) streams. This result is consistent with the findings of the original MHH study because there was no significant relationship between stream size and β-diversity in Finn et al.'s (2011) intra-ecoregional comparisons, as opposed to their interregional comparisons (Finn et al. 2011). Thus, my study supports the idea that the negative stream size – β-diversity relationship is a phenomenon that is absent on small spatial scales. The MHH prediction that β-diversity should be greatest among headwater streams and decline with increasing stream size is based on 2 assumptions, that 1) dispersal is a limiting factor in headwater streams because of high spatial isolation, and 2) habitat dissimilarity is greater among headwater streams than in downstream reaches (Finn et al. 2011). However, study spatial extent is expected to be positively associated with dispersal limitation (Cottenie 2005; Heino 2011) and habitat dissimilarity (Heino et al. 2015) among sites, particularly if sampled sites represent multiple distinct ecoregions. This reason may explain why greater β-diversity occurred in headwater streams than mid-order streams among, but not within ecoregions in the original Finn et al. (2011) study. Last, the comparatively small spatial extent of my study may thus explain why there was not a negative stream size–β-diversity relationship, justifying the need for studies encompassing broader areal coverage (Finn et al., 2011).

My finding of no significant difference in mean habitat dissimilarity among stream size categories, with habitat dissimilarity slightly increasing with stream size, is consistent with habitat dissimilarity patterns in 3 Maryland river networks (Brown and Swan 2010), and also did not support an underlying assumption of the MHH. It is possible that dispersal was less limited in my system than many of systems included in the original MHH study (Finn et al. 2011) because my streams were generally at lower altitudes (250-300 m) with less topographic relief. Topographical distance, a measure that includes Euclidean distance plus elevation gains and losses (sensu Tonkin et al. 2017), can be strongly and negatively associated with macroinvertebrate community similarity indicating that topographic relief exerts potentially strong impacts on macroinvertebrate dispersal (Tonkin et al. 2018). Taken together, the relatively limited topographic relief and associated barriers to dispersal, limited habitat dissimilarity among headwaters relative to larger streams, and the small spatial scale of my study all may decrease β-diversity among headwater streams, thus nullifying the expected negative stream size–β-diversity relationship.

In summary, I found strong support for the RCC prediction of a positive stream size – richness relationship in fishes and Ephemeroptera but not in other macroinvertebrates. Additionally, I found no support for the MHH prediction that β-diversity was negatively correlated with stream size in fish and macroinvertebrate assemblages in an aquatic biodiversity hotspot. The strongly contrasting patterns of biodiversity between fishes and most macroinvertebrates suggest that they largely respond to disparate environmental factors, which may act at different scales. The observed biodiversity patterns can provide guidance for conservation efforts in the region. Positive relationships between stream size and α-diversity as well as nestedness for fish and Ephemeroptera, may suggest that conserving biodiversity in mid-order streams is potentially more important than conservation of headwater streams as these larger streams contain the richest assemblages of these taxa. In contrast, although I found no support for the predictions of the MHH, my results do support a main contention advanced by Finn et al. (2011). Results suggested that headwater streams and higher-order streams both contain high levels of biodiversity (α-diversity and unique species) that contribute to regional diversity and thus headwater are deserving of greater conservation priority and attention than they previously received. Further, because of the dendritic structure of stream networks, the frequency of headwater streams is orders of magnitude higher than those of mid-order streams within a basin (Strahler 1957) and thus headwaters, by their sheer abundance, may contribute more to the diversity of the regional species pool of macroinvertebrates in streams of this and other ecoregions.

LITERATURE CITED

Allan, J. D., and A. S. Flecker. 1993. Biodiversity conservation in running waters. BioScience 43: 32–43.

Araújo, F. G., B. C. T. Pinto, and T. P. Teixeira. 2009. Longitudinal patterns of fish assemblages in a large tropical river in southeastern Brazil: evaluating environmental influences and some concepts in river ecology. Hydrobiologia 618: 89–107.

Atmar, W., and B. D. Patterson. 1993. The measure of order and disorder in the distribution of species in fragmented habitat. Oecologia 96: 373–382.

Barbour, M. T., J. Gerritsen, B. D. Snyder, and J. B. Stribling. 1999. Rapid bioassessment protocols for use in streams and wadeable rivers: periphyton, benthic macroinvertebrates and fish. US Environmental Protection Agency Office of Water Washington DC, 339p.

Barton, K. 2018. MuMIn: Multi-Model Inference. R package version 1.40.4. https://cran.r-project.org/package=MuMIn

Beketov, M. A. 2008. Community structure of Ephemeroptera in Siberian streams. Entomological Science 11: 289–299.

Boyero, L. 2003. The quantification of local substrate heterogeneity in streams and its significance for macroinvertebrate assemblages. Hydrobiologia 499: 161–168.

Boyero, L., and J. Bosch. 2004. The effect of riffle-scale environmental variability on macroinvertebrate assemblages in a tropical stream. Hydrobiologia 524: 125–132.

Brantley, E., B. Helms, A. Ludwig, J. Shaw, G. Jennings, C. Anderson, D. Werneke, and J. Feminella. 2016. Eco-morphological mitigation design and assessment tools for the

Alabama and Tennessee Appalachian Plateau. Atlanta, GA. US EPA Region IV, Grant No. CD 00D01412: Final Report, 76p. http://cses.auburn.edu/eve-brantley/wp-content/uploads/sites/114/2016/10/Final-report_CD00D01412_-093016.pdf

Brittain, J. E., E. Castella, S. Knispel, V. Lencioni, B. Lods-Crozet, B. Maiolini, A. M. Milner, S. J. Saltveit, and D. L. Snook. 2003. Ephemeropteran and Plecopteran communities in glacial rivers. Pages 271–277 in E. Gaino, editor. Research Update on Ephemeroptera & Plecoptera. University of Perugia, Perugia, Italy.

Brown, B. L., and C. M. Swan. 2010. Dendritic network structure constrains metacommunity properties in riverine ecosystems. Journal of Animal Ecology 79: 571–580.

Bruns, D. A., A. B. Hale, and G. W. Minshall. 1987. Ecological correlates of species richness in three guilds of lotic macroinvertebrates. Journal of Freshwater Ecology 4: 163–176.

Burnham, K. P., and D. R. Anderson. 2002. Model selection and multi-model inference: a practical information-theoretic approach, 2nd edition. Springer-Verlag, New York, New York.

Carlisle, D. M., C. P. Hawkins, M. R. Meador, M. Potapova, and J. Falcone. 2008. Biological assessments of Appalachian streams based on predictive models for fish, macroinvertebrate, and diatom assemblages. Journal of the North American Benthological Society 27: 16–37.

Chase, J. M., N. J. B. Kraft, K. G. Smith, M. Vellend, and B. D. Inouye. 2011. Using null models to disentangle variation in community dissimilarity from variation in α-diversity. Ecosphere 2: 1–11.

Clarke, A., R. Mac Nally, N. Bond, and P. S. Lake. 2008. Macroinvertebrate diversity in headwater streams: a review. Freshwater Biology 53: 1707–1721.

Clarke, K., and R. Warwick. 2001. A framework for studying changes in community structure. Page Change in marine communities: an approach to statistical analysis and interpretation, 2nd edition. PRIMER-E Limited, Plymouth, UK.

Cook, R. R., P. L. Angermeier, D. S. Finn, N. L. Poff, and K. L. Krueger. 2004. Geographic variation in patterns of nestedness among local stream fish assemblages in Virginia. Oecologia 140: 639–649.

Costa, S. S., and A. S. Melo. 2008. Beta diversity in stream macroinvertebrate assemblages: Among-site and among-microhabitat components. Hydrobiologia 598: 131–138.

Cottenie, K. 2005. Integrating environmental and spatial processes in ecological community dynamics. Ecology Letters 8: 1175–1182.

Croux, C., and C. Dehon. 2010. Influence functions of the Spearman and Kendall correlation measures. Statistical Methods and Applications 19: 497–515.

Deván, P., and L. Mucinaz. 1986. Structure, zonation, and species diversity of the mayfly communities of the Belá River basin, Slovakia. Hydrobiologia 135: 155–165.

Erman, D. C., and N. A. Erman. 1984. The response of stream macroinvertebrates to substrate size and heterogeneity. Hydrobiologia 108: 75–82.

Feminella, J. W. 1996. Comparison of benthic macroinvertebrate assemblages in small streams along a gradient of flow permanence. Journal of the North American Benthological Society 15: 651–669.

Feminella, J. W. 2000. Correspondence between stream macroinvertebrate assemblages and 4 ecoregions of the southeastern USA. Journal of the North American Benthological Society 19: 442–461.

Finn, D. S., N. Bonada, C. Múrria, and J. M. Hughes. 2011. Small but mighty: headwaters are vital to stream network biodiversity at two levels of organization. Journal of the North American Benthological Society 30: 963–980.

Frimpong, E. A., and P. L. Angermeier. 2010. Trait-based approaches in the analysis of stream fish communities. American Fisheries Society Symposium 73: 109–136.

Giberson, D. J., and R. J. Mackay. 1991. Life history and distribution of mayflies (Ephemeroptera) in some acid streams in south central Ontario, Canada. Canadian Journal of Zoology 69: 899–910.

Gore, J. A. 1996. Discharge measurements and streamflow analysis. Pages 53–74 in G. A. Lamberti and F. R. Hauer, editors. Methods in Stream Ecology. Academic Press, New York, NY.

Gorman, O. T., and J. R. Karr. 1978. Habitat structure and stream fish communities. Ecology 59: 507–515.

Graça, M. A. S., P. Pinto, R. Cortes, N. Coimbra, S. Oliveira, M. Morais, M. J. Carvalho, and J. Malo. 2004. Factors affecting macroinvertebrate richness and diversity in Portuguese streams: a two-scale analysis. International Review of Hydrobiology 89: 151–164.

Haidekker, A., and D. Hering. 2008. Relationship between benthic insects (Ephemeroptera, Plecoptera, Coleoptera, Trichoptera) and temperature in small and medium-sized streams in Germany: a multivariate study. Aquatic Ecology 42: 463–481.

Heino, J. 2011. A macroecological perspective of diversity patterns in the freshwater realm. Freshwater Biology 56: 1703–1722.

Heino, J., A. S. Melo, L. M. Bini, F. Altermatt, S. A. Al-Shami, D. G. Angeler, N. Bonada, C. Brand, M. Callisto, K. Cottenie, O. Dangles, D. Dudgeon, A. Encalada, E. Göthe, M. Grönroos, N. Hamada, D. Jacobsen, V. L. Landeiro, R. Ligeiro, R. T. Martins, M. L. Miserendino, C. S. Md Rawi, M. E. Rodrigues, F. de O. Roque, L. Sandin, D. Schmera, L. F. Sgarbi, J. P. Simaika, T. Siqueira, R. M. Thompson, and C. R. Townsend. 2015. A comparative analysis reveals weak relationships between ecological factors and beta diversity of stream insect metacommunities at two spatial levels. Ecology and Evolution 5: 1235–1248.

Heino, J., H. Mykrä, and T. Muotka. 2009. Temporal variability of nestedness and idiosyncratic species in stream insect assemblages. Diversity and Distributions 15: 198–206.

Heino, J., H. Mykrä, and J. Rintala. 2010. Assessing patterns of nestedness in stream insect assemblages along environmental gradients. Ecoscience 17: 345–355.

Helms, B., J. Zink, D. Werneke, T. Hess, Z. Price, G. Jennings, and E. Brantley. 2016. Development of ecogeomorphological (EGM) stream design and assessment tools for the Piedmont of Alabama, USA. Water 8: 161.

Hurvich, C. M., and C. -L Tsai. 1993. A corrected Akaike information criterion for vector autoregressive model selection. Journal of Time Series Analysis 14: 271–279.

Johnson, R. K., and D. Hering. 2010. Spatial congruency of benthic diatom, invertebrate, macrophyte, and fish assemblages in European streams. Ecological Applications 20: 978–992.

Kuehne, R. 1962. A classification of streams illustrated by fish distribution in an eastern Kentucky Creek. Ecology 43: 608–614.

Lamouroux, N., S. Dolédec, and S. Gayraud. 2004. Biological traits of stream macroinvertebrate communities: effects of microhabitat, reach, and basin filters. Journal of the North American Benthological Society 23: 449–466.

Leibold, M. A., and G. M. Mikkelson. 2002. Coherence, species turnover, and boundary clumping: elements of meta-community structure. Oikos 97: 237–250.

Lloyd, N. J., R. Mac Nally, and P. S. Lake. 2005. Spatial autocorrelation of assemblages of benthic invertebrates and its relationship to environmental factors in two upland rivers in southeastern Australia. Diversity and Distributions 11: 375–386.

Lowe, W. H., and G. E. Likens. 2005. Moving headwater streams to the head of the class. BioScience 55: 196.

Lydeard, C., and R. L. Mayden. 1995. A diverse and endangered aquatic ecosystem of the southeast United States. Conservation Biology 9: 800–805.

Malmqvist, B., Y. Zhang, and P. H. Adler. 1999. Diversity, distribution and larval habitats of North Swedish blackflies (Diptera: Simuliidae). Freshwater Biology 42: 301–314.

Merritt, R. W., K. W. Cummins, and M. B. Berg. 2008. An introduction to the aquatic insects of North America, 4th edition. Kendall Hunt Publishing, Dubuque, IA.

Meyer, J. L., D. L. Strayer, J. B. Wallace, S. L. Eggert, G. S. Helfman, and N. E. Leonard. 2007. The contribution of headwater streams to biodiversity in river networks. Journal of the American Water Resources Association 43: 86–103.

Miranda, L. E., K. J. Killgore, and W. T. Slack. 2019. Spatial organization of fish diversity in a species-rich basin. River Research and Applications 35: 188–196.

Naiman, R. J., J. M. Melillo, M. A. Lock, T. E. Ford, and S. R. Reice. 1987. Longitudinal patterns of ecosystem processes and community structure in a subarctic river continuum. Ecology 68: 1139–1156.

Neteler, M., and H. Mitasova. 2008. Open source GIS: A GRASS GIS approach. Open Source GIS: A GRASS GIS Approach (November): 1–406.

Novak, M., J. W. Moore, and R. A. Leidy. 2011. Nestedness patterns and the dual nature of community reassembly in California streams: A multivariate permutation-based approach. Global Change Biology 17: 3714–3723.

Oksanen, J., F. G. Blanchet, M. Friendly, R. Kindt, P. Legendre, D. McGlinn, P. R. Minchin, R. B. O'Hara, G. L. Simpson, P. Solymos, M. H. H. Stevens, E. Szoecs, and H. Wagner. 2018. vegan: community ecology package. R package version 2.4-6. https://cran.r-project.org/package=vegan

Omernik, J. M. 1995. Ecoregions: a framework for managing ecosystems. The George Wright Forum. 12: 35–50.

Osborne, L. L., and M. J. Wiley. 1992. Influence of tributary spatial position on the structure of warmwater fish communities. Canadian Journal of Fisheries and Aquatic Sciences 49: 671–681.

Paavola, R., T. Muotka, R. Virtanen, J. Heino, and P. Kreivi. 2003. Are biological classifications of headwater streams concordant across multiple taxonomic groups? Freshwater Biology 48: 1912–1923.

Plafkin, J. L., M. T. Barbour, K. D. Porter, S. K. Gross, and R. M. Hughes. 1989. Rapid bioassessment protocols for use in stream and rivers: benthic macroinvertebrates and fish. US EPA, Assessment and Watershed Protection Division, Report EPA/444/4-89-011. Washington, DC.

Poff, N. L. 1997. Landscape filters and species traits: towards mechanistic understanding and prediction in stream ecology. Journal of the North American Benthological society 16: 391–409.

Pritchard, G., L. D. Harder, and R. A. Mutch. 1996. Development of aquatic insect eggs in relation to temperature and strategies for dealing with different thermal environments. Biological Journal of the Linnean Society 58: 221–244.

R Core Team. 2018. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

Revelle, W. 2018. psych: procedures for psychological, psychometric, and personality research. Northwestern University, Evanston, Illinois. R package version 1.8.3. https://cran.r-project.org/package=psych

Rickerson, S. 2004. National forests in Alabama, revised land and resource management plan: environmental impact statement. USDA Forest Service, Montgomery, Alabama USA.

Rodríguez-Gironés, M. A., and L. Santamaría. 2006. A new algorithm to calculate the nestedness temperature of presence-absence matrices. Journal of Biogeography 33: 924–935.

Schlosser, I. J. 1982. Fish community structure and function along two habitat gradients in a headwater stream. Ecological Monographs 52: 395–414.

Sheldon, A. L. 1968. Species diversity and longitudinal succession in stream fishes. Ecology 49: 194–198.

Southwood, T. R. E. 1977. Habitat as the template for ecological strategies? The Journal of animal ecology 46: 336–365.

Strahler, A. N. 1957. Quantitative analysis of watershed geomorphology. Transactions American Geophysical Union 38: 913–920.

Svitok, M. 2006. Structure and spatial variability of mayfly (Ephemeroptera) communities in the upper Hron River basin. Biologia 61: 547–554.

Taylor, C. M., and M. L. Warren. 2001. Dynamics in species composition of stream fish assemblages: environmental variability and nested subsets. Ecology 82: 2320–2330.

Tonkin, J. D., F. Altermatt, D. S. Finn, J. Heino, J. D. Olden, S. U. Pauls, and D. A. Lytle. 2018. The role of dispersal in river network metacommunities: patterns, processes, and pathways. Freshwater Biology 63: 141–163.

Townsend, C. R., and A. G. Hildrew. 1994. Species traits in relation to a habitat templet for river systems. Freshwater Biology 31: 265–275.

Tyers, M. 2017. riverdist: river network distance computation and applications. R package version 0.15.0. https://cran.r-project.org/package=riverdist

Vannote, R. L., W. G. Minshall, K. W. Cummins, J. R. Sedell, and C. E. Cushing. 1980. The River Continuum Concept. Canadian Journal of Fisheries and Aquatic Sciences 37: 130–137.

Vinson, M. R., and C. P. Hawkins. 1996. Effects of sampling area and subsampling procedure on comparisons of taxa richness among streams. Journal of the North American Benthological Society 15: 392–399.

Vinson, M. R., and C. P. Hawkins. 1998. Biodiversity of stream insects: variation at local, basin, and regional scales. Annual Review of Entomology 43: 271–293.

Vorste, R. Vander, P. McElmurray, S. Bell, K. M. Eliason, and B. L. Brown. 2017. Does stream size really explain biodiversity patterns in lotic systems? a call for mechanistic explanations. Diversity 9: 1–21.

Zink, J. M., G. D. Jennings, and G. Alexander Price. 2012. Morphology characteristics of southern Appalachian wilderness streams. Journal of the American Water Resources Association 48: 762–773.

Table 3.1. Site-level habitat and biotic metrics from 17 streams in the Bankhead National Forest.

Site Name	Date	Latitude	Longitude	Drainage Area (km^2)	Strahler Stream Order	Wetted Width (m)	Discharge (m^3/s)	Gradient (%)	pH	Specific Conductivity	Macroinvertebrate Abundance	Macroinvertebrate Richness	Fish Abundance	Fish Richness
Unnamed Tributary of Brushy Creek - 3	6/13/2014	34.25423	-87.25661	0.11	2	1.22	0.0004	8.58860	6.9	15.0	4673.0	54	0	0
Unnamed Tributary of Brushy Creek - 2	6/13/2014	34.25405	-87.25412	0.13	1	1.15	0.0001	7.78890	7.6	14.0	7240.7	64	0	0
Unnamed Tributary of Brown Creek - 1	6/17/2014	34.34296	-87.47037	0.16	2	1.36	0.0002	10.06410	6.3	13.0	10215.0	74	23	1
Unnamed Tributary of West Fork Beech	6/13/2014	34.30202	-87.31618	0.24	3	1.22	0.0003	2.60140	6.3	17.0	14529.3	81	113	1
Elephant Creek	5/13/2015	34.27717	-87.45945	0.25	2	1.13	0.0003	5.13560	6.9	15.8	1399.0	63	4	1
Melody Creek	6/20/2014	34.33031	-87.19704	0.30	3	1.26	0.0007	4.95670	7.6	20.0	6911.0	81	0	0
Unnamed Tributary of Brushy Creek - 1	6/13/2014	34.25193	-87.24786	0.32	2	1.85	0.0002	8.35050	7.7	40.0	4934.0	69	0	0
Unnamed Tributary of Brown Creek - 2	6/17/2014	34.30549	-87.24021	0.77	3	2.07	0.0006	2.81070	6.5	25.0	11297.5	80	183	3
White Oak Creek	5/12/2015	34.34539	-87.45390	0.96	2	4.11	0.0001	8.53780	6.9	19.9	5149.0	66	212	6
Rush Creek	6/20/2014	34.32951	-87.19764	1.14	4	2.42	0.0022	5.78650	7.7	18.0	2359.2	82	0	0
Unnamed Tributary of Capsey Creek	7/10/2014	34.26317	-87.19960	1.36	3	3.10	0.0010	2.04240	7.7	21.0	11729.7	83	302	3
North Fork Caney Creek	6/4/2015	34.26425	-87.42555	13.58	5	7.98	0.0455	1.96370	-	16.4	19721.0	68	308	15
Tedford Creek	6/18/2014	34.34895	-87.47012	14.99	5	7.18	0.0006	0.87150	7.8	80.0	14047.0	68	216	10
Owl Creek	7/10/2014	34.30554	-87.26262	15.69	5	7.88	0.0198	0.43720	7.8	24.0	9497.0	77	426	19
Thompson Creek	6/17/2014	34.34296	-87.47037	38.94	6	10.37	0.0160	0.13060	8.4	70.0	13612.0	68	380	16
Hubbard Creek	6/19/2014	34.26733	-87.45889	48.54	6	10.47	0.1416	0.00001	7.4	23.0	7909.7	61	543	21
Capsey Creek	6/5/2015	34.26917	-87.21083	52.09	6	12.00	0.0175	0.11370	7.45	63.7	20391.0	83	1363	24

Table 3.1. Continued.

Invertebrate Habitat

	Velocity			Depth			Inorganic Substrate							Organic Substrate						
Riffle OM	Mean	Coefficient of Variation	Range	Mean	Coefficient of Variation	Range	Fine	Sand	Gravel	Cobble	Boulder	Bedrock	$H'_{inorganic}$	Rootwad	Moss	CWD	CPOM	FPOM	$H'_{organic}$	$H'_{substrate}$
6.62	0.10	58.70	0.19	0.04	36.08	0.04	0.17	0.18	0.33	0.23	0.08	0.00	1.52	0.00	0.12	0.24	0.33	0.30	1.33	2.85
4.73	0.11	65.55	0.21	0.03	36.49	0.03	0.00	0.13	0.58	0.23	0.05	0.00	1.07	0.00	0.33	0.40	0.13	0.13	1.27	2.34
64.75	0.07	53.36	0.12	0.03	50.20	0.03	0.00	0.50	0.20	0.28	0.02	0.00	1.09	0.00	0.08	0.28	0.64	0.00	0.84	1.94
7.2	0.13	52.52	0.20	0.04	40.05	0.04	0.00	0.15	0.37	0.45	0.03	0.00	1.13	0.00	0.07	0.15	0.78	0.00	0.66	1.78
2.32	0.09	78.68	0.20	0.03	43.27	0.03	0.10	0.13	0.37	0.20	0.20	0.00	1.51	0.00	0.00	0.33	0.67	0.00	0.64	2.15
22.35	0.11	51.98	0.16	0.03	65.43	0.06	0.10	0.43	0.33	0.13	0.00	0.00	1.23	0.24	0.00	0.35	0.35	0.06	1.24	2.47
4.08	0.14	93.66	0.43	0.03	56.25	0.04	0.00	0.03	0.28	0.42	0.27	0.00	1.19	0.00	0.00	0.50	0.25	0.25	1.04	2.23
5.69	0.15	53.38	0.21	0.03	66.67	0.06	0.00	0.23	0.13	0.40	0.18	0.06	1.45	0.18	0.53	0.18	0.12	0.00	1.20	2.65
1.75	0.09	30.52	0.09	0.06	43.64	0.09	0.00	0.12	0.38	0.47	0.03	0.00	1.09	0.00	0.00	0.50	0.50	0.00	0.69	1.78
4.69	0.17	54.53	0.29	0.06	37.89	0.07	0.00	0.25	0.22	0.50	0.03	0.00	1.14	0.08	0.00	0.25	0.58	0.08	1.08	2.21
23.09	0.33	77.87	0.69	0.11	27.33	0.1	0.00	0.10	0.20	0.28	0.08	0.33	1.48	0.00	0.58	0.08	0.33	0.00	0.89	2.37
3.04	0.38	55.40	0.62	0.11	52.68	0.15	0.00	0.13	0.33	0.53	0.00	0.00	0.97	0.00	0.00	0.50	0.50	0.00	0.69	1.66
3.9	0.17	63.57	0.30	0.06	68.01	0.12	0.00	0.10	0.40	0.47	0.03	0.00	1.07	0.09	0.00	0.70	0.21	0.00	0.79	1.86
9.38	0.27	50.19	0.42	0.08	35.71	0.08	0.00	0.08	0.28	0.20	0.43	0.00	1.25	0.00	0.15	0.38	0.46	0.00	1.01	2.26
0.99	0.34	32.27	0.31	0.12	38.83	0.12	0.00	0.02	0.35	0.45	0.18	0.00	1.11	0.00	0.73	0.14	0.14	0.00	0.77	1.88
4.43	0.13	49.18	0.22	0.08	41.93	0.1	0.00	0.53	0.47	0.00	0.00	0.00	0.69	0.10	0.00	0.35	0.55	0.00	0.93	1.62
2	0.32	63.47	0.61	0.15	27.83	0.11	0.00	0.13	0.17	0.20	0.23	0.27	1.58	0.14	0.71	0.14	0.00	0.00	0.80	2.38

Table 3.1. Continued.

Fish Habitat					
Velocity			Depth		
Mean (m/s)	Coefficient of Variation	Maximum	Mean (m)	Coefficient of Variation	Maximum (m)
-	-	-	-	-	-
-	-	-	-	-	-
0.07	101.54	0.29	0.05	61.48	0.15
0.04	139.10	0.21	0.06	70.93	0.17
0.02	135.56	0.08	0.04	101.69	0.17
-	-	-	-	-	-
-	-	-	-	-	-
0.02	109.09	0.09	0.07	48.22	0.14
-	-	-	-	-	-
0.02	214.67	0.25	0.06	102.97	0.23
0.04	128.93	0.20	0.08	62.16	0.22
0.05	119.87	0.23	0.19	60.85	0.49
0.03	166.44	0.22	0.26	117.25	1.53
0.04	147.51	0.28	0.16	75.11	0.57
0.10	192.82	0.97	0.33	78.46	1.02
0.13	66.62	0.34	0.15	41.26	0.27
0.05	159.57	0.42	0.37	77.10	1.12

Table 3.2. Top model or model averaging results for richness of taxonomic groups explained by environmental variables.

Taxa	Variable	Estimate	*z*-value	*p*-value	R^2
Fish					
	Intercept	1.26 (0.21)	6.16	<0.001	0.90
	log(Drainage Area)	0.48 (0.07)	7.53	<0.001	
Cyprinidae					
	Intercept	0.44 (0.30)	1.43	0.15	0.92
	log(Drainage Area)	0.38 (0.10)	3.91	< 0.001	
Centrarchidae					
	Intercept	-0.99 (0.64)	-1.54	0.12	0.69
	log(Drainage Area)	0.64 (0.19)	3.40	< 0.001	
Non-lentic Centrarchidae					
	Intercept	-1.28 (0.74)	-1.73	0.08	0.82
	log(Drainage Area)	0.67 (0.22)	3.07	0.002	
Percidae					
	Intercept	-0.28 (0.45)	-0.63	0.53	0.81
	log(Drainage Area)	0.51 (0.14)	3.72	< 0.001	
Macroinvertebrates					
	Intercept	0	NA	NA	NA
	Gravel (%)	-0.008 (0.004)	2.16	0.031	
	Longitude	0.005 (0.006)	0.76	0.45	
	Substrate H'	-0.003 (0.005)	0.56	0.58	
Coleoptera					
	Intercept	2.50 (0.28)	8.79	< 0.001	0.63
	Gravel (%)	-2.22 (0.92)	-2.41	0.016	
Ephemeroptera					
	Intercept	1.65 (0.11)	14.6	<0.001	0.61
	log(Drainage Area)	0.12 (0.05)	2.76	0.006	

Table 3.3. Test for homogeneity of variance of β-diversity (distance to centroid) for taxonomic groups among stream size categories with significant differences indicated by different letters from high (a) to low (b).

			Stream Size Category			
Taxa	F-value	p-value	< 0.26	0.26 - 2.59	2.59 - 25.9	> 25.9
Fish	5.02	<0.001	*b*	*c*	*a*	*a*
Cyprinidae	5.08	0.02	*b*	*ab*	*a*	*a*
Centrarchidae	1.99	0.18				
Lotic Centrarchidae	2.31	0.11				
Darters	9.06	0.001	*a*	*b*	*a*	*a*
Macroinvertebrates	23.8	< 0.001	*b*	*b*	*b*	*a*
Aquatic Insects	23.8	< 0.001	*b*	*b*	*b*	*a*
Coleoptera	1.45	0.26				
Chironomidae	9.29	0.002	*b*	*c*	*a*	*a*
Non-chironomid Diptera	1.83	0.19				
Diptera	13	< 0.001	*bc*	*c*	*ab*	*a*
Ephemeroptera	15.2	< 0.001	*c*	*bc*	*b*	*a*
Odonata	1.76	0.20				
Plecoptera	1.14	0.35				
Trichoptera	0.73	0.55				

Table 3.4. Nestedness temperature (T), results of test for nestedness using 3 null models, and Kendall's τ and associate p-value for the rank correlation test for correlation between stream size and nestedness rank for various aquatic taxa.

		Nestedness			Correlation with stream size	
Taxa Richness	T	Null Model 1 (p-value)	Null Model 2 (p-value)	Null Model 3 (p-value)	τ	p-value
Fish	6.17	< 0.001	< 0.001	< 0.001	-0.82	< 0.001
Cyprinidae	5.22	0.003	< 0.001	< 0.001	-0.82	< 0.001
Centrarchidae	8.94	< 0.001	0.08	0.02	-0.33	0.19
Lotic Centrarchidae	3.3	<0.001	0.04	0.009	-0.52	0.07
Darters	7.34	0.001	0.05	0.02	-0.67	0.006
Macroinvertebrates	40.3	< 0.001	< 0.001	< 0.001	-0.26	0.08
Aquatic Insects	39.7	< 0.001	< 0.001	< 0.001	-0.21	0.14
Coleoptera	25.5	< 0.001	0.49	0.04	-0.13	0.24
Chironomidae	39.8	< 0.001	0.43	< 0.001	-0.17	0.17
Non-chironomid Diptera	25.7	< 0.001	0.17	0.003	0.42	0.99
Diptera	40.5	< 0.001	0.22	< 0.001	0.13	0.78
Ephemeroptera	17.4	< 0.001	0.02	< 0.001	-0.62	< 0.001
Odonata	10.8	0.002	0.12	0.02	0.25	0.9
Plecoptera	14.8	< 0.001	0.21	0.005	-0.33	0.04
Trichoptera	23.1	< 0.001	0.009	< 0.001	-0.15	0.22

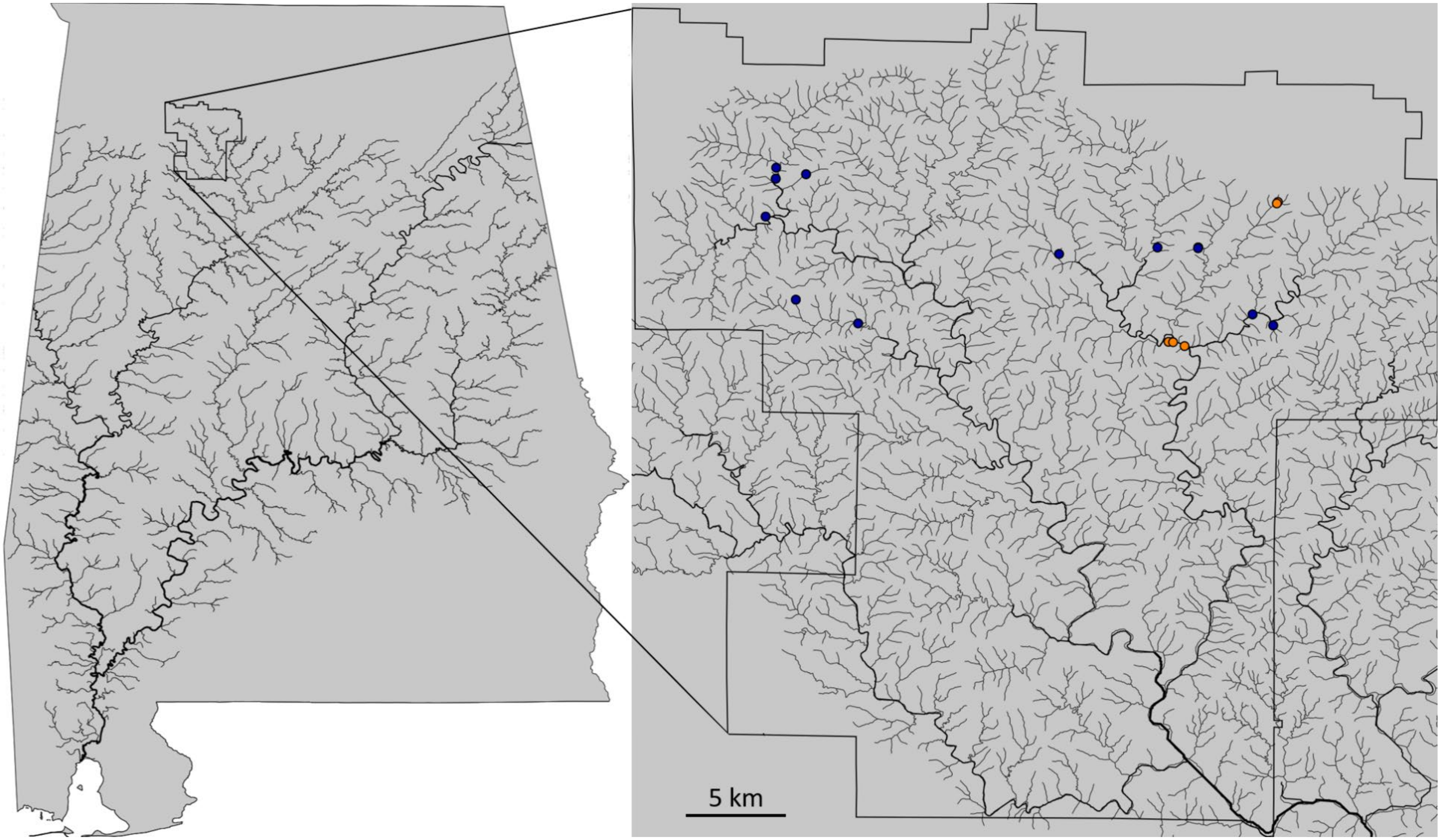


Figure 3.1. Map Alabama and an inset of the Bankhead National Forest with study site locations. Orange-filled circles indicate locations of study stream reaches sampled for invertebrates only and blue-filled circles indicate the locations of stream reaches sampled for fish and invertebrates.

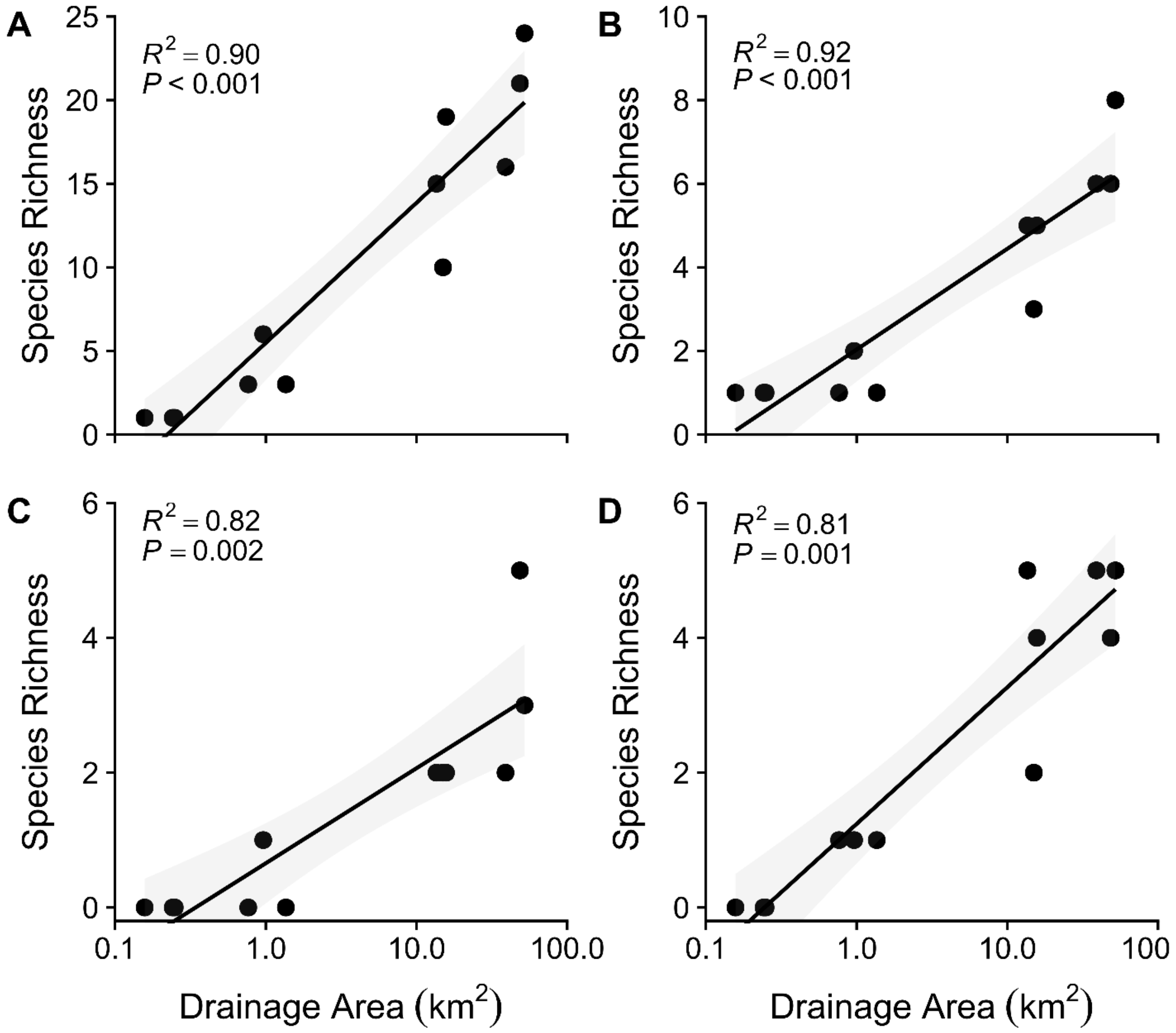


Figure 3.2. Relationships between stream size (as watershed drainage area) and species richness of A) total fish, B) Cyprinidae, C) Centrarchidae, and D) Percidae assemblages in the study reaches.

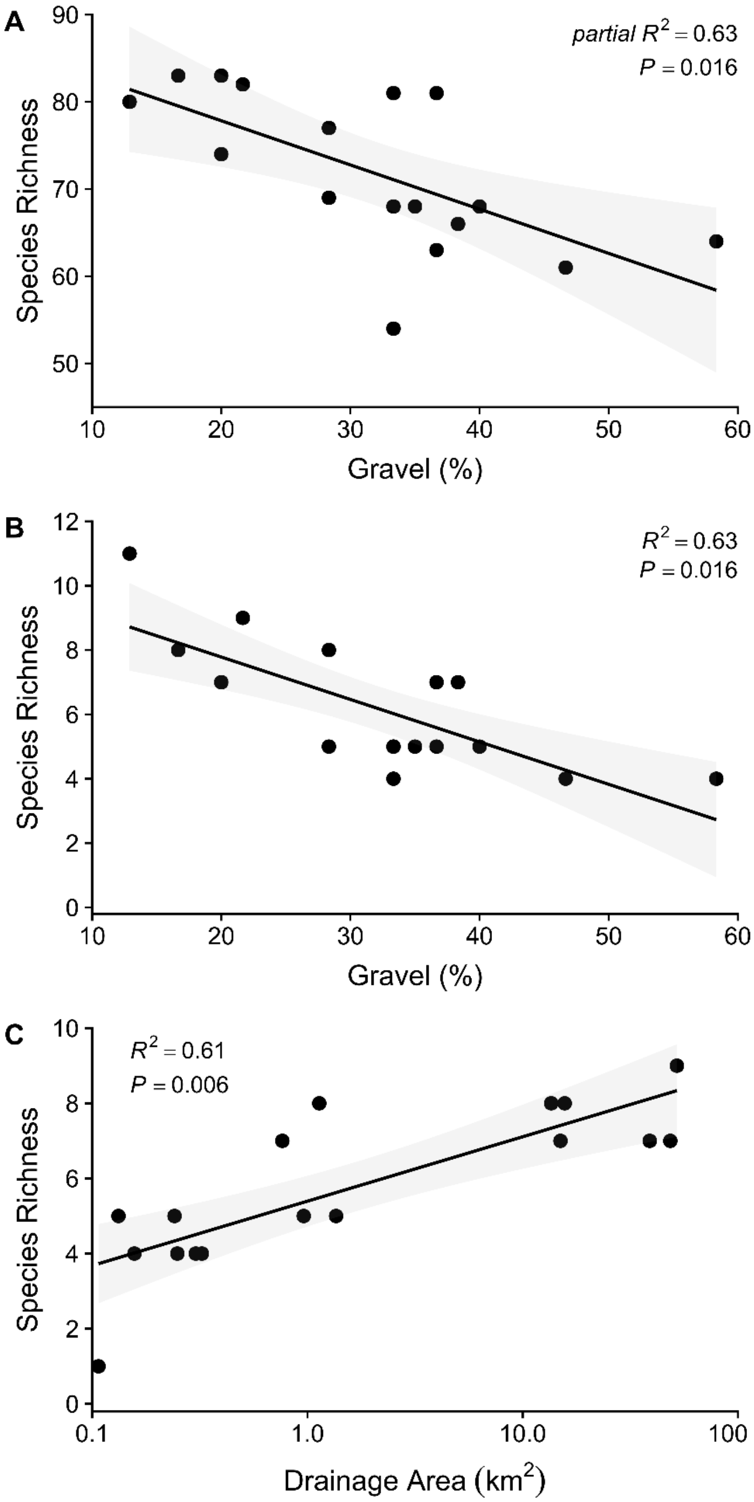


Figure 3.3. Relationships between % gravel and taxa richness of A) macroinvertebrate and B) Coleoptera, as well as between stream size (as drainage area) and C) Ephemeroptera assemblages in riffles of study reaches.

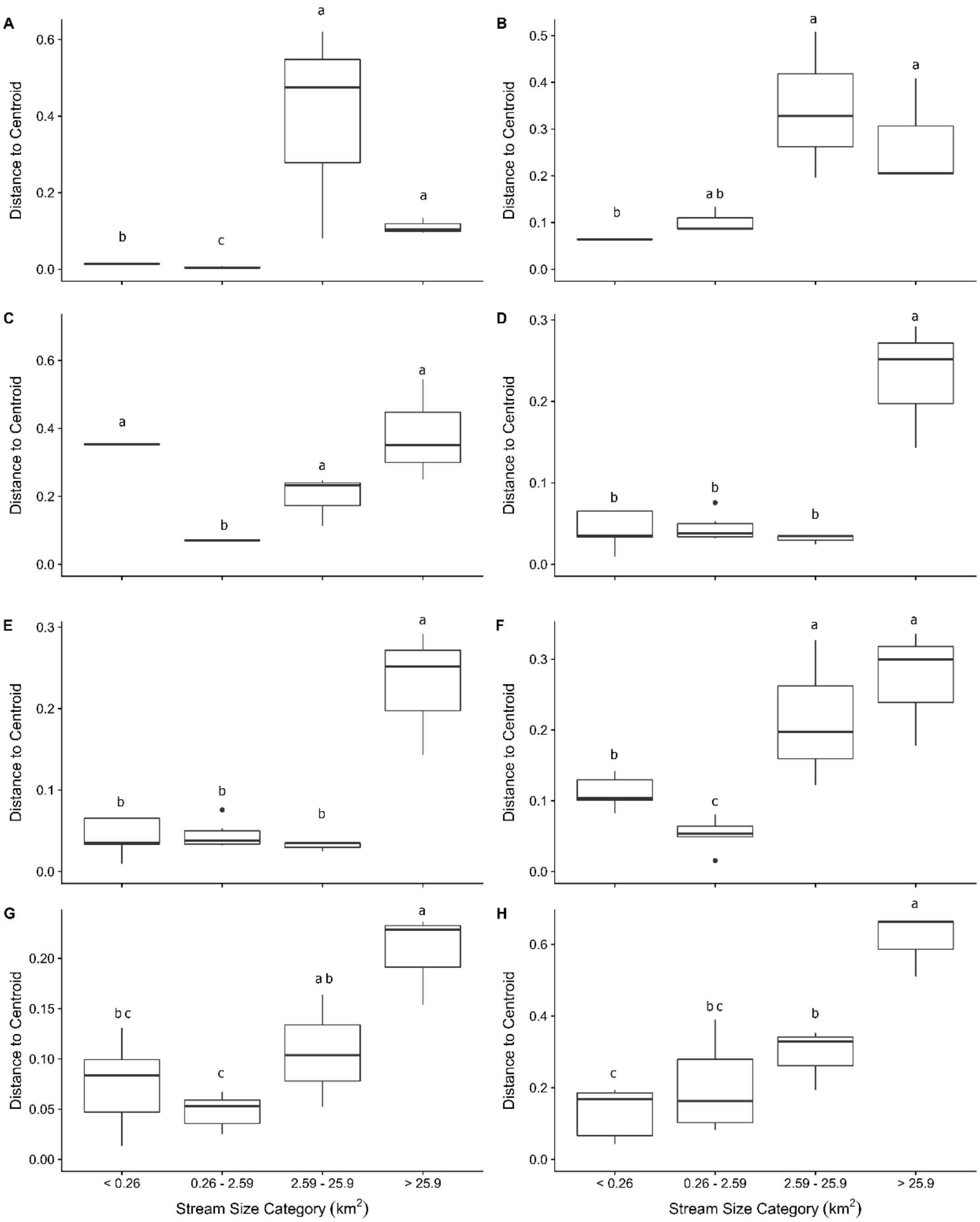


Figure 3.4. Boxplots comparing Raup-Crick β-diversity for A) total fish, B) Cyprinidae, C) Percidae, D) macroinvertebrate, E) aquatic insect, F) Chironomidae, G) Diptera, H) Ephemeroptera assemblages among stream size categories in study reaches. Medians are shown as the midline, 25th and 75th percentiles are shown as the lower and upper limits of the boxes respectively, and whiskers are 1.5 times the interquartile range.

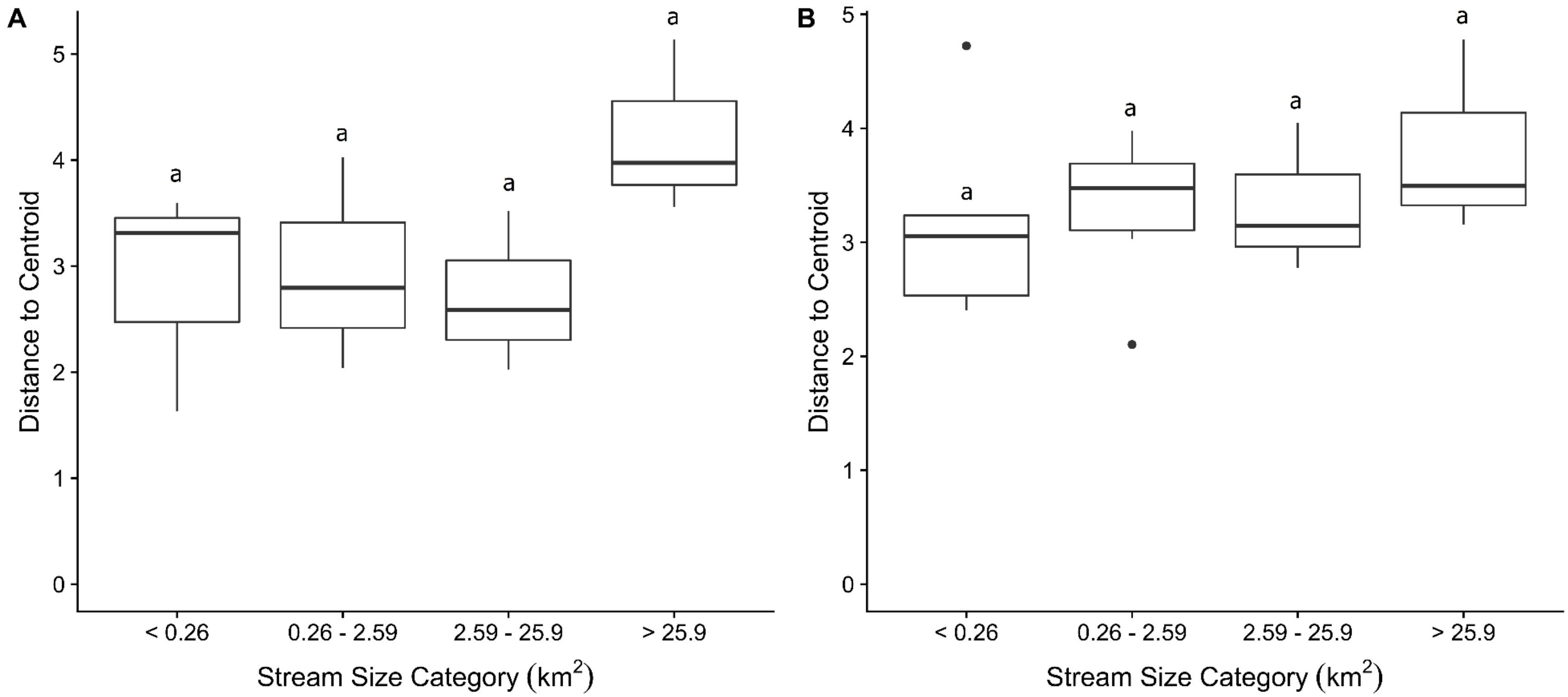


Figure 3.5. Boxplots comparing A) reach-level and B) riffle-level habitat dissimilarity, based on normalized Euclidean distances of habitat variables, among stream size categories in study reaches. Medians are shown as the midline, 25th and 75th percentiles are shown as the lower and upper limits of the boxes respectively, and whiskers are 1.5 times the interquartile range.

Chapter 4. Along for the ride: putting fish parasites on the river continuum

ABSTRACT

Decades ago, the River Continuum Concept (RCC) theorized that physical gradients from headwaters to rivers result in predictable longitudinal changes in species and functional group composition of aquatic communities. More recently, ecologists hypothesized that the spatial isolation and habitat heterogeneity among streams, rather than within a stream along its course, should also result in predictable changes in community composition from headwaters to rivers. I investigated the relationship between stream size and total richness (α-diversity), taxa richness within trait groups, β-diversity, and nestedness of parasite communities of the Redspot Darter (*Etheostoma artesiae*) in forested watersheds of the Bankhead National Forest, Alabama. Using this host-parasite model, I tested the predictions that there would be 1) a positive relationship between stream size and reach-level parasite richness and 2) stream size would be negatively correlated with β-diversity of parasites. Collection and necropsy of 425 Redspot Darters from 15 stream reaches (3rd to 7th order) yielded 24 morphospecies of parasites. Stream size was the only significant predictor of the reach-scale community richness, parasites with 2-host life cycles, microparasites, endoparasites, and parasites with fully aquatic life cycles. Stream size explained 35% of the total variation in reach-scale community richness. Mean richness of parasites among hosts within a reach was also positively related to stream size. There was significant nestedness among reach-scale parasite communities but, contrary to predictions, nestedness rank of individual parasite communities was not negatively correlated with stream size. Last, I found no significant differences in β-diversity among stream size category. These results provide evidence to support the application of the RCC model to fish parasites in stream networks, but failed

support predictions concerning patterns in β-diversity. The strong positive relationship between stream size and richness of reach-scale parasite communities and mean among-host communities suggests that the negative consequences for host health (e.g. condition or fitness) and host populations may increase longitudinally along the river continuum.

INTRODUCTION

The spatial distribution of biological diversity has important implications for conservation (Gering et al. 2003; Clarke et al. 2010; Kanno et al. 2012) and disease ecology (Ostfeld and Keesing 2000; Raymundo et al. 2009). In stream ecosystems, a primary conceptual model used to predict the spatial distribution of aquatic diversity is the River Continuum Concept (RCC; Vannote et al. 1980). RCC posits that longitudinal physical gradients from headwaters to rivers influence stream processes and diversity of resident aquatic biota (Vannote et al. 1980), and makes 2 primary predictions. First, RCC predicts that local species richness (α-diversity) is positively related to stream size (e.g., width, depth, volume, discharge) from low- to intermediate-order streams because increased environmental heterogeneity with stream size allows greater species coexistence (Vannote et al. 1980). Second, RCC predicts that because of stream widening and reduced canopy cover from headwater streams to rivers, shifts in predominant energy sources from allochthonous inputs in headwater streams to autochthonous in cause predictable changes in functional organization of aquatic communities (e.g., longitudinal shifts from shredder- to scraper-dominated macroinvertebrate assemblages; Vannote *et al.*, 1980). However, the RCC framework is primarily concerned with patterns along a streams longitudinal gradient but not patterns among streams within watersheds. More recently, it has also been predicted that there should be a negative relationship between β-diversity and stream size among streams within a watershed because low-order streams are spatially isolated from each other, which may lead to ecological drift in their communities or because the usually low habitat similarity among low-order streams is expected to result in low community similarity (Finn et al. 2011).

Predictions of the RCC have been repeatedly tested using fish and benthic macroinvertebrates (see Tornwall et al. 2015, Vorste et al. 2017), whereas the prediction of a negative relationship between stream size and β-diversity has been primarily tested with invertebrate communities or intraspecific genetic diversity (e.g., Finn *et al.*, 2011; Jyrkänkallio-mikkola *et al.*, 2018; Prunier *et al.*, 2018). Generally, the RCC prediction of a positive relationship between stream size and species richness is equivocal for macroinvertebrate assemblages but well supported for fish (see Vorste *et al.*, 2017). Additionally, the RCC's prediction of longitudinal changes in functional group composition is well supported for both fish and macroinvertebrates (e.g. Hawkins and Sedell, 1981; Schlosser, 1982; Grubaugh *et al.*, 1996; Goldstein and Meador, 2004; Tomanova *et al.*, 2007; Jiang *et al.*, 2011; Terra *et al.*, 2016). In contrast, the predicted negative relationship between β-diversity and stream size is far from universal (Clarke et al. 2010; Vorste et al. 2017) but has been reported for macroinvertebrates (e.g., Finn *et al.*, 2011; Maria *et al.*, 2014) and other taxa such as diatoms (Jyrkänkallio-Mikkola et al. 2018) in many stream networks.

The commonly observed positive richness-stream size relationship often results in species-poor upstream communities that are a non-random nested subsets of the species-rich downstream communities (e.g. Taylor et al. 2006). In a perfectly nested set of communities common species occur in all communities but rare species only occur in species rich communities (Patterson and Atmar 1986). Generally, the presence of such nestedness is interpreted as the result of ordered colonization-extinction dynamics (Patterson and Atmar 1986). In the context of the positive stream size-species richness relationship, nestedness would suggest that small headwater communities have lower colonization and greater extinction rates than larger streams (Taylor and Warren 2001) and may explain why the nestedness of insect communities in Finnish streams has been correlated with stream size (Heino et al. 2009, 2010).

Studies of the spatial distribution of parasite community diversity in streams are uncommon relative to other taxa and the RCC framework has only recently been applied to the abundance of fish parasites (Blasco-Costa et al. 2013). Furthermore, the predicted patterns for α- and β-diversity of stream communities have never been tested in fish-parasite communities. Parasites have a variety of life cycles with different obligate hosts which in turn influence their dispersal capability (Esch et al. 1988). These different life cycles and resulting differences in dispersal ability may be useful in illuminating mechanisms that structure parasite metacommunities in stream networks. For example, parasite communities in isolated headwaters could be dominated by parasites using vagile terrestrial hosts, such as fish-eating birds, because using these hosts is associated with wider parasite ranges than more limited aquatic dispersing hosts (Esch et al. 1988).

Determining the spatial distribution of diversity of fish parasite communities in stream networks is important because it could be useful in predicting disease dynamics (Johnson et al. 2015a, 2015b). Knowledge of parasite diversity patterns in stream networks is extremely limited, but the results of studies from other aquatic systems may inform expectations for parasite community diversity patterns in streams. Fish parasite community diversity has been positively associated with system size (e.g. Kennedy, 1978; Marcogliese and Cone, 1991), host diversity (e.g. Watters, 1992; Thieltges *et al.*, 2011; Negishi *et al.*, 2013), and host trophic level (Luque and Poulin 2008; Locke et al. 2014). More specifically, system size of lakes and ponds is positively linked with parasite richness (Kennedy 1978; Marcogliese and Cone 1991). If system size influences parasite richness in stream networks then, per RCC predictions, parasite richness should increase with stream size. Species richness of hosts also has been positively associated with parasite richness (e.g. Watters, 1992; Thieltges *et al.*, 2011; Negishi *et al.*, 2013), and, on average, host richness accounts for more than one-half of the variation in parasite richness (see Kamiya et al. 2014). Thus, fish parasite richness should increase along the river continuum because of downstream increases in both system size and fish diversity (Vannote et al. 1980; Vorste et al. 2017). These patterns suggest that, in streams, fish parasites should follow the predictions of the RCC. However, these fish parasite diversity patterns mainly come from studies of lentic systems, large rivers, or systems with anthropogenic influences, and less is known about the patterns of community structure of fish parasites in unperturbed small streams.

I investigated the relationship between stream size and diversity of parasites infecting a model fish (Redspot Darter, *Etheostoma artesiae*) in 3rd to 7th order streams of the Bankhead National Forest, Alabama. I hypothesized that parasite community richness and diversity are influenced by longitudinal physical gradients because parasite richness tends to be related to host community diversity and ecosystem size. Specifically, in *E. artesiae* , I predicted that parasite richness at the component community (all parasites infecting the host community at a location; sensu Bush et al. 1997) and mean infracommunity (all parasites infecting a single host sensu Bush et al. 1997) levels would be positively related to stream size. I also predicted that parasite communities of *E. artesiae* would show a high degree of nestedness because the lower diversity of hosts (e.g., fishes) in low-order streams should present a strong local habitat filter that prevents some parasite species from colonizing small streams. Nestedness would indicate that species-poor assemblages are non-random (i.e., similar) subsets of the species occurring in species-rich assemblages and suggest similar structuring environmental forces; I therefore did not expect fish parasites to exhibit a negative relationship between β-diversity and stream size because nestedness and species turnover (β-diversity) are opposing patterns.

METHODS

Study area and host fish species

Two separate watersheds were studied (Sipsey Fork and Brushy Creek) that drained the William B. Bankhead National Forest, northwest Alabama, in the Southwestern Appalachians ecoregion (Omernik 1995; Fig. 1). Study reaches drained catchments that were primarily forested (86.2-100%, mixed deciduous hardwood and hemlocks) with minor land use contributions from agriculture (<6.2%), impervious surface (<0.23%), and developed, open space (<6.5%). Streams in the Bankhead National Forest have bankfull measures of width, mean depth, cross sectional area, and estimated discharge that are strongly positively correlated with drainage area (DA; Brantley et al. 2016). Channel slopes generally range from 0.004 to 0.20 m/m and are negatively related to DA (Brantley et al. 2016). In these streams, fish species richness is strongly and positively correlated with stream size whereas macroinvertebrate richness is not (Chapt. 1). I selected 15 stream reaches for host fish collections based on estimated drainage area and using GRASS GIS (GRASS Development Team, 2017) derived DA and Strahler stream order (Strahler 1957) for each reach. DA was derived for use as a continuous variable in linear models and for creating arbitrary stream size categories of equal representation (DA < 2 km, 2-20 km^2, or > 20 km^2). DA categories and Strahler stream order were created so that β-diversity within stream size categories could be calculated. Stream channel riffles were primarily bedrock, boulders, cobble, and gravel with minor contributions from sand and fine sediments, which were the dominant substrate in pools. Evidence of entrenchment (i.e., bank erosion) was generally minimal throughout the study area (Brantley et al. 2016).

Examination of parasites of a single host species (*E. artesiae*, hereafter fish) allowed comparisons of component communities across sites. This native species was chosen because it is relatively small (< 8 cm standard length, SL), sedentary, and inhabits a wide range of stream sizes across the study area (Boschung and Mayden 2004). While the parasites of other darters have been reported (Scalet 1971; Hoffman 1999), no study has yet examined parasites in the Redspot Darter. Although the home range of *E. artesiae* is unknown, a mark recapture study of its close relative the Orangebelly Darter (*Etheostoma radiosum;* Lang and Mayden 2007) showed that adult darters moved < 30.5 m over the course of a year (Scalet 1973). Other darters also show little movement, traveling to no more than 2 riffles or pools from the original point of capture (Mundahl and Ingersoll 1983). Therefore, I assumed that *E. artesiae* has a similarly small home range and that parasite infracommunities reflected local conditions, thus the potential confounding effects of immigration on community composition were minimized by my use of this model host.

Host collection and necropsies

I collected fish from a representative reach in 15 August to 4 October 2015 from each catchment by working in an upstream direction using a Smith-Root LR-24 electrofisher (Smith-Root Inc., Vancouver, Washington) and a dip-net until I either caught >30 individuals or reached 80 min sampling time. I used total collection time (sec) to estimate catch per unit effort (CPUE; number collected/sampling time) as a proxy for host abundance because host population size or density has been positively associated with community richness (Bagge et al. 2004; Takemoto et al. 2005). After collection, fish were held in Kordon^{TM} breathing bags (Kordon LLC, Hayward, California) until they were transported to Auburn University, where they were euthanized with MS-222, placed in individual bags, and then frozen at 0C until necropsy.

Prior to necropsy, fish were thawed, weighed, and were measured for SL as host size has been found to influence infracommunity richness (e.g., Timi and Poulin 2003; Schabuss et al. 2005). Necropsies comprised external examination of skin, fins, and buccal cavity using a stereomicroscope (40X Olympus, Center Valley, Pennsylvania), and examining the host's eyes, gills, muscle tissue, heart, liver, gallbladder, urinary bladder, spleen, stomach, intestines, and gonads using a Nikon Eclipse E400 compound microscope (400X Nikon Inc., Melville, New York). Collected microscopic parasites (microparasites) and macroscopic parasites (macroparasites) were identified to morphospecies, preserved using standard methods (see Pritchard and Kruse 1982), and quantified for richness estimates. Parasites then were assigned to trait groups based on dispersal strategy, number of hosts, definitive host category, transmission type, life cycle stage, and size using Hoffman (1999) or life cycle descriptions of each parasite from the literature. Once taxa were assigned to trait groups, the number of morphospecies within trait groups per infracommunity and per component community were counted.

Statistical Analyses

All analyses, unless specified, were conducted in R (R Core Team 2014) and statistical significance assessed with an $\alpha = 0.05$. To test for significant relationships between stream size (as DA) and parasite α-diversity while controlling for potential confounding variables, I used a generalized linear model with a Poisson distribution, which is appropriate for non-normal count data (Wilson and Grenfell 1997). DA was log-transformed because it varied by orders of magnitude among sites. In the model, α-diversity was the dependent variable and DA, mean host length, sample size (# hosts examined), and host abundance (as CPUE) were independent variables. I also used the α-diversity of trait-based parasite groups and the same models to determine if parasite richness within particular trait groups increased with DA.

To ensure that my results were not influenced by differences in numbers of hosts sampled at each site, I also used a coverage-based rarefaction/extrapolation method using Hill numbers (Chao and Jost 2012; Colwell et al. 2012) from the iNEXT package (Hsieh et al. 2016) to estimate richness because it is less biased than sample size based rarefaction (Chao and Jost 2012). I then used a general linear model (rarefied estimates are normally distributed), with coverage-based estimates of richness as the dependent variable and the same independent variables (excluding sample size) as the above generalized linear models.

To determine if parasite communities within individual fish become more diverse as stream size increases, I used a general linear model to test for a relationship between mean infracommunity α-diversity and DA with the same predictor variables. A general linear model was appropriate in this case because mean infracommunity richness was normally distributed. I then calculated *adjusted partial* r^2 (hereafter *partial* r^2) values for DA from linear models to determine the amount of variance in parasite richness explained by DA.

To test my prediction of significant nestedness of parasite communities along the river continuum, I used the program BINMATNEST (Rodríguez-Gironés and Santamaría 2006) with 1000 permutations and recommended settings to determine if parasite component communities were nested among sites. BINMATNEST first arranges sites by ordering rows (sites) and columns (species) in descending order of species presence, and then calculates nestedness temperature (T), a measure of unexpected presences and absences in the matrix. BINMATNEST then compares *T* of the original matrix with *T* calculated for randomly generated matrices using 3 separate null models with different constraints to determine if the original matrix is more nested than expected by chance (Rodríguez-Gironés and Santamaría 2006). To test my prediction that upstream parasite communities would be non-random nested subsets of more species rich downstream communities, I tested for a negative correlation between stream size and nestedness rank (low rank indicates more species present at a site) from BINMATNEST using a Kendall's rank order correlation.

To investigate the relationship between stream size and β-diversity, I used a null model-derived pairwise Raup-Crick β-diversity metric, because it controls for the influence of differences in α-diversity and nestedness (Chase et al. 2011), and a permutational analysis of variance (ANOVA) test. I created a distance matrix of pairwise Raup-Crick dissimilarity metrics and then used the betadisper function (vegan package; Oksanen et al. 2018) in R to calculate the distance from each site to the centroid for its respective DA category and Strahler stream order. There was only one 7th-order stream, so I ran analyses where I either assigned this stream to the 6th order stream group or excluded it from the analysis. I then used a permutational ANOVA test (1000 permutations) to compare the pairwise average distances to group centroids among the 3 DA categories, or 4 Strahler stream order levels, to determine if β-diversity was significantly related to stream size.

RESULTS

DA of stream sites ranged from 1.01 (unnamed tributary of Brown Creek) to 217.51 km^2 (Brushy Creek; Table 1). The number of fish hosts collected ranged from 20 (Hubbard Creek) to

33 (upper Rush Creek; Table 1). Mean host size ranged from 37.8 to 45.8 mm SL and was not related to DA ($r = -0.09$, $p = 0.74$; Table 1) in contrast to prior reports for *E. artesiae*. I collected 24 morphospecies of parasites, which were assigned to 21 trait groups (Appendix 1). Parasite richness of ranged from 5 at Collier Creek to 15 at Turkey, Capsey, and Thompson creeks (Table 1).

DA was the only predictor variable significantly related to parasite richness at the infracommunity, component community, or trait-group levels. As DA increased there was an increase in richness of total component communities ($z = 2.52$, $p = 0.0117$, Fig 2A), 2-host parasites ($z = 2.54$, $p = 0.0111$, Fig. 2B), endoparasites ($z = 2.19$, $p = 0.0285$, Fig 2C), autogenic parasites ($z = 2.63$, $p = 0.0085$, Fig. 2D), and microparasites ($z = 2.67$, $p = 0.0075$, Fig. 2E). Stream size explained 35% of the variation in total parasite component community richness (*partial* $r^2 = 0.347$), 22% of the variation in microparasite richness (*partial* $r^2 = 0.215$), 40% of the variation in endoparasite richness (*partial* $r^2 = 0.399$), 47% of the variation in 2-host parasite richness (*partial* $r^2 = 0.471$), and 29% of the variation in autogenic parasite richness (*partial* $r^2 = 0.288$). Mean SL, CPUE, and sample size explained <10% of the variance in richness measures and were all nonsignificant. There was no relationship between DA and macroparasites ($z = 1.40$, $p = 0.16$), allogenic parasites ($z = 0.44$, $p = 0.66$), larval parasites ($z = 0.81$, $p = 0.42$), adult parasites ($z = 1.38$, $p = 0.17$), ectoparasites ($z = 1.26$, $p = 0.21$), intestinal helminths ($z = 1.38$, $p = 0.17$), 3-host parasites ($z = 0.82$, $p = 0.41$), or trophically transmitted parasites ($z = 1.22$, $p = 0.22$). There was also no relationship between DA and parasites that use birds ($z = 0.57$, $p = 0.57$), insects ($z = 0.25$, $p = 0.81$), fish ($z = 1.45$, $p = 0.15$), piscivorous fish ($z = 0.81$, $p = 0.42$), snails ($z = 1.72$, $p = 0.08$), or mollusks ($z = 0.99$, $p = 0.32$) as hosts.

Mean infracommunity richness was positively related to DA ($t = 3.23$, $p = 0.009$). Rarefaction estimated that at least 94% of the species occurring at each reach were contained in the samples (coverage), and thus richness patterns for 94% coverage was compared among all sites. Component community richness was still positively related to DA ($t = 2.29$, $p = 0.043$). Parasite component communities among sites were significantly nested ($T = 22.8$, $p < 0.001$) irrespective of which of the 3 null models were used. The relationship between stream size and nestedness rank order was negative but only marginally significant ($\tau = -0.37$, $p = 0.059$). There were no significant differences in β-diversity among stream size categories ($F = 1.54$, $p = 0.25$), Strahler stream orders excluding the largest (7th-order) stream (Brushy Creek, $F = 1.36$, $p = 0.31$), or Strahler stream orders when Brushy Creek was included in the 6th-order stream group ($F = 1.29$, $p = 0.37$).

DISCUSSION

In this study, I demonstrated that richness of parasite component communities and mean infracommunities in a stream-dwelling host fish was strongly and positively related to stream size, and that significant nestedness was observed among parasite component communities where species in poor communities were a non-random subset of those occurring in richer communities. Furthermore, richness of several trait-based categories of parasite taxa (2-host life cycles, endoparasites, microparasites, and autogenic parasites) were positively related to stream size. These findings provide foundational evidence to support the application of the River Continuum Concept model to fish parasites in stream networks. In contrast, the lack of a relationship between β-diversity and stream size did not support the prediction that β-diversity declines with increasing stream size and connectivity, at least at the watershed scale. My finding that richness within some trait groups increased with stream size while others did not suggests that life history traits, particularly those related to potential for dispersal, may be a useful means of exploring potential drivers of local parasite diversity among streams in a watershed.

Parasites on the River Continuum

Stream size (as DA) explained 35% of the variation among sites in total richness of parasite component communities. These results are similar to importance of the pattern of species additions found along the longitudinal gradient of a single stream (Basin Creek, North Carolina; Barger and Esch 2001), but documents this pattern across multiple streams in a stream network. My results also suggest that, in watersheds with minimal anthropogenic impacts, low-order streams are naturally depauperate in parasite species relative to downstream reaches. It also is important to note that stream size was a better predictor of parasite richness than either host size or host abundance (CPUE), as neither were significant in the analyses. This contrasts with the finding that CPUE was a stronger predictor of endoparasite community richness than host size, social behavior, reproductive behavior, spawning type, trophic position, trophic behavior, habitat preference, or native/exotic status among 53 fishes collected the upper Paraná River, Brazil (Takemoto et al. 2005). The relatively strong relationship between richness and stream size but not host size or abundance also is noteworthy because host size and abundance often are positively related to parasite richness (Guégan and Hugueny 1994; Morand et al. 2000; Poulin and Valtonen 2001; Arneberg 2002). However, part of this discrepancy may be that intraspecific host size is more important in determining infracommunity richness within a host population than among populations of hosts.

The pattern of increasing total parasite richness with increasing stream size appears to be driven by concomitant increases in endoparasites, microparasites, and autogenic parasites, and parasite species with 2-host life cycles. Parasites possessing all 4 of these traits, and thus were major contributors to downstream increase in richness, were the myxozoan parasites (*Myxobolous* sp. A, *Myxobolus* sp. B, *Myxidium* sp., and *Myxobilatus* sp.), which ranged from 0-4 species along the stream size gradient. These parasites also might have important pathological consequences for host health because many myxozoans are causative agents of consequential fish diseases, such as whirling disease, and can impact various organs of their hosts (Ellis et al. 1985; Hoffman 1990; Kent et al. 2001; Ferguson et al. 2011; Whipps 2011). Therefore, future studies should examine the host health consequences of increases in parasite richness downstream, and the degree to which myxozoans (vs. other species) promote pathogenetic impacts for host fish.

The observed longitudinal patterns of parasite diversity may have important implications for host health. Parasite richness is an important, but often overlooked, attribute of parasitic infections that may have negative effects on host populations (Bordes and Morand 2009). For example, parasite richness may drive genetic diversity in the major histocompatibility complex (Wegner et al. 2003; Šimková et al. 2006) and increase host basal metabolic rate (Morand and Harvey 2000) which shows energetic investment to adapt to and fight diverse parasitic infections. Additionally, parasite diversity has been shown to be negatively associated with reproductive investments (gonad size) and positively associated with immune system investments (e.g., spleen size) in fish (Šimková et al. 2008). Therefore, my results suggest that downstream host populations are more likely to suffer negative health consequences than upstream populations as a result of their comparatively diverse parasitic infections. Such impacts result from diverse parasitic infections may add to the consequences of increased abundance of individual parasites downstream which are more traditionally studied and considered (Bordes and Morand 2009). For example, *Uvulifer ambloplitis*, a parasite documented to have negative effects on host health (Lemly and Esch 1984), was more abundant on darters in larger streams where the abundance of their intermediate host snails also was high (E. Bauer, pers. obs.).

Mechanisms and metacommunity theory

My study was not designed to directly test metacommunity theory predictions for stream communities; however, metacommunity theory may be the best way to understand the mechanisms that could potentially explain the patterns I observed. Metacommunity theory ascribes to the view that local community structure is driven by both local environment (e.g., habitat) and regional (e.g., dispersal) factors, with the relative importance of either factor determined by the strength of community-environment interactions and degree of connectivity among communities (Leibold et al. 2004; Brown and Swan 2010). Metacommunity theory is a composite of four frameworks with different model assumptions; patch dynamic, neutral models, species sorting and mass effects. For my purposes, patch-dynamics and neutral model frameworks have assumptions that appear to be violated by freshwater parasite communities in stream networks. Specifically, both models assume that habitat patches are identical in their environmental conditions (Leibold et al. 2004; Brown and Swan 2010) but habitat patches in my study area are not identical. For example, I collected trophically transmitted parasites that require piscivorous-fish hosts (e.g., *Neoechinorhynchus* sp.) that do not occur in low-order streams in my study area (E. Bauer, pers. obs.) which suggests that there are differences in habitat for parasites (fish assemblages) among sites. In contrast, the species-sorting and mass effects paradigms assume that differences among habitat patches exist (Leibold et al. 2004; Brown and Swan 2010) which is more in line with observed habitat conditions in my study area. In the species sorting framework community similarity is expected to positively correspond with similarity in environmental conditions (Brown and Swan 2010). Therefore, the fact that fish assemblages in low-order streams of the Bankhead National Forest are a consistent subset (primarily *Semotilus atromaculatus* and *E. artesiae*) of comparatively richer downstream assemblages (E. Bauer, pers. obs.) should result in similar parasite communities among these low-order streams. In contrast, the mass effects framework expects a weakened community similarity – habitat similarity relationship due to strong dispersal from neighboring habitats that swamps the effects of local environmental conditions (and spatial auto-correlation) and species occur in suboptimal habitats due to high dispersal (Brown and Swan 2010).

Several lines of evidence suggest that local environment is the primary factor structuring parasite communities among reaches in stream networks, which is consistent with the species sorting paradigm of metacommunity theory. First, the predicted and observed positive relationship between stream size and parasite community richness may be explained by local host diversity or richness. Like parasite community richness, the richness of fish assemblages increases with stream size in my study area (Brantley et al. 2016) and suggests that fish assemblage diversity could be a primary structuring force on fish parasite communities in stream networks because the habitats for fish parasites are the host fish assemblages they. Additionally, the observed relationship between stream size and parasite richness, if driven by host diversity, would be consistent with studies reporting that parasite diversity is positively and directly related to host diversity (Hechinger and Lafferty 2005; Hechinger et al. 2007; Kamiya et al. 2014). Second, the species sorting model predicts anticipates nestedness in a metacommunity when habitats are also nested (Driscoll 2008). Fish assemblages, the primary habitat for fish parasites, are nested in the Bankhead National Forest along the stream size gradient with *Semotilus atromaculatus* and *E. artesiae* consistently the dominant inhabitants of low-order streams to which other species of fish are added downstream (Chapter 2). Therefore, the negative and marginally significant relationship between parasite community nestedness and stream size could be explained by the parallel nestedness patterns among fish assemblages in the stream network. However, future studies should do more to try to disentangle the direct and indirect effects of stream size from effects of host community richness. Additionally, parasites with complex life cycles often have obligate invertebrate hosts that are also part of their habitat requirements. In contrast to fishes, macroinvertebrate communities do not increase in richness along the stream size gradient in the Bankhead National Forest (Chapter 2) but some important invertebrate hosts (e.g. snails) do not occur in low-order streams of this region (E. Bauer, pers. obs.). The lack of snails may therefore represent an additional habitat filter (sensu Wright et al. 1998) in low-order streams that reduces parasite community richness and increases metacommunity nestedness along the stream size gradient.

A third indication of the influence local factors is that the low β-diversity among streams of similar size suggests local factors (e.g., habitat similarity) determine parasite community composition in similarly sized streams. This pattern again might reflect similar fish assemblages exist in streams of similar sizes (e.g., low-order stream assemblages consisting of *S. atromaculatus* and *E. artesiae*). The low β-diversity among streams of similar size is consistent with the predictions of the species sorting paradigm because it predicts that community similarity increases with increasing environmental similarity (Brown and Swan 2010). Therefore, the patterns of richness, nestedness, and community similarity of fish parasites in the Bankhead National Forest as suggesting that the species-sorting model from metacommunity theory is the most applicable model to fish-parasite communities in stream networks, at least in relatively undisturbed streams. Thus my data support the hypothesis that the species-sorting model is the most likely to apply in low-order streams which are expected to be more spatially isolated than high-order streams (Brown and Swan 2010).

My study is the first to document nestedness among component communities of a stream fish. Nestedness in parasite communities in other environments however tends to be inconsistent over time and space (Vidal-Martínez and Poulin 2003; Poulin 2007; Kennedy 2009). For example, only one-third of parasite communities of global marine fishes examined are nested (Rohde et al. 1998). However, most of these studies examined nestedness among individual hosts (infracommunities) in the same component community and not among component communities, where biogeographical colonization-extinction dynamics (e.g. dispersal) are more likely to occur (González and Poulin 2005). Consistent nested patterns of fish parasite communities have been reported for populations of 9 species of marine fishes, which were suggested to be structured by species sorting mechanisms (e.g., host population size, environmental conditions for ectoparasites, changes in the host prey or available intermediate hosts for endoparasites, González and Oliva 2009). Thus, I also interpret the nestedness pattern among fish parasite communities in this study to be evidence of species sorting.

In contrast to my other predictions which were strongly supporter, the prediction that nestedness rank would be negatively correlated with stream size was only weakly supported and marginally significant ($\tau = -0.37$, $p = 0.059$). I interpret this weaker evidence for a relationship between stream size and nestedness rank to be consistent with the influence of dispersal from mass-effects (sensu Leibold et al. 2004) because dispersal is expected to decrease nestedness when habitats are heterogeneous (Gianuca et al. 2016). For example, my sample reach on White Oak Creek, a low-order (3rd order) tributary of Thompson Creek (6th order), was <0.5 km from the confluence with Thompson Creek and had a richer fish assemblage (including centrarchids) than expected for its size suggesting relatively high connectivity between these streams (Chapter 2). This may explain why White Oak Creek had high parasite richness for a relatively small stream and could have weakened the stream size – nestedness relationship. In fact, excluding White Oak Creek resulted in the predicted significant negative relationship between stream size and nestedness ($\tau = -0.47$, $p = 0.019$). It also should be noted that non-parametric tests tend to have lower power than parametric tests (Siegel and Castellan 1988), which reduced my ability to detect a significant relationship when White Oak Creek was included. The influence of dispersal on parasite communities in stream networks maybe elucidated in future studies, in part, by examining the influence of connectivity or network position, as measured by downstream link magnitude (sensu Osborne and Wiley 2008). Measures of connectivity or network position like link magnitude have been found to correlate with fish community richness and composition (Gorman 1986, Osborne and Wiley 2008).

Autogenic (i.e., completely aquatic) species richness was positively correlated with stream size, whereas allogenic (i.e., partially terrestrial) species richness showed no relationship with stream size. This disparity in richness patterns between allogenic and autogenic parasites may be a result of varying parasite dispersal or because there were fewer allogenic species than autogenic species collected. There were consistently 4 to 8 allogenic parasites among sites along the stream size gradient whereas autogenic parasite species richness ranged from 1 to 9 among sites, with higher in larger streams. One explanation for this pattern is that isolated low-order streams are less of a barrier to dispersal for allogenic parasites as they are for autogenic parasites because the former uses highly vagile hosts such as fish-eating birds. This inference is consistent with the finding that autogenic parasites of Arctic char (*Salvelinus alpinus*) were restricted to the lower portions of a linear catchment of interconnected lakes, whereas allogenic parasites were ubiquitous throughout the catchment (Paterson et al. 2018). The contrasting patterns between allogenic and autogenic species in my study also suggests that mass effects (increased community due to dispersal) are more likely to occur in the allogenic element of parasite communities, because of their high capacity for dispersal, than in the allogenic element of parasites communities. This potential explanation is consistent with the results of prior studies that report allogenic species to be more widespread than autogenic species (Esch et al. 1988; Karvonen and Valtonen 2004; Fellis and Esch 2005; Paterson et al. 2018). Thus, parasites, because of their innate differences in dispersal ability (Esch et al. 1988), may provide fertile ground for testing predictions from metacommunity theory.

In summary, this study is the first to document a consistent and strong positive relationship between stream size and parasite community richness and the presence of significant nestedness in a parasite metacommunity in a stream network. These patterns are likely to be common in streams because of the natural branching spatial structure of streams and fish species richness commonly increases with stream size. Thus, studies of fish parasites in lotic ecosystems should report and account for stream size. However, more parasite metacommunity and long-term studies in streams are required to confirm the predicted ubiquity of the stream size-parasite richness and nestedness patterns. Ultimately, the potential mechanistic causes of the metacommunity patterns observed in this study need to be disentangled and systematically tested, but trait-based analyses of parasite communities, particularly those focused on contrasting dispersal abilities, may offer a path forward. Additionally, the positive relationship between parasite richness and stream size has important implications for host health and populations in fluvial systems and this natural variation in parasite richness among host populations may offer opportunities to investigate the effects of parasite diversity on host health.

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LITERATURE CITED

Arneberg, P. 2002. Host population density and body mass as determinants of species richness in parasite communities: comparative analyses of directly transmitted nematodes of mammals. Ecography 25(1): 88–94.

Bagge, A. M., R. Poulin, and E. T. Valtonen. 2004. Fish population size, and not density, as the determining factor of parasite infection: a case study. Parasitology 128: 305–313.

Barger, M. A, and G. W. Esch. 2001. Downstream changes in the composition of the parasite community of fishes in an Appalachian stream. The Journal of parasitology 87(2): 250–255.

Blasco-Costa, I., A. V. Koehler, A. Martin, and R. Poulin. 2013. Upstream-downstream gradient in infection levels by fish parasites: a common river pattern? Parasitology 140: 266–274.

Bordes, F., and S. Morand. 2009. Parasite diversity: an overlooked metric of parasite pressures? Oikos 118(6): 801–806.

Boschung, H., and R. L. Mayden. 2004. Fishes of Alabama. Smithsonian Books, Washington D.C.

Brantley, E., B. Helms, A. Ludwig, J. Shaw, G. Jennings, C. Anderson, D. Werneke, and J. Feminella. 2016. Eco-morphological mitigation design and assessment tools for the Alabama and Tennessee Appalachian Plateau. US EPA Region IV Atlanta, GA. Grant No. CD00D01412. http://cses.auburn.edu/eve-brantley/wp-content/uploads/sites/114/2016/10/Final-report_CD00D01412_-093016.pdf

Brown, B. L., and C. M. Swan. 2010. Dendritic network structure constrains metacommunity properties in riverine ecosystems. Journal of Animal Ecology 79(3): 571–580.

Bush, A. O., K. D. Lafferty, J. M. Lotz, and A. W. Shostak. 1997. Parasitology meets ecology on its own terms: Margolis et al. revisited. The Journal of Parasitology 83(4): 575–583.

Chao, A., and L. Jost. 2012. Coverage-based rarefaction and extrapolation: standardizing samples by completeness rather than size. Ecology 93(12): 2533–2547.

Chase, J. M., N. J. B. Kraft, K. G. Smith, M. Vellend, and B. D. Inouye. 2011. Using null models to disentangle variation in community dissimilarity from variation in α-diversity. Ecosphere 2(2): 1–11.

Clarke, A., R. Mac Nally, N. R. Bond, and P. S. Lake. 2010. Conserving macroinvertebrate diversity in headwater streams: the importance of knowing the relative contributions of α and β diversity. Diversity and Distributions 16(5): 725–736.

Colwell, R. K., A. Chao, N. J. Gotelli, S. Y. Lin, C. X. Mao, R. L. Chazdon, and J. T. Longino. 2012. Models and estimators linking individual-based and sample-based rarefaction, extrapolation and comparison of assemblages. Journal of Plant Ecology 5(1): 3–21.

Driscoll, D. 2008. The frequency of metapopulations, metacommunities and nestedness in a fragmented landscape. Oikos 117(2): 297–309.

Ellis, A. E., A. H. McVicar, and A. L. S. Munro. 1985. Proliferative kidney disease in brown trout, *Salmo trutta* L., and Atlantic salmon, *Salmo salar* L., parr: histopathological and epidemiological observations. Journal of Fish Diseases 8(2): 197–208.

Esch, G. W., C. R. Kennedy, A. O. Bush, and J. M. Aho. 1988. Patterns in helminth communities in freshwater fish in Great Britain: alternative strategies for colonization. Parasitology 96: 519–532.

Fellis, K. J., and G. W. Esch. 2005. Autogenic-allogenic status affects interpond community similarity and species area relationship of macroparasites in the bluegill sunfish, *Lepomis macrochirus*, from a series of freshwater ponds in the Piedmont area of North Carolina. The Journal of parasitology 91(4): 764–7.

Ferguson, J. A., W. Koketsu, I. Ninomiya, P. A. Rossignol, K. C. Jacobson, and M. L. Kent. 2011. Mortality of coho salmon (*Oncorhynchus kisutch*) associated with burdens of multiple parasite species. International Journal for Parasitology 41(11): 1197–1205.

Finn, D. S., N. Bonada, C. Múrria, and J. M. Hughes. 2011. Small but mighty: headwaters are vital to stream network biodiversity at two levels of organization. Journal of the North American Benthological Society 30(4): 963–980.

Gering, J. C., T. O. Crist, and J. A. Veech. 2003. Regional conservation of biodiversity additive partitioning of species diversity across multiple spatial scales: implications for regional conservation of biodiversity. Conservation Biology 17(2): 488–499.

Gianuca, A. T., S. A. J. Declerck, P. Lemmens, and L. De Meester. 2016. Effects of dispersal and environmental heterogeneity on the replacement and nestedness components of β - diversity. Ecology 98(2): 525–533.

Goldstein, R. M., and M. R. Meador. 2004. Comparisons of fish species traits from small streams to large rivers. Transactions of the American Fisheries Society 133(4): 971–983.

González, M. T., and M. E. Oliva. 2009. Is the nestedness of metazoan parasite assemblages of marine fishes from the southeastern Pacific coast a pattern associated with the geographical distributional range of the host? Parasitology 136(4): 401–9.

González, M. T., and R. Poulin. 2005. Nested patterns in parasite component communities of a marine fish along its latitudinal range on the Pacific coast of South America. Parasitology 131(Pt 4): 569–577.

Göthe, E., N. Friberg, M. Kahlert, J. Temnerud, and L. Sandin. 2014. Headwater biodiversity among different levels of stream habitat hierarchy. Biodiversity and Conservation 23(1): 63–80.

Grubaugh, J. W., J. B. Wallace, and E. S. Houston. 1996. Longitudinal changes of macroinvertebrate communities along an Appalachian stream continuum. Canadian Journal of Fisheries and Aquatic Sciences 53(4): 896–909.

Guégan, J. F., and B. Hugueny. 1994. A nested parasite species subset pattern in tropical fish: host as major determinant of parasite infracommunity structure. Oecologia 100(1–2): 184–189.

Hawkins, C. P., and J. R. Sedell. 1981. Longitudinal and seasonal changes in functional organization of macroinvertebrate communities in four Oregon streams. Ecology 62(2): 387–397.

Hechinger, R. F., and K. D. Lafferty. 2005. Host diversity begets parasite diversity: bird final hosts and trematodes in snail intermediate hosts. Proceedings of the Royal Society Biology 272(1567): 1059–1066.

Hechinger, R. F., K. D. Lafferty, T. C. Huspeni, A. J. Brooks, and A. M. Kuris. 2007. Can parasites be indicators of free-living diversity? Relationships between species richness and the abundance of larval trematodes and of local benthos and fishes. Oecologia 151(1): 82–92.

Heino, J., H. Mykrä, and T. Muotka. 2009. Temporal variability of nestedness and idiosyncratic species in stream insect assemblages. Diversity and Distributions 15(2): 198–206.

Heino, J., H. Mykrä, and J. Rintala. 2010. Assessing patterns of nestedness in stream insect assemblages along environmental gradients. Ecoscience 17(4): 345–355.

Hoffman, G. L. 1990. Myxobolus cerebralis, a worldwide cause of Salmonid whirling disease. Journal of Aquatic Animal Health 2(2): 30–3730.

Hoffman, G. L. 1999. Parasites of North American freshwater fishes, 2nd edition. Cornell University Press, Ithaca, NY.

Hsieh, T. C., K. H. Ma, and A. Chao. 2016. iNEXT: An R package for interpolation and extrapolation in measuring species diversity. Available at: http://chao.stat.nthu.edu.tw/blog/software-download/inext-r-package (accessed 1 January 2018).

Jiang, X., J. Xiong, Z. Xie, and Y. Chen. 2011. Longitudinal patterns of macroinvertebrate functional feeding groups in a Chinese river system: a test for river continuum concept (RCC). Quaternary International 244(2): 289–295.

Johnson, P. T. J., R. S. Ostfeld, and F. Keesing. 2015a. Frontiers in research on biodiversity and disease. Ecology Letters 18(10): 1119–1133.

Johnson, P. T. J., J. C. de Roode, and A. Fenton. 2015b. Why infectious disease research needs community ecology. Science 349(6252): 1259504.

Jyrkänkallio-Mikkola, J., M. Siljander, V. Heikinheimo, P. Pellikka, and J. Soininen. 2018. Tropical stream diatom communities – the importance of headwater streams for regional diversity. Ecological Indicators 95: 183–193.

Kamiya, T., K. O'Dwyer, S. Nakagawa, and R. Poulin. 2014. Host diversity drives parasite diversity: meta-analytical insights into patterns and causal mechanisms. Ecography 37(7): 689–697.

Kanno, Y., W. T. Russ, C. J. Sutherland, and S. B. Cook. 2012. Prioritizing aquatic conservation areas using spatial patterns and partitioning of fish community diversity in a near-natural temperate basin. Aquatic Conservation: Marine and Freshwater Ecosystems 22(6): 799–812.

Karvonen, A., and E. T. Valtonen. 2004. Helminth assemblages of whitefish (*Coregonus lavaretus*) in interconnected lakes: similarity as a function of species-specific parasites and geographical separation. The Journal of parasitology 90(3): 471–476.

Kennedy, C. R. 1978. An analysis of the metazoan parasitocoenoses of brown trout *Salmo trutta* from British Lakes. Journal of Fish Biology 13(2):255–263.

Kennedy, C. R. 2009. The ecology of parasites of freshwater fishes: the search for patterns. Parasitology 136(12):1653–1662.

Kent, M. L., K. B. Andree, J. L. Bartholomew, M. El-Matbouli, S. S. Desser, R. H. Devlin, S. W. Feist, R. P. Hedrick, R. W. Hoffmann, J. Khattra, S. L. Hallett, R. J. G. Lester, M.

Longshaw, O. Palenzeula, E. Siddall, and C. Xiao. 2001. Recent advances in our knowledge of the Myxozoa. The Journal of Eukaryotic Microbiology 48(4): 395–413.

Lang, N. J., and R. L. Mayden. 2007. Systematics of the subgenus Oligocephalus (Teleostei: Percidae: Etheostoma) with complete subgeneric sampling of the genus *Etheostoma*. Molecular Phylogenetics and Evolution 43(2): 605–615.

Leibold, M. A., M. Holyoak, N. Mouquet, P. Amarasekare, J. M. Chase, M. F. Hoopes, R. D. Holt, J. B. Shurin, R. Law, D. Tilman, M. Loreau, and A. Gonzalez. 2004. The metacommunity concept: a framework for multi-scale community ecology. Ecology Letters 7(7): 601–613.

Lemly, A. D., and G. W. Esch. 1984. Effects of the trematode *Uvulifer ambloplitis* on juvenile Bluegill sunfish , *Lepomis macrochirus*: ecological implications. The Journal of Parasitology 70(4): 475–492.

Locke, S. A., D. J. Marcogliese, and E. Tellervo Valtonen. 2014. Vulnerability and diet breadth predict larval and adult parasite diversity in fish of the Bothnian Bay. Oecologia 174(1): 253–262.

Luque, J. L., and R. Poulin. 2008. Linking ecology with parasite diversity in Neotropical fishes. Journal of Fish Biology 72(1): 189–204.

Marcogliese, D. J., and D. K. Cone. 1991. Importance of lake characteristics in structuring parasite communities of salmonids from insular Newfoundland. Canadian Journal of Zoology 69(12): 2962–2967.

Morand, S., T. H. Cribb, M. Kulbicki, M. C. Rigby, C. Chauvet, V. Dufour, E. Faliex, R. Galzin, C. M. Lo, A. Lo-Yat, S. Pichelin, and P. Sasal. 2000. Endoparasite species richness of new caledonian butterfly fishes: host density and diet matter. Parasitology 121: 65–73.

Morand, S., and P. H. Harvey. 2000. Mammalian metabolism, longevity and parasite species richness. Proceedings of the Royal Society B: Biological Sciences 267(1456): 1999–2003.

Mundahl, N. D., and C. G. Ingersoll. 1983. Early autumn movements and densities of Johnny (*Etheostoma nigrum*) and fantail (*E. flabellare*) darters in a southwestern Ohio stream. Ohio Journal of Science 83(3): 103–108.

Negishi, J. N., S. Nagayama, M. Kume, S. Sagawa, Y. Kayaba, and Y. Yamanaka. 2013. Unionoid mussels as an indicator of fish communities: A conceptual framework and empirical evidence. Ecological Indicators 24:127–137.

Oksanen, J., F. G. Blanchet, M. Friendly, R. Kindt, P. Legendre, D. McGlinn, P. R. Minchin, R. B. O'Hara, G. L. Simpson, P. Solymos, M. H. H. Stevens, E. Szoecs, and H. Wagner. 2018. vegan: community ecology package. R package version 2.4-6. https://cran.r-project.org/package=vegan

Omernik, J. M. 1995. Ecoregions: a framework for managing ecosystems. The George Wright Forum. 12(1): 35–50.

Osborne, L. L., and M. J. Wiley. 2008. Influence of tributary spatial position on the structure of warmwater fish communities. Canadian Journal of Fisheries and Aquatic Sciences 49(4): 671–681.

Ostfeld, R. S., and F. Keesing. 2000. Biodiversity and disease risk: the case of lyme disease. Conservation Biology 14(3): 722–728.

Paterson, R. A., R. Knudsen, A. M. Dunn, and S. Hytterød. 2018. Determinants of parasite distribution in Arctic char populations: catchment structure versus dispersal potential. Journal of Helminthology 93: 559–566.

Patterson, B. D., and W. Atmar. 1986. Nested subsets and the structure of insular mammalian faunas and archipelagos. Biological Journal of the Linnean Society 28: 65–82.

Poulin, R. 2007. Are there general laws in parasite ecology? Parasitology 134: 763–776.

Poulin, R., and E. T. Valtonen. 2001. Nested assemblages resulting from host size variation: the case of endoparasite communities in fish hosts. International Journal for Parasitology 31(11): 1194–1204.

Pritchard, M. H., and G. O. Kruse. 1982. The collection and preservation of animal parasites. The Harold W. Manter Laboratory, University of Nebraska Press, Lincoln, Nebraska. Technical Bulletin No. 1. 141p

Prunier, J. G., V. Dubut, G. Loot, L. Tudesque, and S. Blanchet. 2018. The relative contribution of river network structure and anthropogenic stressors to spatial patterns of genetic diversity in two freshwater fishes: a multiple-stressors approach. Freshwater Biology 63(1): 6–21.

R Core Team. 2014. R: A language and environment for statistical computing (version 3.2.0). R Foundation for Statistical Computing. http://www.r-project.org/.

Raymundo, L. J., A. R. Halford, A. P. Maypa, and A. M. Kerr. 2009. Functionally diverse reef-fish communities ameliorate coral disease. Proceedings of the National Academy of Sciences 106(40): 17067–17070.

Rodríguez-Gironés, M. a., and L. Santamaría. 2006. A new algorithm to calculate the nestedness temperature of presence-absence matrices. Journal of Biogeography 33(5): 924–935.

Rohde, K., W. B. Worthen, M. Heap, B. Hugueny, and J. F. Guégan. 1998. Nestedness in assemblages of metazoan ecto- and endoparasites of marine fish. International Journal for Parasitology 28(4): 543–549.

Scalet, C. G. 1971. Parasites of the orangebelly darter, *Etheostoma radiosum* (Pisces: Percidae). The Journal of Parasitology 57(4): 900.

Scalet, C. G. 1973. Stream movements and population density of the Orangebelly Darter, *Etheostoma radiosum cyanorum* (Osteichthyes: Percidae). The Southwestern Naturalist 17(4): 381–387.

Schabuss, M., C. R. Kennedy, R. Konecny, B. Grillitsch, F. Schiemer, and A. Herzig. 2005. Long-term investigation of the composition and richness of intestinal helminth communities in the stocked population of eel, *Anguilla anguilla*, in Neusiedler See, Austria. Parasitology 130: 185–194.

Schlosser, I. J. 1982. Fish community structure and function along two habitat gradients in a headwater stream. Ecological Monographs 52(4): 395–414.

Siegel, S., and N. J. Castellan. 1988. Non-parametric statistics for the behavioral sciences, 2nd edition. MacGraw-Hill International, New York, New York.

Šimková, A., T. Lafond, M. Ondračková, P. Jurajda, E. Ottová, and S. Morand. 2008. Parasitism, life history traits and immune defense in cyprinid fish from Central Europe. BMC Evolutionary Biology 8(1): 1–11.

Šimková, A., E. Ottová, and S. Morand. 2006. MHC variability, life-traits and parasite diversity of European cyprinid fish. Evolutionary Ecology 20(5): 465–477.

Strahler, A. N. 1957. Quantitative analysis of watershed geomorphology. Transactions American Geophysical Union 38(6): 913–920.

Takemoto, R. M., G. C. Pavanelli, M. A. P. Lizama, J. L. Luque, and R. Poulin. 2005. Host population density as the major determinant of endoparasite species richness in floodplain fishes of the upper Paraná River, Brazil. Journal of Helminthology 79(1): 75–84.

Taylor, C. M., T. L. Holder, R. A. Fiorillo, L. R. Williams, R. B. Thomas, and M. L. Warren, Jr. 2006. Distribution, abundance, and diversity of stream fishes under variable environmental conditions. Canadian Journal of Fisheries and Aquatic Sciences 63(1): 43–54.

Taylor, C. M., and M. L. Warren. 2001. Dynamics in species composition of stream fish assemblages: environmental variability and nested subsets. Ecology 82(8): 2320–2330.

Terra, B. D. F., R. M. Hughes, and F. G. Araújo. 2016. Fish assemblages in Atlantic Forest streams: the relative influence of local and catchment environments on taxonomic and functional species. Ecology of Freshwater Fish 25(4): 527–544.

Thieltges, D. W., C. Hof, D. M. Dehling, M. Brändle, R. Brandl, and R. Poulin. 2011. Host diversity and latitude drive trematode diversity patterns in the European freshwater fauna. Global Ecology and Biogeography 20(5): 675–682.

Timi, J. T., and R. Poulin. 2003. Parasite community structure within and across host populations of a marine pelagic fish: how repeatable is it? International Journal for Parasitology 33(12): 1353–1362.

Tomanova, S., P. A. Tedesco, M. Campero, P. A. Van Damme, N. Moya, and T. Oberdorff. 2008. Longitudinal and altitudinal changes of macroinvertebrate functional feeding groups in neotropical streams: a test of the River Continuum Concept. Fundamental and Applied Limnology, Archiv für Hydrobiologie 170(3): 233–241.

Tornwall, B., E. Sokol, J. Skelton, and B. L. Brown. 2015. Trends in stream biodiversity research since the river continuum concept. Diversity 7(1): 16–35.

Vannote, R. L., G. W. Minshall, K. W. Cummins, J. R. Sedell, and C. E. Cushing. 1980. The River Continuum Concept. Canadian Journal of Fisheries and Aquatic Sciences 37(1): 130–137.

Vidal-Martínez, V. M., and R. Poulin. 2003. Spatial and temporal repeatability in parasite community structure of tropical fish hosts. Parasitology 127: 387–398.

Vorste, R. Vander, P. McElmurray, S. Bell, K. M. Eliason, and B. L. Brown. 2017. Does stream size really explain biodiversity patterns in lotic systems? a call for mechanistic explanations. Diversity 9(26): 1–21.

Watters, G. T. 1992. Unionids, fishes, and the species-area curve. Journal of Biogeography 19(5): 481–490.

Wegner, K. M., T. B. H. Reusch, and M. Kalbe. 2003. Multiple parasites are driving major histocompatibility complex polymorphism in the wild. Journal of Evolutionary Biology 16(2): 224–232.

Whipps, C. M. 2011. Interrenal disease In Bluegills (*Lepomis macrochirus*) caused by a new genus and species of Myxozoan. Journal of Parasitology 97(6): 1159–1165.

Wilson, K., and B. T. Grenfell. 1997. Generalized linear modelling for parasitologists. Parasitology Today 13(1): 33–38.

Wright, D. H., B. D. Patterson, G. M. Mikkelson, A. Cutler, and W. Atmar. 1998. A comparative analysis of nested subset patterns of species composition. Oecologia 113(1): 1–20.

Table 4.1. Reach-level stream parameters for 15 streams in the Bankhead National Forest, AL including stream metrics (drainage area, size category, Strahler stream order), sampling time, host metrics, and parasite community metrics. Host parameters include # hosts collected, an estimate of host abundance as catch per unit effort (CPUE), and mean standard length (mean SL). Parasite community parameters include component community richness (species richness), average parasite diversity among hosts at each site (mean infracommunity richness), an estimate of species richness using coverage-based rarefaction (rarefied richness), number of host samples used in the rarefaction (# host samples), and nestedness rank of component communities derived from a packed (maximized nestedness temperature) matrix. Nestedness ranks were derived from an ordered matrix in the BINMATNEST program where low ranks are associated with more species presences in contrast with higher ranks which have fewer.

Site	Latitude	Longitude	Sampling Date	Drainage Area (km^2)	Size Category	Strahler Order	Sample Time (min)	# Hosts	CPUE (Individuals/s)	Mean SL	Species Richness	Mean Infracommunity Richness	Rarefied Richness	# Host Samples	Nestedness Rank
UT Brown Creek	34.305670	-87.238474	9/13/2015	1.01	< 2 km^2	3	23.4	30	0.021	43.4	6	1.90	4.8	11	14
Upper Rush Creek	34.328370	-87.198850	9/13/2015	1.17	< 2 km^2	4	24.2	33	0.023	37.8	6	1.70	4.9	16	12
White Oak Creek	34.339590	-87.460955	8/16/2015	1.86	< 2 km^2	3	34.1	30	0.015	42.1	13	3.57	11.1	12	2
UT 3 Capsey	34.284471	-87.206193	8/15/2015	1.97	< 2 km$_2$	3	25.4	25	0.016	45.2	9	2.08	8.8	23	11
Collier Creek	34.272624	-87.325112	10/4/2015	3.73	2-20 km^2	4	38.9	31	0.013	44.2	5	1.71	3.7	8	15
Turkey Creek	34.312360	-87.168484	10/4/2015	3.92	2-20 km^2	5	21.3	30	0.023	40.9	15	2.80	15	30	3
Upper NF Caney Creek	34.267516	-87.459212	9/12/2015	5.23	2-20 km^2	4	59.3	24	0.007	38.5	6	1.46	5.7	19	13
Tedford Creek	34.348232	-87.469803	9/12/2015	13.42	2-20 km^2	5	33.8	28	0.014	41.6	14	4.96	10.8	7	8
Lower NF Caney Creek	34.265368	-87.446378	9/6/2015	13.58	2-20 km^2	5	80.0	27	0.006	38.9	11	4.67	7.9	6	9
Owl Creek	34.306184	-87.261811	9/7/2015	16.52	2-20 km^2	5	43.5	29	0.011	42.2	12	3.24	9.9	17	5
Hubbard Creek	34.307938	-87.500099	9/6/2015	29.05	> 20 km^2	6	24.2	20	0.014	45.8	8	4.10	6.6	5	10
Lower Rush Creek	34.274103	-87.251481	10/3/2015	30.25	> 20 km^2	5	63.6	30	0.008	38.6	12	4.03	9.3	10	6
Thompson Creek	34.342654	-87.470721	9/12/2015	39.09	> 20 km^2	6	75.8	31	0.007	39.4	15	5.35	10.3	9	1
Capsey Creek Mainstem	34.269714	-87.210822	9/13/2015	46.70	> 20 km^2	6	39.6	31	0.013	42.5	15	4.48	12.3	15	7
Brushy Creek	34.248199	-87.244709	10/3/2015	217.51	> 20 km^2	7	70.8	26	0.006	40.7	14	5.08	11.2	10	4

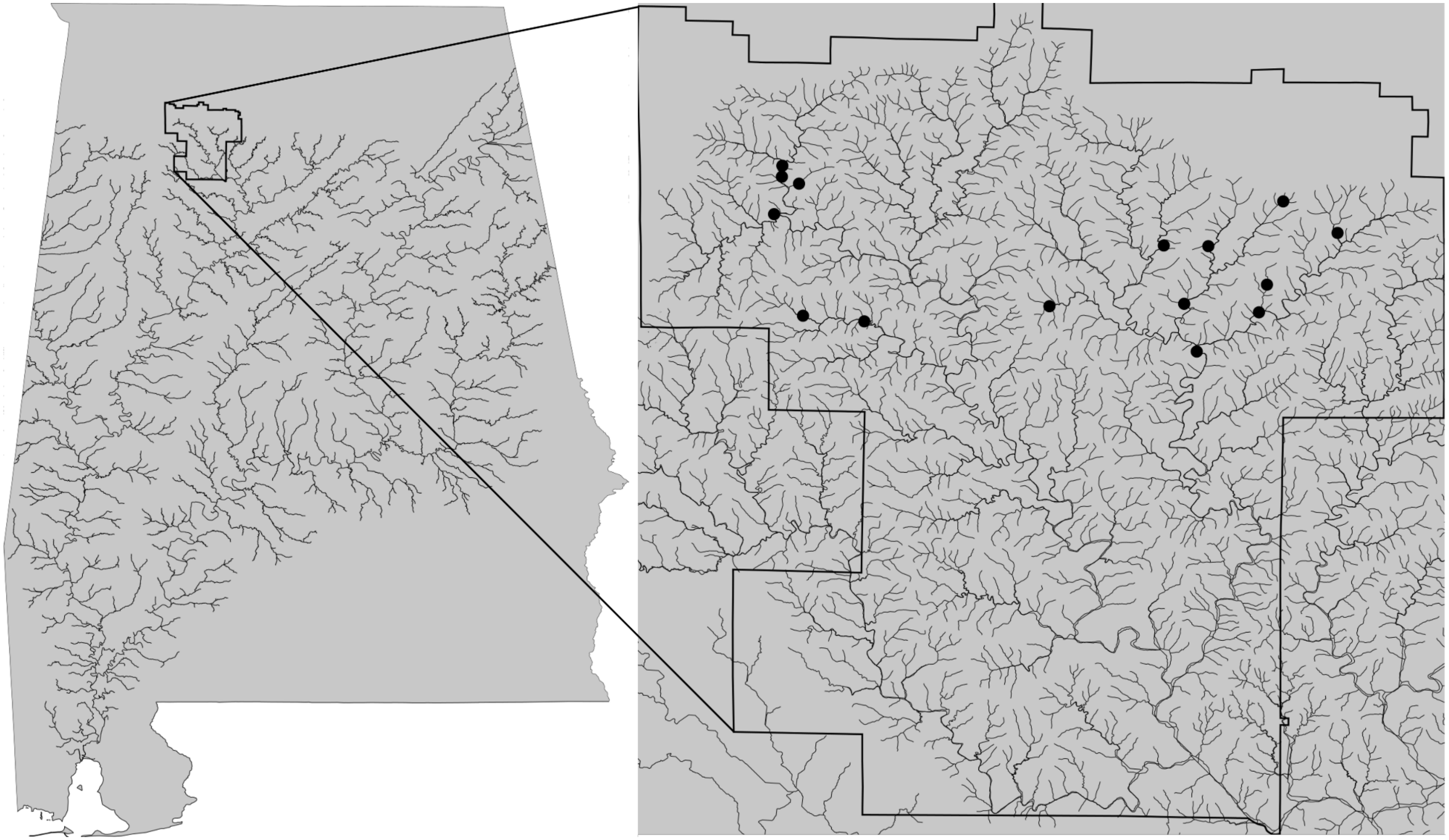

Figure 4.1. The major streams in the Mobile River Basin and the location of the William B. Bankhead National Forest in Alabama (left) with inset of the national forest and location of sampling locations in the Brushy Creek and Sipsey Fork watersheds.

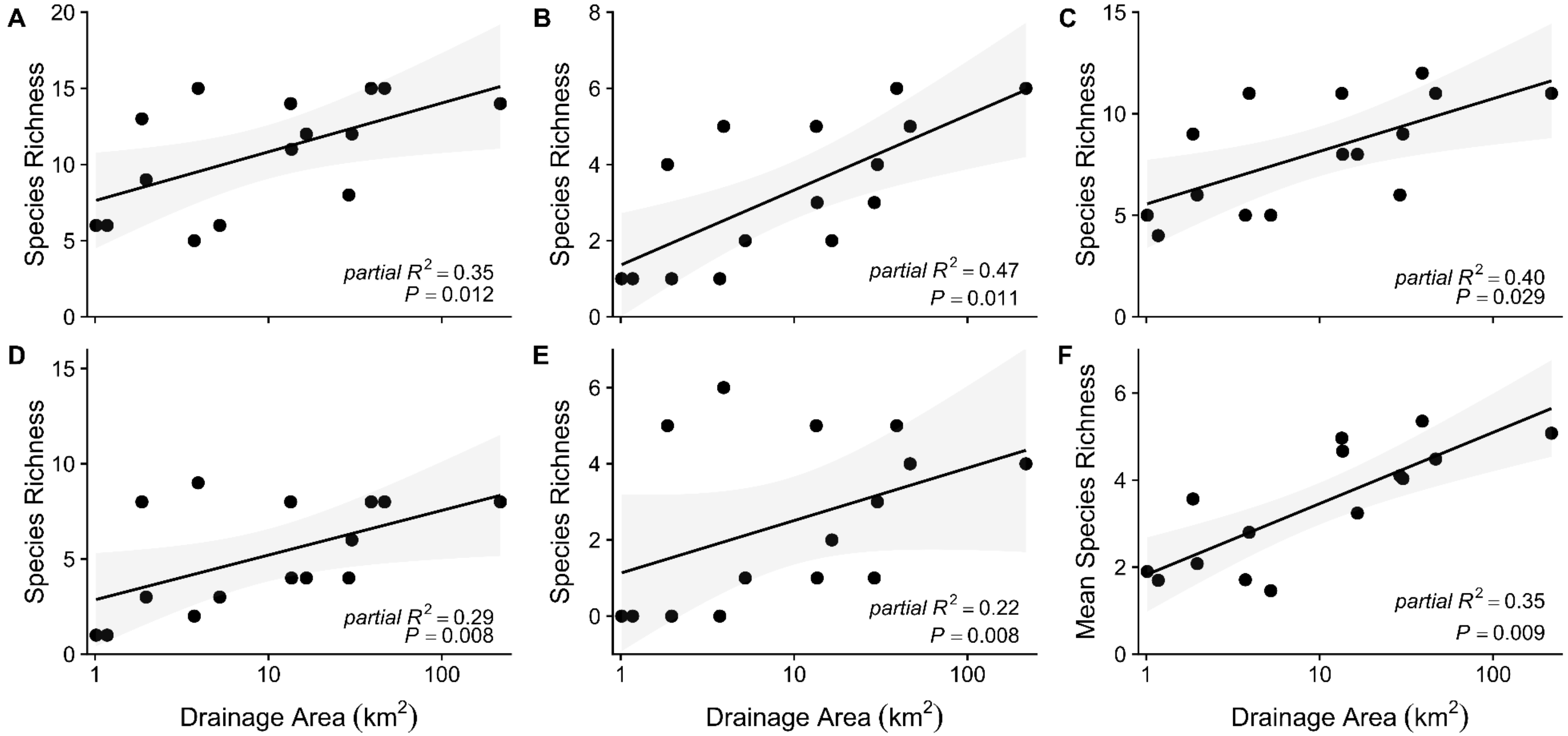

Figure 4.2. The relationships between drainage area and species richness of A) parasite component communities, B) parasites with 2-host life cycles, C) endoparasites, D) autogenic parasites, E) microparasites, and F) mean infracommunities of *Etheostoma artesiae* collected from streams in the William B. Bankhead National Forest, AL.

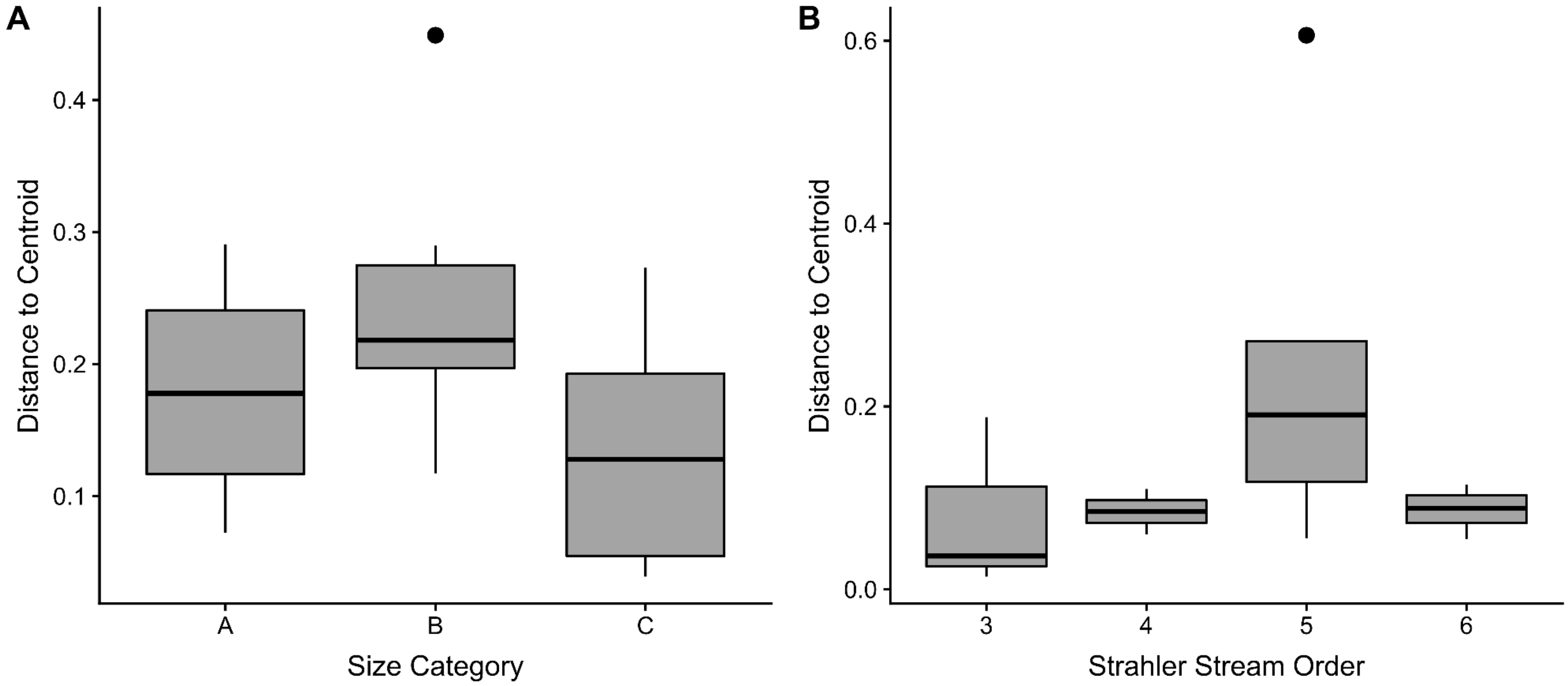

Figure 4.3. Boxplot of β-diversity (distance to group centroid) within A) within stream size categories of < 2.0 km^2 (A), 2.0-20.0 km^2 (B), and > 20.0 km^2 (C) and B) Strahler Stream Order categories where Brushy Creek, the only 7th order stream was included in the 6th order stream group.

Appendix 4.1. Taxa list and life history characteristics for parasites collected from *Etheostoma artesiae* in the Bankhead National Forest in the August to October of 2015.

	Parasite	# of Hosts	Microparasite/Macroparasite	Endo- or Ectoparasite	Larval stage	Intestinal Helminth	Autogenic/Allogenic	Intermediate host 1	Intermediate host 2	Definitive host	Non-aquatic host	*Etheostoma artesiae* definitive host	Trophically transmitted	Piscivore Host
Protozoa														
	Oodinium sp	1	Micro	Ecto	No	No	Auto	NA	NA	Fish	NA	Yes	No	No
	Protozoa sp.	1	Micro	Ecto	No	No	Auto	NA	NA	Fish	NA	Yes	No	No
Myxozoa														
	Myxobolus sp. A	2	Micro	Endo	No	No	Auto	Fish	NA	Oligochaete	NA	No	No	No
	Myxobolus sp. B	2	Micro	Endo	No	No	Auto	Fish	NA	Oligochaete	NA	No	No	No
	Myxidium sp.	2	Micro	Endo	No	No	Auto	Fish	NA	Oligochaete	NA	No	No	No
	Myxobilatus sp.	2	Micro	Endo	No	No	Auto	Fish	NA	Oligochaete	NA	No	No	No
Ciliates														
	Trichodina sp.	1	Micro	Ecto	No	No	Auto	NA	NA	Fish	NA	Yes	No	No
Acanthocephala														
	Neoechinorhynchus sp.	3	Macro	Endo	Yes	No	Auto	Amphipod/Isopod	Small fish	Piscivorous fish	NA	No	Yes	Yes
Nematoda														
	Eustrongylides sp.	3	Macro	Endo	Yes	No	Allo	Oligochaete	Small fish	Bird	Bird	No	Yes	Yes
	Spinitectus sp.	3	Macro	Endo	Yes	No	Allo	Aquatic insect	NA	Fish	Insect	Yes	Yes	No
Nematomorpha														
	Paragordius varius	2	Macro	Endo	Yes	No	Allo	Aquatic insects	NA	Orthoptera	Insect	No	Yes	No
Digenea														
	Echinostomatidae sp.	3	Macro	Endo	Yes	No	Allo	Snail	Fish	Bird	Bird	No	Yes*	Yes
	Diplostomulum sp.	3	Macro	Ecto	Yes	No	Allo	Snail	Fish	Bird	Bird	No	No	Yes
	Postodiplostomum sp.	3	Macro	Endo	Yes	No	Allo	Snail	Fish	Bird	Bird	No	No	Yes
	Uvulifer ambloplitis	3	Macro	Ecto	Yes	No	Allo	Snail	Fish	Bird	Bird	No	No	Yes
	Crepidostomum sp.	3	Macro	Endo	No	Yes	Allo	Sphaeriidae	Crayfish and aquatic insects	Fish	Insect	Yes	Yes	No
	Phyllodistomum superbum cf	3	Macro	Endo	No	Yes	Allo	Sphaeriidae	Aquatic insects	Fish	Insect	Yes	Yes	No
	Pisciamphistoma stunkardi	Unknown	Macro	Endo	No	Yes	Unknown	Unknown	Unknown	Fish	Unknown	Yes	Yes	No
	Plagioporus sp.	3	Macro	Endo	No	Yes	Allo	Snail	Aquatic insects	Fish	Insect	Yes	Yes	No
	Proterometra sp.	2	Macro	Endo	No	Yes	Auto	Snail	NA	Fish	NA	Yes	Yes	No
Cestodea														
	Bothriocephalus sp.	2	Macro	Endo	No	Yes	Auto	Copepod	NA	Fish	NA	Yes	Yes	No
	Protecephalus sp.	3	Macro	Endo	Yes	No	Auto	Copepod	Small fish	Piscivorous fish	NA	No	Yes	Yes
Huridinea														
	Piscicola reducta	1	Macro	Ecto	No	No	Auto	NA	NA	Fish	NA	Yes	No	No
Unionidae														
	Unionidae	1	Macro	Ecto	No	No	Auto	Fish	NA	NA	NA	No	No	No

Chapter 5. Contrasting patterns of population structure in sympatric southeastern US crayfishes

ABSTRACT

Trait-based frameworks have been successfully used to create predict spatial patterns of genetic diversity and population connectivity of aquatic biota. In crayfishes, these frameworks often have overestimated population connectivity because they assume all crayfishes can disperse terrestrially. I compared the genetic population structure of 2 sympatric crayfishes in upland streams of the Bankhead National Forest of Alabama, *Cambarus striatus*, a secondary burrower and common inhabitant of intermittent streams, and *Faxonius validus*, a tertiary burrower (obligate stream-dweller) and permanent stream. I hypothesized that these contrasting burrowing traits influence population structure (in Mitochondrial cytochrome oxidase subunit I, COI) between the 2 species because traits are linked to habitat requirements and the spatial distribution of their populations. I included 15 sites within and among 4 drainages (Sipsey Fork, Brushy Creek, Flint Creek, and Bear Creek) in 2 river basins (Mobile and Tennessee rivers). Most of the genetic variation (82%) was contained among drainages and there was a correlation between genetic and stream distance in *F. validus*. In contrast, in *C. striatus*, most of the genetic variation (54.5%) was contained within populations and there was no correlation between genetic and stream or geographic distance. Spatial patterns of genetic diversity for *F. validus* and *C. striatus* resemble the Stream Hierarchy Model and Panmictic model, respectively. Thus, population structure was stronger in *F. validus* than *C. striatus*, which is a finding consistent with my predictions and hypotheses based on their distinct burrowing traits. My results suggest that burrowing traits of crayfishes, which may determine their ability to occupy intermittent upper reaches of streams, may be a useful heuristic for predicting their population structure.

INTRODUCTION

Quantifying genetic diversity among populations of stream organisms can play an important informative role in conservation (Hughes et al. 2009) because diversity often is linked to fitness at the population level (Reed and Frankham 2003). Additionally, population genetic structure (genetic diversity within and among populations) is associated population connectivity (Hughes et al. 2009). Organismal traits, particularly those related to reproduction (e.g., generation time), habitat (e.g. generalists or specialists), and dispersal mode (e.g., swimming, crawling, flying, aquatic or terrestrial) are increasingly used to predict population genetic structure (Hughes et al. 2013, Paz et al. 2015, Mims et al. 2018). These trait-based approaches enable *a priori* predictions about spatial patterns of genetic diversity and thus population connectivity using a few organismal traits and habitat characteristics (Hughes et al. 2013; Paz et al. 2015; Mims et al. 2018). When validated, such approaches can aid conservation efforts because they accurately predict population connectivity and genetic diversity of species for which no genetic data exists (Mims et al. 2018).

Freshwater crayfishes are one of the most-threatened taxonomic groups in the world as a result of habitat degradation, disease, and losses from invasive species (Lodge et al. 2000; Capinha et al. 2013; Richman et al. 2015). As of 2016, published genetic information exists for only ~44% of North American crayfish species, with even fewer having data describing population genetic diversity and connectivity (Figiel 2016). This paucity of information suggests that a trait-based phylogeographic approach may be especially useful for crayfish conservation as it could prioritize particular species for conservation. For example, species anticipated to show greater population connectivity could be deprioritized relative to those anticipated to show lower population connectivity.

A trait-based predictive framework has been developed for Australian stream invertebrates, including crayfishes, using site features (i.e., connected by freshwater, upland vs lowland), organismal dispersal (e.g., aquatic vs terrestrial, crawling vs flying terrestrial dispersal), and habitat characteristics (upland specialist) to predict population genetic structure (Hughes et al. 2013). For example, lowland populations are predicted to be more genetically homogenous because of greater population connectivity than upland sites, and organisms capable of flight should be more genetically homogenous among populations than those that only crawl or swim (Hughes et al. 2013). However, for crayfishes, the trait-based phylogeographic approach of Hughes et al., (2013) misclassified 35.3% (12 of 34 studies) species or populations, primarily in upland habitats, by generally overestimating population connectivity, suggesting that some species have more limited terrestrial dispersal than previously thought (Hughes et al. 2013). The relatively high misclassification rate for crayfishes suggests that accuracy of this trait-based framework could be refined by identifying other crayfish traits linked to probability of terrestrial dispersal and therefore degree of population connectivity.

One of the most notable traits varies among crayfish species is burrowing behavior. Although all crayfishes are believed to be capable of burrowing (Berrill and Chenoweth 1982), Hobbs (1942, 1981) classified crayfishes into three categories primary, secondary, and tertiary burrower categories; primary burrowers are semi-terrestrial and largely restricted to their burrows, secondary burrowers spend much of their lives in burrows but also are regularly found in surface waters, and tertiary burrowers primarily inhabit permanent waters (Hobbs 1942, 1981; Bouchard 1978). Burrowing classification is a trait that could be useful in predicting population connectivity because these classifications often relate to habitat requirements. For example, primary burrowers specialize in terrestrial areas with shallow water tables (e.g. bogs, roadside ditches etc.), secondary burrowers often have burrows in banks of streams, ponds, and lakes and can burrow to the water table during drying events, whereas tertiary burrowers are largely restricted to surface waters (Hobbs Jr. 1942, 1981; Bouchard 1978). Burrowing classification is thought to be associated with dispersal opportunities and thus geographic range in Tasmanian crayfishes, with primary burrowing species having more restricted range sizes than those associated with streams and rivers (Richardson et al. 2006). I proposed that crayfish burrowing classification and associated habitat requirements are traits that relate to terrestrial dispersal and thus population genetic structure.

Faxonius validus (Faxon) and *Cambarus striatus* (Hay) inhabit upland streams of the Bankhead National in Forest, Alabama (Brantley et al. 2016). Both crayfishes are sympatric in headwater streams along ridges between the Mobile River and Tennessee River basins and between two rivers dividing the Bankhead National Forest (Sipsey Fork and Brushy Creek; Brantley et al. 2016). *Cambarus striatus* is a secondary burrower (Bouchard 1978), whereas *F. validus* is classified as a tertiary burrower (stream-dweller; Schuster and Taylor 2004). This contrast in burrowing behaviors also may be associated with differential habitat use and longitudinal distribution in streams. Although the 2 species occur sympatrically, previous surveys suggest that *C. striatus* is more of a habitat generalist that occurs along the longitudinal stream gradient, including intermittent upper reaches, whereas *F. validus* is a habitat specialist of larger and more permanently flowing reaches further downstream (Bauer, personal observation). Thus, overland distances between *C. striatus* populations in less permanent upper stream reaches in adjacent drainages is generally shorter than distances between populations of *F. validus* in more permanent downstream reaches of the same streams. The relatively shorter distance between *C. striatus* populations relative to *F. validus* populations should increase the likelihood of terrestrial dispersal between populations of *C. striatus* relative to *F. validus*.

This study was designed to determine if population genetic structure in *C. striatus* and *F. validus* were predictably associated with their contrasting habitat use and burrowing behaviors. I hypothesized that burrowing classification and habitat use are associated with patterns of crayfish population genetic structure because these traits affect overland dispersal between headwater populations. Thus, I predicted that *C. striatus* would show minimal genetic differentiation among drainages and basins, and that genetic distances would be correlated with overland distances between populations. In contrast, I predicted that *F. validus* would show strong genetic differentiation among drainages and basins, and that genetic distances would be correlated with stream (vs. overland) distances between populations. I also predicted that most genetic diversity would be found within populations of *C. striatus* and among drainages for *F. validus*.

METHODS

Study area, crayfish sampling and tissue collection

The study area was streams in the Bankhead National Forest, northwest Alabama (Fig. 5.1) and within the Southwestern Appalachians ecoregion (Omernik 1987). The climate of Alabama is humid subtropical (Köppen 1918; Peel et al. 2007). A karstic ridge between the Tennessee River and Mobile River basins (Veni 2002) runs through the forest; on the Mobile River side, the ridge is drained by Brushy Creek and Sipsey Fork (Fig. 5.1), which flow into the

Black Warrior River. Study streams were relatively high gradient and drained heavily forested watersheds dominated by mixed-hardwood forests with a combination of bigleaf magnolia (*Magnolia macrophylla* Michaux), white oak (*Quercus alba* L.), American beech, chestnut oak (*Quercus prinus* Willd), and red maple (*Acer rubrum* L.) (Brantley et al., 2016).

I collected crayfish from 15 streams, with 2 to 3 adjacent streams separated by ridges dividing minor and major drainages (Fig. 5.1, Table 5.1). Preliminary crayfish collections were made in 4 streams in Fall 2015 to estimate genetic diversity within populations. I revisited and sampled these 4 streams, along with 11 additional streams, in Fall 2018 (Table 5.1). I chose specific sampling locations by hiking along each stream where they came closest to the ridge, and began sampling where I first observed surface water.

Crayfish were sampled by 1- or 2-person field crews, flipping rocks and dip-netting individuals until 2 h passed or 12 individuals were collected. In 2015, I collected tissue samples from euthanized specimens by removing a cheliped and cracking the chela to expose tissue, which I then field-preserved in 95% EtOH for subsequent genetic analyses. However, polymerase chain reaction (PCR) amplification failed from some of these 2015 specimens, with the preservation protocol being the suspected issue. Thus, for specimens collected in 2018, I instead used tail tissue removed from euthanized crayfish, placed directly into 95% EtOH. Voucher specimens were preserved in 70% EtOH and deposited in the Auburn University Museum of Natural History.

DNA extractions, amplification, and sequencing

I extracted total genomic DNA from tissues using a DNeasy Tissue and Blood Kit (Qiagen, Inc.), which was used as template to amplify an ~670-bp fragment of the mitochondrial (mtDNA) cytochrome oxidase I (COI) gene with PCR. I completed reactions using a PTC-100™ thermocycler (MJ Research) in 25 µl volumes containing ~10–30 ng of template DNA, 10 mM Tris–HCl (pH 8.3), 50 mM KCl, 0.001% gelatin, 2.0 mM MgCl2, 200 µM of each deoxynucleotide triphosphate (i.e., dATP, dCTP, dGTP, and dTTP), 1 U Taq polymerase, and 0.4 µM each of primers LCO1490 and HCO2198 (Folmer et al. 1994). Thermocycler temperature profile settings followed Santos (2006). I confirmed amplification success by gel electrophoresis of 3 µl from each reaction in 1% agarose with ethidium bromide staining and viewing with shortwave (265 nm) UV light. I then sent amplicons to Eton Bioscience Inc. (Raleigh-Durham, NC) for purification and sequencing. I trimmed chromatograms and corrected ambiguities by comparing complementary DNA strands in Sequencher v4.7 (Gene Codes Corporation, Ann Arbor, MI, USA). Last, I manually aligned finalized sequences in SE-AL version 2.0a11 (available at http:// evolve.zoo.ox.ac.uk/) and deposited into GenBank.

Haplotype diversity, genetic structure, and demographic analyses

I estimated nucleotide (π) and haplotype (h) diversity of *C. striatus* and *F. validus* populations with DnaSP v6 (Rozas et al. 2017) using methods of Nei (1987). I visualized relationships among COI haplotypes (i.e. unique sequences) using networks generated in TCS v1.21 (Clement et al. 2000) using the cladogram estimation algorithm of Templeton et al. (1992) under the default settings of 95% parsimony plausible branch connections between haplotypes. I used the criteria of Crandall et al. (1994) to resolve network reticulations representing ambiguous connections between haplotypes. I determined consistency of COI sequence variations in *C. striatus* and *F. validus* with the predictions of the neutral model using Tajima's D (Tajima 1989) and Fu's F_S (Fu 1997); significance of these neutrality tests for each population was assessed using 10000 permutations in ARLEQUIN v3.5 (Excoffier and Lischer 2010).

I conducted pairwise Φ_{ST} (based on haplotype frequencies and molecular divergence) and an analysis of molecular variance (AMOVA; Excoffier et al. 1992) to test for genetic differentiation among populations and quantify spatial distribution of genetic variance, respectively, using ARLEQUIN v3.5 (Excoffier and Lischer 2010). In the AMOVA, I used Φ-statistics to estimate the relative contribution of molecular variance at 3 spatial levels: among major drainages (Φ_{CT}), among populations within major drainages (Φ_{SC}), and within populations (Φ_{ST}). I used Tamura and Nei's (1993) model of evolution with rate variation among sites [TN + Γ ($\alpha = 0.76$)] for pairwise Φ_{ST} and AMOVA statistics, with significance assessed by 10000 permutations. I used this model of evolution as it was the most similar to the HKY + I model selected by the Akaike information criterion (AIC_C) in jModeltest (Guindon and Gascuel 2003; Posada 2008)] that could be implemented in ARLEQUIN. Additionally, I calculated Wright's F_{ST} (based on haplotype frequencies; Wright 1943) between populations in ARLEQUIN for comparison with my Φ_{ST} estimates.

I used partial Mantel tests to determine whether overland (Euclidean) or river distances between streams correlated with population genetic distance (Φ_{ST} and F_{ST}) for each crayfish species. For these analyses, overland and river distances were calculated using the fields v9.8-3 (Nychka et al. 2017) and riverdist v0.15.0 (Tyers 2017) packages, respectively, in R v1.1.442 (R Core Team 2018). I then conducted Mantel tests using the vegan v2.4-6 package (Oksanen et al. 2018) in R with $\alpha = 0.05$.

RESULTS

Genetic diversity of Cambarus striatus *and* Faxonius validus

COI mtDNA was successfully amplified from 222 *C. striatus* and 227 *F. validus* individuals. Purification and sequencing of mtDNA COI amplicons yielded 620 and 614-bp COI gene fragments from *C. striatus* and *F. validus*, respectively. A total of 28 and 11 haplotypes were identified from *C. striatus* and *F. validus*, respectively. Translation of these unique haplotype sequences into amino acids revealed no stop codons, with most substitutions being silent in nature. In the two cases of non-synonymous substitutions in *C. striatus*, these resulted in changes to amino acids with similar biochemical properties. Such patterns imply that amplicons originated from mtDNA COI and not COI-like pseudogenes isolated from nuclear copies of mitochondrial-derived genes (i.e. numts), which can be common in arthropods (Buhay 2009).

Comparisons between species, *C. striatus* showed considerably higher *h* and π diversity than *F. validus* given the higher number of haplotypes in *C. striatus* than *F. validus* (*nh* = 28 and 11, respectively; Table 5.2). Across populations, *h* and π diversity were considerably lower in Sipsey Fork than Brushy Creek for both species, with Sipsey Fork showing fewer *F. validus* haplotypes (*nh* = 1) than Brushy Creek (*nh* = 7; Table 5.2). TCS analyses resulted in a single haplotype network for *C. striatus* (Fig. 5.2) and 2 discrete networks for *F. validus* (Fig. 5.3). In the latter case, western (i.e., Sipsey Fork and Bear Creek) and eastern (i.e., Brushy Creek and Flint Creek) populations of *F. validus* generally were segregated into different networks. The one exception was Collier Creek, the southernmost population in the Brushy Creek drainage, which had a single haplotype that was shared with all Sipsey Fork individuals (Fig. 5.3).

Genetic structure of Cambarus striatus *and* Faxonius validus

For *C. striatus*, one haplotype occurred in all but one population, being numerically dominant in 10 of 15 (67%) populations and widely distributed geographically among all 4 drainages (Fig. 5.4). For the other 27 haplotypes, 9 were unique to a single population whereas the other 18 were shared between at least 2 populations. Similar haplotype frequencies were recovered from Bear Creek and neighboring Ross Branch in Sipsey Fork, whereas Indian Creek (Flint Creek drainage) was dominated by its own unique haplotype and had dissimilar haplotype frequencies from neighboring Turkey and Rush creek populations in the Brushy Creek drainage (Fig. 5.4). In contrast to *C. striatus*, most (e.g., 8 of 11, 73%) *F. validus* haplotypes were unique to single populations. Notably, all *F. validus* individuals in Sipsey Fork and Collier Creek (n=83) shared the same haplotype (Fig. 5.5) whereas most Brushy Creek populations (except for Collier and Turkey creeks) were dominated by a 2nd common haplotype. The 3rd most common *F. validus* haplotype was shared by only 2 populations, in upper and lower East Fork Beech Creek (Fig. 5.5). Populations of *F. validus* from the 2 tributaries of the Tennessee River showed contrasting patterns; the Bear Creek population had dissimilar haplotype frequencies from neighboring Ross Branch (Sipsey Fork tributary) whereas Indian Creek had similar haplotype frequencies as neighboring Rush Creek (Brushy Creek tributary; Fig. 5.5).

Significance of pairwise comparisons for genetic differentiation among populations were similar between F_{ST} and Φ_{ST} measures for both crayfish species, with F_{ST} values being slightly more conservative than those of Φ_{ST}. Thus, only Φ_{ST} pairwise comparisons are discussed here whereas F_{ST} values are given in Tables 5.3 and 5.4. Temporal comparisons between 2015 and 2018 samples from the same population did not differ for *C. striatus* (Table 5.3). In contrast, temporal comparisons within populations of *F. validus* were not different in Sipsey Fork but did differ in populations from East Fork Beech Creek (Brushy Creek drainage; Table 5.4). Most pairwise comparisons of differentiation among populations of *C. striatus* were significant (114 of 171, 67%; Table 5.3), whereas a higher proportion was significant in *F. validus* (124 of 171, 73%; Table 5.4). For *C. striatus*, the number of significant pairwise comparisons among Sipsey Fork populations (13 of 28, 46%) was much lower than among Brushy Creek populations (24 of 36, 67%; Table 5.3). Similarly, for *F. validus*, the proportion of significant pairwise comparisons was higher among Brushy Creek populations (29 of 36, 81%) than among Sipsey Fork populations (0 of 28, 0.0%; Table 5.4).

AMOVAs identified strongly contrasting patterns in distribution of genetic variation within and between *C. striatus* and *F. validus* populations (Table 5.5). For example, a small and non-significant amount of genetic variation was found among drainages for *C. striatus* (~14%, $\Phi_{CT} = 0.146$; $P > 0.05$), whereas a significant amount occurred for *F. validus* (~82%; $\Phi_{CT} = 0.821$; $P < 0.05$; Table 5.5). Furthermore, there was significant genetic variation within drainages for both species, although genetic variation was about 2x higher for *C. striatus* (~31%, $\Phi_{SC} = 0.363$, $P < 0.05$) than *F. validus* (~16.5%, $\Phi_{SC} = 0.918$, $P < 0.05$; Table 5.5). The amount of variation contained within populations also was higher in *C. striatus* (~54.4%, $\Phi_{SC} = 0.456$, $P < 0.05$) than *F. validus* (~1.5%, $\Phi_{SC} = 0.985$, $P < 0.05$; Table 5.5).

Partial Mantel tests of genetic and geographic distances were consistent when using either F_{ST} and Φ_{ST}; thus, only the results for Φ_{ST} are presented as this measure incorporates both haplotype frequencies and molecular divergence. For *F. validus*, positive correlations were detected between genetic distance (as F_{ST} and Φ_{ST}) and river distance ($r = 0.20$, $P = 0.026$), but not between genetic distance and overland (Euclidean) distance ($r = 0.14$, $P = 0.064$; Fig. 5.6). In contrast, no significant correlations occurred between genetic (F_{ST} and Φ_{ST}) and either overland ($r = 0.04$, $P = 0.340$) or river ($r = 0.02$, $P = 0.381$) distances for *C. striatus* (Fig. 5.6).

Demography of Cambarus striatus *and* Faxonius validus

For neutrality tests, Tajima's D values were significantly negative for 3 populations and significantly positive for 1 population of *C. striatus* (Table 5.2), with negative and positive values suggestive of population expansions and contractions, respectively (Tajima 1989). Notably, only a single population of *C. striatus* had a significant negative value for Fu's F_S, suggestive of population expansion, but this was not one of the populations with a significant Tajima's D value (Table 5.2). No population of *F. validus* had significant values for either Tajima's D or Fu's F_S neutrality tests (Table 5.2).

DISCUSSION

Overall, my results are consistent with the hypothesis that population genetic structure among neighboring drainages is associated with burrowing and habitat use traits of crayfishes. Population genetic structure was greater in the habitat generalist *Cambarus striatus* (secondary burrower) than in the obligate stream-dweller *Faxonius validus* (tertiary burrower). Most of the genetic diversity (54%) in *C. striatus* was contained within populations, whereas most (82%) occurred among drainages for *F. validus.* The population structure of *F. validus* confirmed the prediction of an obligate freshwater life history, with genetic distance being positively associated with stream distance; however, patterns of population structure in *C. striatus* varied from predictions. Specifically, population structure in *C. striatus* showed no correlation between genetic and overland or stream distances, indicating a lack of genetic isolation by distance

(Wright 1946). Population structure of *F. validus* and *C. striatus* most closely conform to the Stream Hierarchy (Meffe and Vrijenhoek 1988; Hughes et al. 2009, 2013) and Panmixia models (Wright 1943), respectively. Specifically, the Stream Hierarchy Model predicts genetic population differentiation among, but not within, drainages (Meffe and Vrijenhoek 1988; Hughes et al. 2009, 2013) whereas the Panmixia model (Wright 1943) predicts no patterns of differentiation among populations. Furthermore, the results of my study indicated the potential for cryptic species diversity in *F. validus* with genetically distinct western and eastern lineages.

Crayfish genetic diversity and population structure

Genetic diversity of *C. striatus* populations (average $\pi = 0.0041$, $nh = 28$) was substantially higher than *F. validus* populations ($\pi = 0.0006$, $nh = 11$); there are at least 3 potential explanations for this difference. First, lower diversity of *F. validus* could result from a shorter evolutionary history than *C. striatus*, as the genus *Faxonius* is thought to be derived from a *Cambarus*-like ancestor (Crandall and De Grave 2017). In this context, *C. striatus* might be an older species that has had more time to diversify genetically. Second, higher genetic diversity in *C. striatus* may be because of larger effective population sizes relative to *F. validus*; large population sizes are expected to increase maintenance of genetic diversity and reduction of losses from genetic drift under assumptions of neutrality (Kimura and Crow 1964; Kimura 1983; Piganeau and Eyre-Walker 2009). Indeed, mean density of *C. striatus* (0.85 individuals/m^2) was substantially higher than *F. validus* (0.21/m^2) in headwater streams (< 1.4 km^2 drainage area, n = 11) in the study area (E. Bauer, unpublished data). If population size does drive the disparity in genetic diversity between species, then this would suggest that results might have differed if larger downstream reaches (> 1.4 km^2) had been sampled exclusively where *F. validus* had higher densities than *C. striatus* (0.08 vs. 0.03 individuals/m^2, respectively; unpublished data).

Additionally, in the study area, density of both species declined with stream size (E. Bauer, unpublished data), patterns also noted in other species (e.g. Helms et al. 2016). Therefore, if crayfish population size strongly influences genetic diversity in crayfishes, then diversity of *F. validus* and *C. striatus* also would be expected to decrease downstream. The pattern of decreasing genetic diversity with increasing stream size would contrast with models previously developed for stream organisms predicting increased diversity downstream because streamflow biases dispersal in a downstream direction (Finn et al. 2011). Given that genetic diversity of aquatic organisms often increases with stream size (Finn et al. 2011; Whelan et al. 2019), future studies focused on the elucidating causes of genetic diversity in crayfishes should sample populations along stream size gradients to test the relative roles of population size and stream size.

A third potential explanation for lower genetic diversity in *F. validus* relative to *C. striatus* is that *F. validus* could be a recent colonizer of streams in the Bankhead National Forest, with lower genetic diversity reflecting founder effects or population bottlenecks (e.g., Huchon et al. 1999). However, neutrality test results suggested no evidence for bottlenecks or founder events, as no population had significant values for both neutrality tests; however, my analyses may be constrained by the fact that most *F. validus* populations had a single haplotype thus nullifying calculation of Fu's F_s for most populations. Parsimony analyses also support the possibility of multiple colonization events explaining the spatial distribution of genetic diversity in *F. validus*. Colonization of the Sipsey Fork and Brushy Creek could have occurred independently from different sources because these populations belonged to separate lineages, with ancestral haplotypes occurring in the Bear and Flint Creek drainages of the Tennessee River, respectively (Fig. 5.3). This pattern is consistent with evidence suggesting the genus

Faxonius originated in the Eastern Highlands (Highland Rim and Cumberland Plateau) of Tennessee and dispersed south (Hobbs 1988; Crandall and Templeton 2010; Taylor et al. 2014).

It was surprising that genetic diversity was lower for both species' populations in the Sipsey Fork than in Brushy Creek. No clear explanations exist for this pattern, although observations from 2014-2018 suggested that streams in the Sipsey Fork drainage have flashier stormflows and also are more prone to drying than those in the Brushy Creek, potentially because of less consolidated stream bed materials and steeper canyons in the Sipsey Fork. This difference would suggest that whereas *F. validus* and *C. striatus* are vulnerable to effects of stream drying, their contrasting burrowing behavior renders them differentially vulnerable to such impacts. As a secondary burrower, *C. striatus* is more able to track the retreating water table and survive extensive stream drying during droughts than *F. validus*, which only excavates shallow depressions in the streambed under large rocks. Tracking a receding water table also has been observed in other burrowing crayfishes (*Cambarus harti* and *Creaserinus fodiens*) (Williams and Hynes 1976, 1977; Helms et al. 2013), suggesting that burrowing behavior may be important for species survival in disturbance-prone environments. For example, short-term decreases in water level were associated with increased burrowing activity in *C. harti* (Helms et al. 2013), a close relative of *C. striatus*.

My study also identified potential cryptic diversity in *F. validus* because parsimony analyses suggested that western (Sipsey Fork and Bear Creek) and eastern (Brushy and Flint Creek) populations were distinct genetic lineages of *F. validus* with many fixed mutational differences between them, indicating historical divergence between populations. Within these lineages, private (unique population) haplotypes generally only were a few mutational differences away from ancestral haplotypes, implying more recent divergence. Cryptic diversity also has been reported for *C. englishi* and *C. halli* in eastern Alabama, which also corresponded to particular drainages; parsimony analyses of COI mtDNA in those species resulted in 3 historically divergent clades (Helms et al. 2015). My findings of cryptic diversity in *F. validus* may have conservation implications as most major drainages contain genetically unique populations, which need to be conserved to maintain already low genetic diversity.

Population structure and models of population connectivity

Most pairwise Φ_{ST} comparisons between samples from the same populations of *C. striatus* and *F. validus* collected in 2015 and 2018 were non-significant, suggesting little to no change in haplotype frequencies occurring over a 3-y span. Exceptions to this general pattern were *F. validus* populations in upper and lower East Fork Beech Creek, both of which changed from 2 haplotypes in 2015 to a single haplotype in 2018. The cause of this reduction in genetic diversity is unclear, although one explanation is that the reduction resulted from genetic drift after a population decline. This region of Alabama experienced a prolonged period of moderate to exceptional drought (late May 2016 to early May 2017, NDMC 2019), which occurred between the 2 sampling periods. If true, then populations of *F. validus* may have experienced greater negative effects relative to *C. striatus*, as *C. striatus* can track the receding water table (above). Drought was reported to have strong negative effects on populations of *Procambarus spiculifer*, a tertiary burrower, but not on *C. striatus* in sympatry (Taylor 1983). Tertiary burrowers, like *F. validus*, would appear potentially at greater risk of population and genetic diversity losses under such drought disturbances. Another reason for the reduction in genetic diversity could be sampling error, although this explanation is unlikely for at least 3 reasons. First, between 2015 and 2018 sample size was either equal or increased between years; this should have increased the probability of detecting the second haplotype not decreased it. Second, the lost haplotype occurred in 5 of 10 individuals in one population and 9 of 12 individuals in the other population, thus the reduction in diversity was not the result of losing a rare haplotype. Third, the reduction in haplotype diversity was not the result of using different preservation protocols between years because successful amplification occurred for all individuals collected in 2018, the year that showed lower haplotype diversity.

Contrary to predictions, there was no relationship between genetic and geographic distances in *C. striatus*; thus, there was no apparent spatial pattern to population differentiation despite two-thirds of pairwise comparisons of genetic distance being significant. In addition, the ancestral haplotype identified by parsimony analysis was shared among individuals at all sampling sites. These results are consistent with recent or ongoing and extensive connectivity among populations of *C. striatus* and the prediction of the Panmictic model of no population structure (Wright 1943). Apparent population connectivity in *C. striatus* is greater than that expected using the trait-based framework of Hughes et al. (2013), as an isolation by distance relationships is expected for an upland stream inhabitant capable of terrestrial dispersal by crawling (Hughes et al. 2013). In fact, panmixia was only predicted for organisms capable of crawling terrestrial dispersal if they inhabit lowland streams (Hughes et al. 2013). *C. striatus* may be the first crayfish species occupying upland habitats reported to show evidence of panmixia; however, this could be a result of my study design. Lack of isolation by distance for *C. striatus* could be because of the small geographic scale of this study compared to the wide range of *C. striatus*, which includes 9 of the major rivers of Alabama and parts of Florida, Georgia, Kentucky, Mississippi, South Carolina, and Tennessee (Hobbs 1989). It is possible that a study conducted at a larger scale (> 26.6 km^2) may, in fact, find isolation by distance. It is also possible that a larger-scale study may find cryptic diversity in *C. striatus* as this species appears not to be monophyletic (Glon et al. 2018). Last, alternative genetic markers or newer genomic techniques (e.g., restriction site-associated DNA sequencing) may detect isolation by distance and finer-scale population structure than COI mtDNA as has been found in other species (e.g., Rahman et al. 2018).

In contrast to the weak population differentiation among drainages for *C. striatus*, there was strong differentiation among most drainages for *F. validus*. Additionally, patterns of genetic diversity of *C. striatus* in the Bankhead National Forest were consistent with models assuming extensive population connectivity and imply efficient dispersal through terrestrial and aquatic routes. In contrast, diversity patterns in *F. validus* were consistent with models assuming low terrestrial and high aquatic dispersal. Most of the genetic diversity (82.1%) in *F. validus* was contained among drainages, with little diversity within major drainages or populations and genetic distance was positively correlated with stream distance. There was little gene flow and strong population differentiation among larger rivers, even those connected hydrologically (i.e., Sipsey Fork, Brushy Creek, Turkey Creek). These results are consistent with the Stream Hierarchy Model and low levels of terrestrial dispersal but high levels of aquatic dispersal (Meffe and Vrijenhoek 1988; Hughes et al. 2009, 2013). Furthermore, the large percentage of genetic variation contained among (vs. within) drainages, or within populations, was virtually identical to genetic patterns of 2 other Alabama crayfishes (*Cambarus englishi* and *C. halli*), which, like *F. validus*, are tertiary burrowers (Helms et al. 2015). Related, over one-third of Australian crustaceans (primarily crayfishes) showed population genetic patterns that also fit the Stream Hierarchy Model, suggesting that an obligatory aquatic life history with minimal terrestrial dispersal may be common among freshwater crayfishes (Hughes et al. 2013).

Patterns of population structure in *F. validus* also suggest that geology of the Bankhead National Forest may promote gene flow among particular populations, through specific dispersal routes. For example, Collier and Indian creek populations were notable exceptions to otherwise strong differentiation between *F. validus* populations in neighboring drainages. The Collier Creek population was genetically indistinguishable from populations in the Sipsey Fork despite occurring in the Brushy Creek Drainage. This pattern suggests that the Collier Creek population has had recent and strong gene flow from the Sipsey Fork, to the point where only the single haplotype from the Sipsey Fork was present. Perhaps the lack of differentiation between Collier Creek and neighboring Hurricane Creek occurred because these streams were the southernmost sampled, and which had more gradual slopes and were lower in elevation than more northern streams. As such, landscape resistance (i.e., slope and distance to ridge) to overland dispersal between these streams was lower than other pairs of neighboring streams. Landscape resistance may have large effects on population structure of crayfishes as it is positively associated with population structuring as in other semi-aquatic species (salamanders; Richardson 2012; Emel and Storfer 2014), although these likely show greater terrestrial dispersal than crayfishes. As such, additional studies that directly quantify the relationships between landscape resistance and genetic differentiation in crayfishes are needed to assess the generality of this pattern.

Geology of the Bankhead National Forest and the ridge between the Tennessee and Mobile basins also may explain the genetic similarity between Indian Creek (Tennessee River drainage) and Rush Creek (Brushy Creek drainage) populations of *F. validus*. Landscape resistance between these streams is likely high (higher elevation and steeper slopes) relative to Collier and Hurricane creeks. However, the ridge between the Tennessee River and the Sipsey River lies in a karst area with numerous caves (Veni 2002) and subterranean connections between drainages have been hypothesized as a dispersal corridor by which non-indigenous fishes (*Chrosomus erythrogaster* and *Rhinichthys obtusus*) colonized the Mobile Basin (Ray et al. 2014; Bauer and Hayes 2017). Therefore, it is possible that the lack of differentiation between *F. validus* populations in Indian and Rush creeks also could have resulted from subterranean inter-basin transfer.

In summary, population genetic structure was stronger in *F. validus* than *C. striatus*, a finding consistent with my predictions and hypotheses based on their contrasting burrowing traits and habitat use. Spatial patterns of genetic diversity for *F. validus* and *C. striatus* resemble those predicted by the Stream Hierarchy and Panmixia models, respectively. These results suggest that burrowing traits, and associated habitat use, of stream crayfishes may be a useful heuristic for predicting their population structure. However, before practical application of this heuristic is possible, its usefulness must be tested by examining population structure in other species of crayfishes including primary burrowers which were not examined in this study. Future studies of crayfishes should examine other biological and landscape traits (e.g., body size, geographic range, biogeographic origin, reproductive traits, and landscape resistance) that have been associated with population genetic structure in other semi-aquatic taxa (salamanders and anurans; Richardson 2012; Emel and Storfer 2014; Paz et al. 2015). Furthermore, my study lends additional evidence that crayfishes are a cryptically diverse group (Mathews et al. 2008; Bentley et al. 2010; Taylor et al. 2014), especially in the southeastern United States (Helms et al. 2015), and future studies are likely to find more cryptic diversity in obligate stream-dwelling species.

LITERATURE CITED

Bauer, E., and M. M. Hayes. 2017. First report of a population of western Blacknose Dace (*Rhinichthys obtusus*) in the Brushy Creek system of the Black Warrior River drainage, Alabama. Southeastern Fishes Council Proceedings 56: 42–49.

Bentley, A. I., D. J. Schmidt, and J. M. Hughes. 2010. Extensive intraspecific genetic diversity of a freshwater crayfish in a biodiversity hotspot. Freshwater Biology 55: 1861–1873.

Berrill, M., and B. Chenoweth. 1982. The burrowing ability of nonburrowing crayfish. American Midland Naturalist 108: 199–201.

Bouchard, R. W. 1978. Taxonomy, ecology and phylogeny of the subgenus Depressicambarus, with the description of a new species from Florida and redescriptions of *Cambarus graysoni*, *Cambarus latimanus* and *Cambarus striatus* (Decapoda: Cambaridae). Bulletin of the Alabama Museum of Natural History : 27–60.

Brantley, E., B. Helms, A. Ludwig, J. Shaw, G. Jennings, C. Anderson, D. Werneke, and J. Feminella. 2016. Eco-morphological mitigation design and assessment tools for the Alabama and Tennessee Appalachian Plateau. US EPA Region IV Atlanta, GA. Grant No. CD00D01412. http://cses.auburn.edu/eve-brantley/wp-content/uploads/sites/114/2016/10/Final-report_CD00D01412_-093016.pdf

Buhay, J. E. 2009. “COI-like” sequences are becoming problematic in molecular systematic and DNA barcoding studies. Journal of Crustacean Biology 29: 96–110.

Capinha, C., E. R. Larson, E. Tricarico, J. D. Olden, and F. Gherardi. 2013. Effects of climate change, invasive species, and disease on the distribution of native European crayfishes. Conservation Biology 27: 731–740.

Clement, M., D. Posada, and K. A. Crandall. 2000. TCS: A computer program to estimate gene genealogies. Molecular Ecology 9: 1657–1659.

Crandall, K. A., and S. De Grave. 2017. An updated classification of the freshwater crayfishes (Decapoda: Astacidea) of the world, with a complete species list. Journal of Crustacean Biology 37: 615–653.

Crandall, K. A., and A. R. Templeton. 2010. The zoogeography and centers of origin of the crayfish subgenus *Procericambarus* (Decapoda:Cambaridae). Evolution 53: 123–134.

Crandall, K. A., A. R. Templeton, and C. F. Sing. 1994. Intraspecific phylogenetics: problems and solutions. Pages 273–296 *in* Models in Phylogeny Reconstruction. Clarendon Press, Oxford, UK.

Emel, S. L., and A. Storfer. 2014. Landscape genetics and genetic structure of the southern torrent salamander, *Rhyacotriton variegatus*. Conservation Genetics 16: 209–221.

Excoffier, L., and H. E. L. Lischer. 2010. Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. Molecular Ecology Resources 10: 564-567.

Excoffier, L., P. E. Smouse, and J. M. Quattro. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics 131: 479–491.

Figiel, C. R. 2016. Crayfish population genetic studies: assessment and trends. Freshwater Crayfish 22: 19–42.

Finn, D. S., N. Bonada, C. Múrria, and J. M. Hughes. 2011. Small but mighty: headwaters are vital to stream network biodiversity at two levels of organization. Journal of the North American Benthological Society 30: 963–980.

Folmer, O., M. Black, W. Hoeh, R. Lutz, and R. Vrijenhoek. 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Molecular marine biology and biotechnology 3: 294–299.

Fu, Y. X. 1997. Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. Genetics 147: 915–925.

Glon, M. G., R. F. Thoma, C. A. Taylor, M. Daly, and J. V. Freudenstein. 2018. Molecular phylogenetic analysis of the devil crayfish group, with elevation of *Lacunicambarus* Hobbs, 1969 to generic rank and a redescription of the devil crayfish, *Lacunicambarus diogenes* (Girard, 1852) comb. Nov. (Decapoda: Astacoidea: Cambaridae). Journal of Crustacean Biology 38: 600–613.

Guindon, S., and O. Gascuel. 2003. A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. Systematic Biology 52: 696–704.

Helms, B. S., C. Figiel, J. Rivera, J. Stoeckel, G. Stanton, and T. A. Keller. 2013. Life-history observations, environmental associations, and soil preferences of the Piedmont Blue Burrower (*Cambarus* [*Depressicambarus*] *harti*) Hobbs. Southeastern Naturalist 12: 143–160.

Helms, B. S., R. C. Vaught, S. K. Suciu, and S. R. Santos. 2015. Cryptic diversity within two endemic crayfish species of the Southeastern US revealed by molecular genetics and geometric morphometrics. Hydrobiologia 755: 283–298.

Helms, B., J. Zink, D. Werneke, Z. Price, G. Jennings, and E. Brantley. 2016. Development of ecogeomorphological (EGM) stream design and assessment tools for the Piedmont of Alabama, USA. Water 8: 1–25.

Hobbs Jr., H. H. 1981. Crayfishes of Georgia. Smithsonian Contributions to Zoology, 318p.

Hobbs Jr., H. H. 1988. Crayfish distribution, adaptive radiation and evolution. Pages 52–82 in D. M. H. and R. S. Lowery, editor. Freshwater crayfish: biology, management and exploitation. Croom Helm, London, England.

Hobbs Jr., H. H. 1942. The crayfishes of Florida. University of Florida Publications, Biological Science Series 3: 1–79.

Hobbs Jr., H. H. 1989. An illustrated checklist of the American crayfishes (Decapoda: Astacidae, Cambaridae, and Parastacidae). Smithsonian Contributions to Zoology 480: 1–236.

Huchon, D., F. Delsuc, F. M. Catzeflis, and E. J. P. Douzery. 1999. Armadillos exhibit less genetic polymorphism in North America than in South America: Nuclear and mitochondrial data confirm a founder effect in *Dasypus novemcinctus* (Xenarthra). Molecular Ecology 8: 1743–1748.

Hughes, J. M., J. A. Huey, and D. J. Schmidt. 2013. Is realised connectivity among populations of aquatic fauna predictable from potential connectivity? Freshwater Biology 58: 951–966.

Hughes, J. M., D. J. Schmidt, and D. S. Finn. 2009. Genes in streams: using DNA to understand the movement of freshwater fauna and their riverine habitat. BioScience 59: 573–583.

Kimura, M. 1983. The neutral theory of molecular evolution. Cambridge University Press, New York, New York.

Kimura, M., and J. F. Crow. 1964. The number of alleles that can be maintained in finite populations. Genetics 49: 725–738.

Köppen, W. 1918. Klassifikation der Klimate nach Temperatur, Niederschlag und Jahresablauf (Classification of climates according to temperature, precipitation and seasonal cycle). Petermanns geographische Mitteilungen 64: 193–248.

Lodge, D. M., C. A. Taylor, D. M. Holdich, and J. Skurdal. 2000. Nonindigenous crayfishes threaten North American freshwater biodiversity: lessons from Europe. Fisheries 25: 7–20.

Mathews, L. M., L. Adams, E. Anderson, M. Basile, E. Gottardi, and M. A. Buckholt. 2008. Genetic and morphological evidence for substantial hidden biodiversity in a freshwater crayfish species complex. Molecular Phylogenetics and Evolution 48: 126–135.

Meffe, G. K., and R. C. Vrijenhoek. 1988. Conservation genetics management of desert fishes. Conservation Biology 2: 157–169.

Mims, M. C., E. E. Hartfield Kirk, D. A. Lytle, and J. D. Olden. 2018. Traits-based approaches support the conservation relevance of landscape genetics. Conservation Genetics 19: 17–26.

NDMC. 2019. United States Drought Monitor: Map Archive. https://droughtmonitor.unl.edu/Maps/MapArchive.aspx.

Nei, M. 1987. Molecular Evolutionary Genetics. Columbia University Press, New York, New York.

Nychka, D., R. Furrer, J. Paige, and S. Sain. 2017. fields: tools for spatial data. University Corporation for Atmospheric Research, Boulder, CO, USA. https://github.com/NCAR/Fields

Oksanen, J., F. G. Blanchet, M. Friendly, R. Kindt, P. Legendre, D. McGlinn, P. R. Minchin, R. B. O'Hara, G. L. Simpson, P. Solymos, M. H. H. Stevens, E. Szoecs, and H. Wagner. 2018. vegan: community ecology package. R package version 2.4-6. https://cran.r-project.org/package=vegan

Omernik, J. M. 1987. Ecoregions of the conterminous United States. Annals of the Association of American Geographers 77: 118–125.

Paz, A., R. Ibáñez, K. R. Lips, and A. J. Crawford. 2015. Testing the role of ecology and life history in structuring genetic variation across a landscape: a trait-based phylogeographic approach. Molecular Ecology 24: 3723–3737.

Peel, M. C., B. L. Finlayson, and T. A. McMahon. 2007. Updated world map of the Köppen-Geiger climate classification. Hydrology and Earth System Sciences 11: 1633–1644.

Piganeau, G., and A. Eyre-Walker. 2009. Evidence for variation in the effective population size of animal mitochondrial DNA. PLoS ONE 4: 2–9.

Posada, D. 2008. jModelTest: Phylogenetic model averaging. Molecular Biology and Evolution.

R Core Team. 2018. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.r-project.org

Rahman, S., D. Schmidt, and J. Hughes. 2018. De novo SNP discovery and strong genetic structuring between upstream and downstream populations of *Paratya australiensis* Kemp, 1917 (Decapoda: Caridea: Atyidae). Journal of Crustacean Biology 38: 166–172.

Ray, C. K., M. Tan, and J. W. Armbruster. 2014. First record of *Chrosomus erythrogaster* (Cypriniformes: Cyprinidae) in the Mobile Basin. Southeastern Naturalist 13: N33–N36.

Reed, D. H., and R. Frankham. 2003. Correlation between fitness and genetic diversity. Conservation Biology 17: 230–237.

Richardson, A., N. Doran, and B. Hansen. 2006. The geographic ranges of Tasmanian crayfish: extent and pattern. Freshwater Crayfish 15: 347–364.

Richardson, J. L. 2012. Divergent landscape effects on population connectivity in two co-occurring amphibian species. Molecular Ecology 21: 4437–4451.

Richman, N. I., M. Böhm, S. B. Adams, F. Alvarez, E. A. Bergey, J. J. S. Bunn, Q. Burnham, J. Cordeiro, J. Coughran, K. A. Crandall, K. L. Dawkins, R. J. Distefano, N. E. Doran, L. Edsman, A. G. Eversole, L. Füreder, J. M. Furse, F. Gherardi, P. Hamr, D. M. Holdich, P. Horwitz, K. Johnston, C. M. Jones, J. P. G. Jones, R. L. Jones, T. G. Jones, T. Kawai, S. Lawler, M. López-Mejía, R. M. Miller, C. Pedraza-Lara, A. M. M. Richardson, M. B. Schultz, G. A. Schuster, P. J. Sibley, C. Souty-Grosset, C. A. Taylor, R. F. Thoma, J. Walls, T. S. Walsh, and B. Collen. 2015. Multiple drivers of decline in the global status of freshwater crayfish (Decapoda: Astacidea). Philosophical Transactions of the Royal Society B: Biological Sciences 370: 1–11.

Rozas, J., A. Ferrer-Mata, J. C. Sánchez-DelBarrio, S. Guirao-Rico, P. Librado, S. E. Ramos-Onsins, and A. Sánchez-Gracia. 2017. DnaSP v6: DNA sequence polymorphism analysis of large datasets. Molecular Biology and Evolution 34: 3299–3302.

Santos, S. R. 2006. Patterns of genetic connectivity among anchialine habitats: a case study of the endemic Hawaiian shrimp *Halocaridina rubra* on the island of Hawaii. Molecular Ecology 15: 2699–2718.

Schuster, G. A., and C. A. Taylor. 2004. Report on the crayfishes of Alabama: literature and museum database review, species list with abbreviated notations and proposed conservation statuses. Illinois Natural History Survey, Center for Biodiversity Technical Report 12: 1–46.

Tajima, F. 1989. Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. Genetics 123: 585–595.

Tamura, K., and M. Nei. 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Molecular Biology and Evolution 10: 512–526.

Taylor, C. A., S. B. Adams, and G. A. Schuster. 2014. Systematics and biogeography of *Orconectes*, subgenus *Trisellescens*, in the southeastern United States, a test of morphology-based classification. Journal of Crustacean Biology 34: 1–14.

Taylor, R. C. 1983. Drought-induced changes in crayfish populations along a stream continuum. American Midland Naturalist 110: 286–298.

Templeton, a R., K. a Crandall, and C. F. Sing. 1992. A cladistic analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping and DNA sequence data. III. Cladogram estimation. Genetics 132: 619–633.

Tyers, M. 2017. riverdist: River Network Distance Computation and Applications. R package version 0.15.0. https://cran.r-project.org/package=riverdist

Veni, G. 2002. Revising the karst map of the United States. Journal of Cave and Karst Studies 64: 45–50.

Whelan, N. V., M. P. Galaska, B. N. Sipley, J. M. Weber, P. D. Johnson, K. M. Halanych, and B. S. Helms. 2019. Riverscape genetic variation, migration patterns, and morphological variation of the threatened Round Rocksnail, *Leptoxis ampla*. Molecular Ecology 28: 1593–1610.

Williams, D. D., and H. B. N. Hynes. 1976. The ecology of temporary streams I. The faunas of two Canadian streams. Internationale Revue der Gesamten Hydrobiologie und Hydrographie 61: 761–787.

Williams, D. D., and H. B. N. Hynes. 1977. The ecology of temporary streams II. General remarks on temporary streams. Internationale Revue der gesamten Hydrobiologie und Hydrographie 62: 53–61.

Wright, S. 1943. Isolation by distance. Genetics 28: 114–138.

Wright, S. 1946. Isolation by distance under diverse systems of mating. Genetics 31: 39–59.

Table 5.1. Study streams and major drainages sampled for genetic analyses of the crayfish *Cambarus striatus* and *Faxonius validus* in Fall 2015 and 2018 and the number of each species collected during each sampling event.

					C. striatus		*F. validus*	
Stream	Code	Latitude	Longitude	Major Drainage	2015	2018	2015	2018
Hurricane Creek	HCC	34.26891	-87.34395	Sipsey Fork	12	13	14	11
Montgomery Creek Tributary	MGT	34.37217	-87.33309	Sipsey Fork	14	12	13	12
Horse Creek	HRC	34.35225	-87.34033	Sipsey Fork	-	12	-	12
North Fork Caney Creek	NFC	34.26891	-87.48888	Sipsey Fork	-	12	-	12
Whitman Creek	WNC	34.27706	-87.49704	Sipsey Fork	-	11	-	12
Ross Branch	RSB	34.38123	-87.50700	Sipsey Fork	-	12	-	12
East Fork Beach Creek (Lower)	EFL	34.34953	-87.32946	Brushy Creek	10	11	10	11
Collier Creek	COL	34.27525	-87.33399	Brushy Creek	-	12	-	12
West Fork Beech Creek	WFB	34.34319	-87.33490	Brushy Creek	-	12	-	12
Brushy Creek Tributary	BRS	34.37489	-87.32222	Brushy Creek	-	11	-	12
East Fork Beach Creek (Upper)	EFU	34.36312	-87.32493	Brushy Creek	10	11	12	12
Turkey Creek	TRK	34.33141	-87.18000	Brushy Creek	-	12	-	12
Rush Creek	RSH	34.32960	-87.19450	Brushy Creek	-	12	-	12
Bear Creek Tributary	BRC	34.37942	-87.51334	Bear Creek	-	11	-	12
Indian Creek Tributary	IND	34.34047	-87.18816	Flint Creek	-	12	-	12

Table 5.2. Indices of genetic diversity and tests of neutrality for each population of *Cambarus striatus* and *Faxonius validus* using mitochondrial (mtDNA) cytochrome oxidase subunit I (COI) haplotypes recovered from the Brushy Creek, Sipsey Fork, and Tennessee River drainages in the Bankhead National Forest, AL.

	Cambarus striatus						*Faxonius validus*					
Locations	*n*	*nh*	*π*	*h*	Tajima's *D*	Fu's *Fs*	*n*	*nh*	*π*	*h*	Tajima's *D*	Fu's *Fs*
HCC ^{2015}	12	1	0.0000	0.000	0.00	NA	14	1	0.0000	0.000	0.00	NA
HCC ^{2018}	13	4	0.0003	0.167	-1.14	-0.48	11	1	0.0000	0.000	0.00	NA
MGT ^{2015}	14	4	0.0036	0.626	**-1.61**	1.51	13	1	0.0000	0.000	0.00	NA
MGT ^{2018}	12	4	0.0040	0.682	**-1.61**	1.44	12	1	0.0000	0.000	0.00	NA
HRC ^{2018}	12	2	0.0073	0.409	0.98	7.82	12	1	0.0000	0.000	0.00	NA
NFC ^{2018}	12	4	0.0021	0.636	-0.06	-0.05	12	1	0.0000	0.000	0.00	NA
WNC ^{2018}	11	3	0.0033	0.473	**-1.67**	2.25	12	1	0.0000	0.000	0.00	NA
RSB ^{2018}	12	2	0.0005	0.303	-0.19	0.30	12	1	0.0000	0.000	0.00	NA
EFL ^{2015}	10	4	0.0068	0.533	-0.02	2.48	10	2	0.0009	0.556	1.46	1.10
EFL ^{2018}	11	4	0.0089	0.636	1.66	4.05	11	1	0.0000	0.000	0.00	NA
COL ^{2018}	12	4	0.0013	0.682	0.75	-1.01	12	1	0.0000	0.000	0.00	NA
WFB ^{2018}	12	5	0.0061	0.667	-0.52	1.37	12	2	0.0000	0.000	0.00	NA
BRS ^{2018}	11	2	0.0090	0.509	**2.11**	8.61	12	3	0.0013	0.667	0.55	0.22
EFU ^{2015}	10	4	0.0052	0.733	0.06	1.73	12	2	0.0067	0.409	0.54	0.74
EFU ^{2018}	11	6	0.0105	0.873	1.59	1.41	12	1	0.0000	0.000	0.00	NA
TRK ^{2018}	12	3	0.0013	0.439	-0.73	0.18	12	2	0.0010	0.303	-0.25	1.38
RSH ^{2018}	12	4	0.0012	0.561	-0.83	-1.26	12	2	0.0007	0.409	0.54	0.74
BRC ^{2018}	11	3	0.0006	0.345	-1.43	**-1.25**	12	2	0.0003	0.167	-1.14	-0.48
IND ^{2018}	12	5	0.0064	0.803	1.33	1.50	12	2	0.0005	0.303	-0.19	0.30

n = number of individuals, nh = number of haplotypes, π = nucleotide diversity, h = haplotype (gene) diversity.

Table 5.3. Pairwise φ_{ST} (below diagonal) and haplotype frequency based F_{ST} (above diagonal) estimates of genetic differentiation between populations of *Cambarus striatus* from the Sipsey Fork, Brushy Creek, and Tennessee River drainages in the Bankhead National Forest, AL. Bold values indicate significantly different populations ($P < 0.05$) and superscripts indicate year that a sample was collected.

Locations	HCC ^{2015}	HCC ^{2018}	MGT ^{2015}	MGT ^{2018}	HRC ^{2018}	NFC ^{2018}	WNC ^{2018}	RSB ^{2018}	EFL ^{2015}	EFL ^{2018}	COL ^{2018}	WFB ^{2018}	BRS ^{2018}	EFU ^{2015}	EFU ^{2018}	TRK ^{2018}	RSH ^{2018}	BRC ^{2018}	IND ^{2018}
HCC ^{2015}	-	0.000	**0.247**	**0.318**	0.182	**0.236**	**0.722**	0.091	**0.727**	**0.576**	**0.628**	**0.200**	**0.314**	**0.660**	**0.577**	0.121	0.159	0.060	**0.518**
HCC ^{2018}	0.000	-	**0.156**	**0.217**	0.079	0.137	**0.622**	0.005	**0.629**	**0.479**	**0.519**	0.104	0.196	**0.568**	**0.482**	0.030	0.065	-0.022	**0.428**
MGT ^{2015}	0.055	0.053	-	-0.067	0.061	0.053	**0.381**	0.107	**0.346**	**0.226**	**0.314**	0.013	0.067	**0.304**	**0.241**	0.065	0.040	0.080	**0.169**
MGT ^{2018}	**0.077**	**0.073**	-0.074	-	0.097	0.070	**0.362**	0.135	**0.315**	**0.202**	**0.289**	0.029	0.085	**0.269**	**0.200**	0.103	0.068	0.127	**0.138**
HRC ^{2018}	0.182	0.177	0.041	0.030	-	0.071	**0.490**	0.051	**0.376**	0.216	**0.418**	-0.033	-0.063	**0.374**	**0.317**	0.030	0.030	0.023	**0.307**
NFC ^{2018}	0.218	**0.199**	**0.123**	**0.122**	**0.183**	-	**0.378**	0.086	**0.376**	**0.255**	**0.307**	0.012	0.088	**0.317**	**0.248**	0.044	0.021	0.058	**0.203**
WNC ^{2018}	**0.340**	**0.321**	**0.170**	**0.167**	0.150	**0.102**	-	**0.546**	**0.489**	**0.417**	**0.411**	**0.314**	**0.445**	**0.400**	**0.327**	**0.472**	**0.411**	**0.519**	**0.339**
RSB ^{2018}	0.091	0.061	0.045	0.043	0.152	**0.175**	**0.301**	-	**0.552**	0.407	**0.471**	0.057	0.138	**0.494**	**0.410**	0.010	0.028	-0.019	**0.358**
EFL ^{2015}	**0.780**	**0.772**	**0.637**	**0.618**	**0.393**	**0.723**	**0.658**	**0.758**	-	-0.049	**0.384**	**0.270**	**0.236**	0.069	**0.118**	**0.477**	**0.413**	**0.525**	**0.315**
EFL ^{2018}	**0.622**	**0.615**	**0.479**	**0.457**	0.222	**0.577**	**0.506**	**0.598**	-0.052	-	**0.327**	0.139	0.088	0.068	0.088	**0.338**	**0.282**	**0.379**	**0.249**
COL ^{2018}	**0.667**	**0.582**	**0.329**	**0.343**	**0.296**	**0.487**	**0.469**	**0.599**	**0.746**	**0.604**	-	**0.286**	**0.369**	**0.294**	**0.146**	**0.402**	**0.342**	**0.444**	**0.247**
WFB ^{2018}	0.094	0.091	-0.014	-0.015	-0.067	**0.106**	0.088	0.071	**0.493**	**0.324**	**0.261**	-	-0.036	**0.251**	**0.195**	0.005	-0.004	0.029	**0.186**
BRS ^{2018}	**0.314**	**0.308**	0.149	0.131	-0.063	**0.288**	**0.226**	**0.283**	0.239	0.080	**0.360**	0.003	-	**0.276**	**0.233**	0.094	0.071	0.109	**0.263**
EFU ^{2015}	**0.835**	**0.827**	**0.690**	**0.674**	**0.459**	**0.774**	**0.712**	**0.813**	-0.053	0.005	**0.795**	**0.553**	**0.309**	-	0.059	**0.421**	**0.357**	**0.466**	**0.231**
EFU ^{2018}	**0.582**	**0.574**	**0.443**	**0.417**	**0.192**	**0.539**	**0.468**	**0.557**	-0.024	-0.072	**0.551**	**0.289**	0.062	0.024	-	**0.349**	**0.286**	**0.391**	**0.163**
TRK ^{2018}	0.072	0.061	0.050	0.046	0.145	**0.152**	**0.267**	-0.019	**0.737**	**0.581**	**0.519**	0.065	**0.269**	**0.790**	**0.540**	-	0.000	-0.017	**0.290**
RSH ^{2018}	0.109	0.091	**0.074**	**0.086**	**0.174**	**0.184**	**0.287**	0.105	**0.747**	**0.596**	**0.527**	**0.098**	**0.294**	**0.800**	**0.557**	0.092	-	0.001	**0.221**
BRC ^{2018}	0.008	0.003	0.044	0.061	0.161	0.173	**0.291**	0.044	**0.754**	**0.597**	**0.577**	0.078	**0.287**	**0.811**	**0.556**	0.047	0.073	-	**0.330**
IND ^{2018}	**0.494**	**0.481**	**0.308**	**0.295**	**0.186**	**0.436**	**0.384**	**0.452**	**0.494**	**0.355**	**0.456**	**0.214**	**0.176**	**0.525**	**0.314**	**0.429**	**0.453**	**0.461**	-

Location abbreviations follows Table 1 designations

Table 5.4. Pairwise φST (below diagonal) and haplotype frequency based FST (above diagonal) estimates of genetic differentiation between populations of *Faxonius validus* from the Sipsey Fork, Brushy Creek, and Tennessee River drainages in the Bankhead National Forest, AL. Bold values indicate significantly different populations ($P < 0.05$) and superscripts indicate year that a sample was collected.

Locations	HCC ^{2015}	HCC ^{2018}	MGT ^{2015}	MGT ^{2018}	HRC ^{2018}	NFC ^{2018}	WNC ^{2018}	RSB ^{2018}	EFL ^{2015}	EFL ^{2018}	COL ^{2018}	WFB ^{2018}	BRS ^{2018}	EFU ^{2015}	EFU ^{2018}	TRK ^{2018}	RSH ^{2018}	BRC ^{2018}	IND ^{2018}
HCC ^{2015}	-	0.000	0.000	0.000	0.000	0.000	0.000	0.000	**0.763**	**1.000**	0.000	**1.000**	**0.687**	**0.810**	**1.000**	**0.860**	**0.810**	**0.923**	**0.860**
HCC ^{2018}	0.000	-	0.000	0.000	0.000	0.000	0.000	0.000	**0.734**	**1.000**	0.000	**1.000**	**0.655**	**0.787**	**1.000**	**0.842**	**0.787**	**0.913**	**0.842**
MGT ^{2015}	0.000	0.000	-	0.000	0.000	0.000	0.000	0.000	**0.754**	**1.000**	0.000	**1.000**	**0.677**	**0.803**	**1.000**	**0.854**	**0.803**	**0.920**	**0.854**
MGT ^{2018}	0.000	0.000	0.000	-	0.000	0.000	0.000	0.000	**0.744**	**1.000**	0.000	**1.000**	**0.667**	**0.795**	**1.000**	**0.848**	**0.795**	**0.917**	**0.848**
HRC ^{2018}	0.000	0.000	0.000	0.000	-	0.000	0.000	0.000	**0.744**	**1.000**	0.000	**1.000**	**0.667**	**0.795**	**1.000**	**0.848**	**0.795**	**0.917**	**0.848**
NFC ^{2018}	0.000	0.000	0.000	0.000	0.000	-	0.000	0.000	**0.744**	**1.000**	0.000	**1.000**	**0.667**	**0.795**	**1.000**	**0.848**	**0.795**	**0.917**	**0.848**
WNC ^{2018}	0.000	0.000	0.000	0.000	0.000	0.000	-	0.000	**0.744**	**1.000**	0.000	**1.000**	**0.667**	**0.795**	**1.000**	**0.848**	**0.795**	**0.917**	**0.848**
RSB ^{2018}	0.000	0.000	0.000	0.000	0.000	0.000	0.000	-	**0.734**	**1.000**	0.000	**1.000**	**0.655**	**0.787**	**1.000**	**0.842**	**0.787**	**0.913**	**0.842**
EFL ^{2015}	**0.984**	**0.981**	**0.983**	**0.982**	**0.982**	**0.982**	**0.982**	**0.981**	-	**0.461**	**0.744**	**0.476**	**0.183**	0.038	**0.476**	**0.579**	**0.233**	**0.653**	**0.273**
EFL ^{2018}	**1.000**	**1.000**	**1.000**	**1.000**	**1.000**	**1.000**	**1.000**	**1.000**	**0.461**	-	**1.000**	0.000	**0.320**	**0.717**	0.000	**0.842**	0.170	**0.913**	0.081
COL ^{2018}	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	**0.982**	**1.000**	-	**1.000**	**0.667**	**0.795**	**1.000**	**0.848**	**0.795**	**0.917**	**0.848**
WFB ^{2018}	**1.000**	**1.000**	**1.000**	**1.000**	**1.000**	**1.000**	**1.000**	**1.000**	**0.476**	0.000	**1.000**	-	**0.333**	**0.727**	0.000	**0.848**	0.182	**0.917**	0.091
BRS ^{2018}	**0.976**	**0.972**	**0.975**	**0.974**	**0.974**	**0.974**	**0.974**	**0.972**	**0.323**	**0.199**	**0.974**	**0.211**	-	**0.385**	**0.333**	**0.515**	0.139	**0.583**	**0.169**
EFU ^{2015}	**0.986**	**0.984**	**0.986**	**0.985**	**0.985**	**0.985**	**0.985**	**0.984**	0.038	**0.717**	**0.985**	**0.727**	**0.521**	-	**0.727**	**0.644**	**0.497**	**0.712**	**0.550**
EFU ^{2018}	**1.000**	**1.000**	**1.000**	**1.000**	**1.000**	**1.000**	**1.000**	**1.000**	**0.476**	0.000	**1.000**	0.000	**0.211**	**0.727**	-	**0.848**	0.182	**0.917**	0.091
TRK ^{2018}	**0.982**	**0.979**	**0.981**	**0.980**	**0.980**	**0.980**	**0.980**	**0.979**	**0.612**	**0.686**	**0.980**	**0.697**	**0.535**	**0.710**	**0.697**	-	**0.644**	**0.765**	**0.697**
RSH ^{2018}	**0.987**	**0.985**	**0.987**	**0.986**	**0.986**	**0.986**	**0.986**	**0.985**	**0.362**	0.170	**0.986**	0.182	**0.202**	**0.590**	0.182	**0.594**	-	**0.712**	0.051
BRC ^{2018}	**0.929**	**0.920**	**0.926**	**0.923**	**0.923**	**0.923**	**0.923**	**0.920**	**0.977**	**0.994**	**0.923**	**0.995**	**0.970**	**0.981**	**0.995**	**0.977**	**0.982**	-	**0.765**
IND ^{2018}	**0.989**	**0.989**	**0.989**	**0.989**	**0.989**	**0.989**	**0.989**	**0.989**	**0.365**	0.081	**0.989**	0.091	**0.181**	**0.612**	0.091	**0.611**	0.146	**0.985**	-

Location abbreviations follows Table 1 designations

* = $P < 0.05$

Table 5.5. Analyses of molecular variance (AMOVA's) for *Cambarus striatus* and *Faxonius validus* from the Brushy Creek, Sipsey Fork, and Tennessee River drainages of the Bankhead National Forest, AL.

Source of Variation	df	Sum of squares	Variance component	% Variation	φ statistic
C. striatus					
Among drainages	3	74.309	$0.34277\ V_a$	14.58	$\varphi_{CT} = 0.146$
Among populations within a drainage	15	147.044	$0.72880\ V_b$	31.00	$\varphi_{SC} = 0.363$*
Within populations	203	259.682	$1.27922\ V_c$	54.42	$\varphi_{ST} = 0.456$*
Total	221	481.034	2.35079		
F. validus					
Among drainages	3	657.433	$4.64378\ V_a$	82.07	$\varphi_{CT} = 0.821$*
Among populations within a drainage	15	167.083	$0.9311\ V_b$	16.46	$\varphi_{SC} = 0.918$*
Within populations	207	17.301	$0.08358\ V_c$	1.48	$\varphi_{ST} = 0.985$*
Total	225	841.816	5.65847		

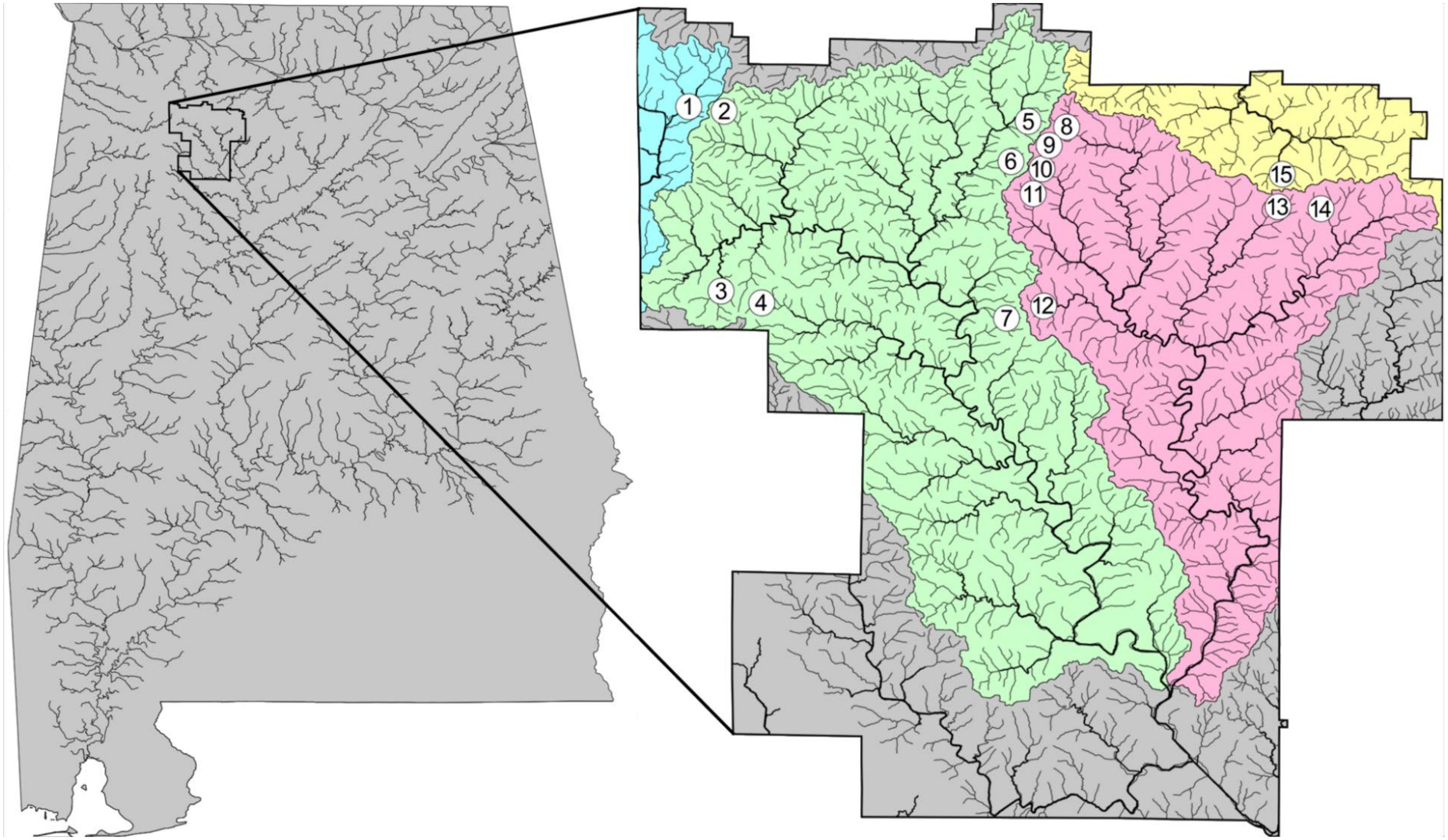

Figure 5.1. Map of Alabama with inset of the Bankhead National Forest and the 15 locations *Cambarus striatus* and *F. validus* were collected. Highlighted watersheds represent the major drainages that were sampled: Bear Creek (blue; Tennessee River), Sipsey Fork (green; Mobile River), Brushy Creek (pink; Mobile River), and Flint Creek (yellow; Tennessee River).

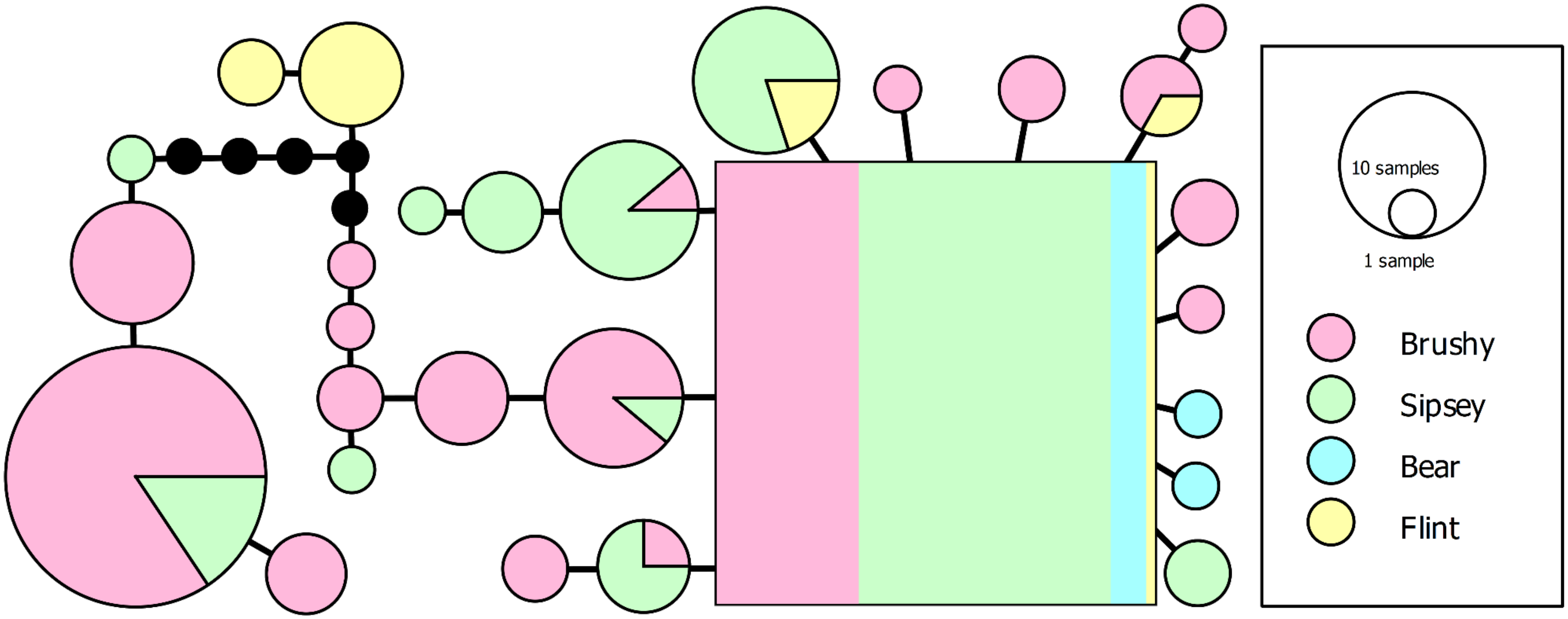

Figure 5.2. Parsimony networks depicting relationships between *Cambarus striatus* mitochondrial (mtDNA) cytochrome c oxidase subunit I (COI) haplotypes recovered from drainages in the Bankhead National Forest, Alabama. For each network, black dots represent unsampled (missing) haplotypes whereas a rectangle represents the haplotype with the highest outgroup probability. Size of circles and rectangles is proportional to haplotype frequency. Irrespective of lengths, each branch implies a single mutational difference between haplotypes.

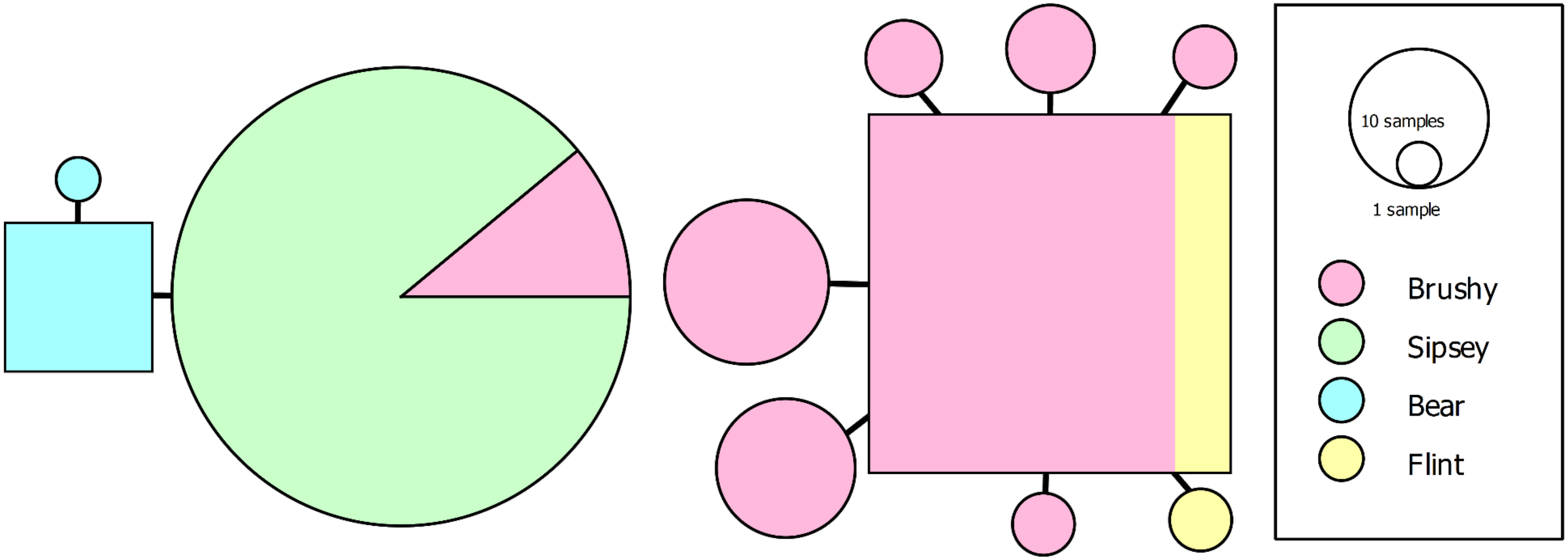

Figure 5.3. Parsimony networks depicting relationships between *Faxonius validus* mitochondrial (mtDNA) cytochrome c oxidase subunit I (COI) haplotypes recovered from drainages in the Bankhead National Forest, Alabama. For each network, black dots represent unsampled (missing) haplotypes whereas rectangles represent the haplotype with the highest outgroup probability according to the analysis. The size of circles and rectangles is proportional to haplotype frequency. Irrespective of lengths, each branch implies a single mutational difference between haplotypes.

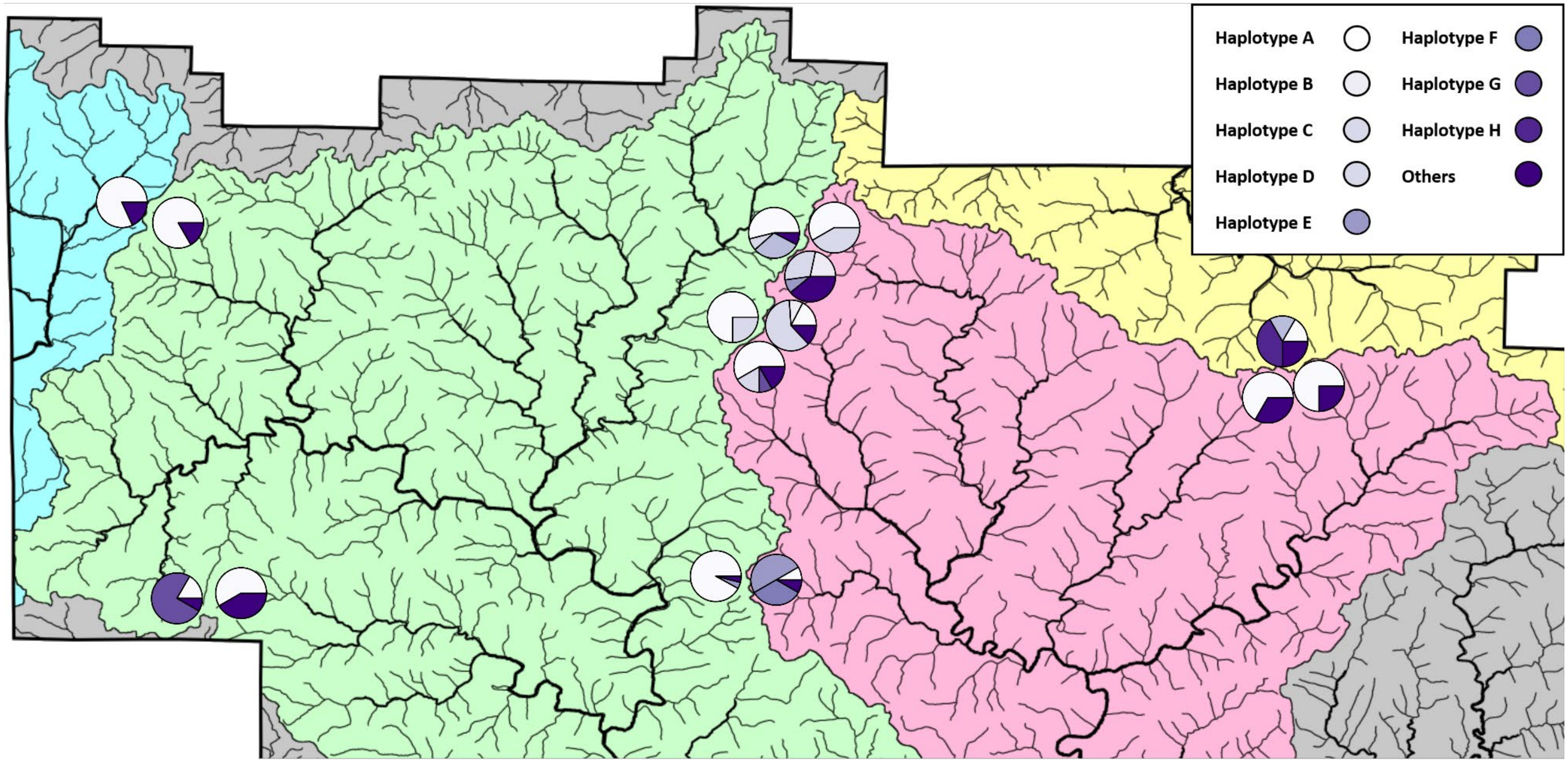

Figure 5.4. Map of the northern part of the Bankhead National Forest and the 15 locations where *Cambarus striatus* was collected. Highlighted watersheds represent major drainages sampled: Bear Creek (blue), Sipsey Fork (green), Brushy Creek (pink), and Flint Creek (yellow). Pie charts represent frequencies of mitochondrial (mtDNA) cytochrome c oxidase subunit I (COI) haplotypes recovered at each sampling location. Colors in pie charts represent individual haplotypes (see legend).

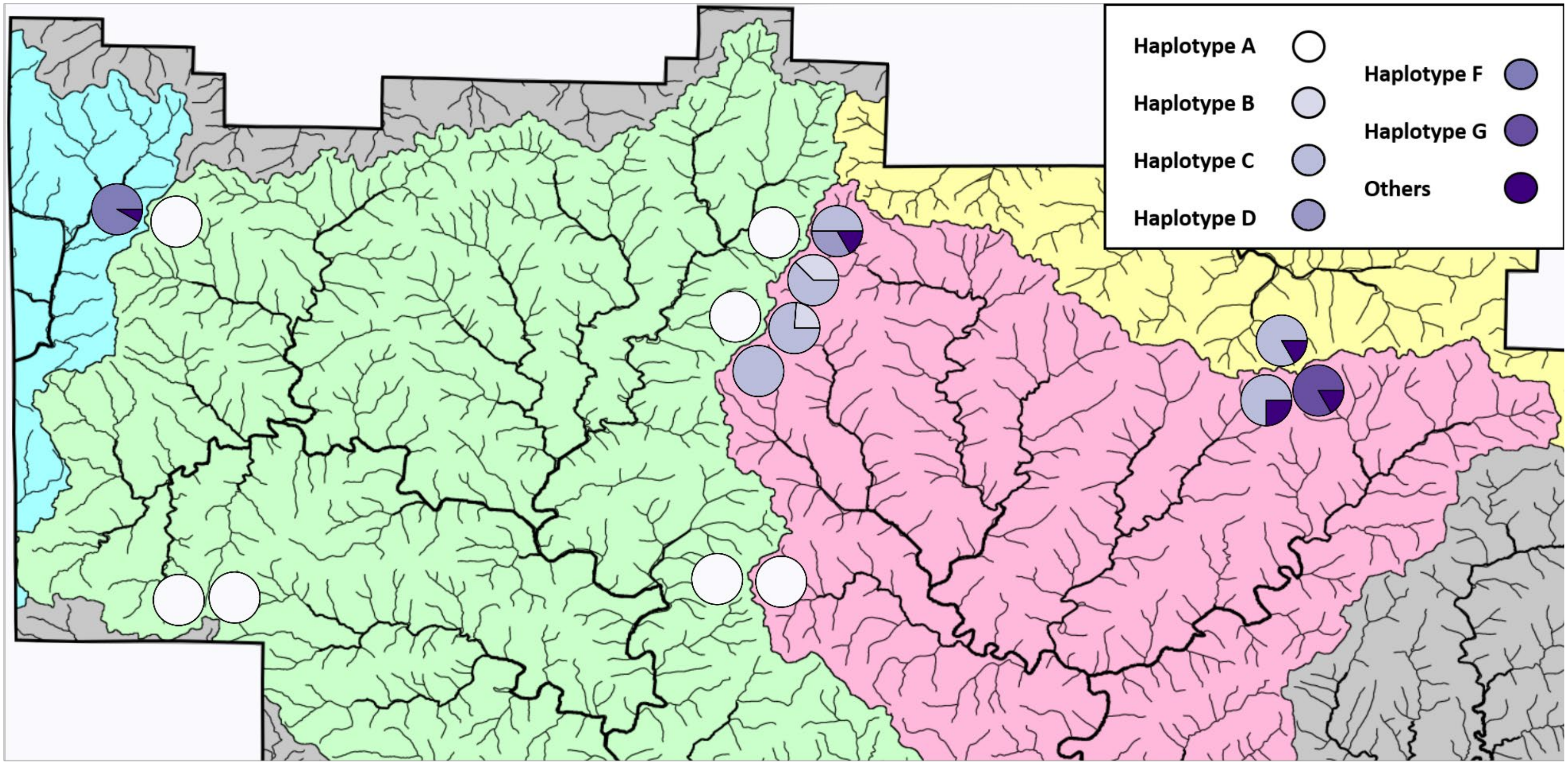

Figure 5.5. Map of the northern part of the Bankhead National Forest showing 15 locations where *Faxonius validus* was collected. Highlighted watersheds represent major drainages sampled: Bear Creek (blue), Sipsey Fork (green), Brushy Creek (pink), and Flint Creek (yellow). Pie charts represent frequencies of mitochondrial (mtDNA) cytochrome c oxidase subunit I (COI) haplotypes recovered at each sampling location. Colors in pie charts represent individual haplotypes (see legend).

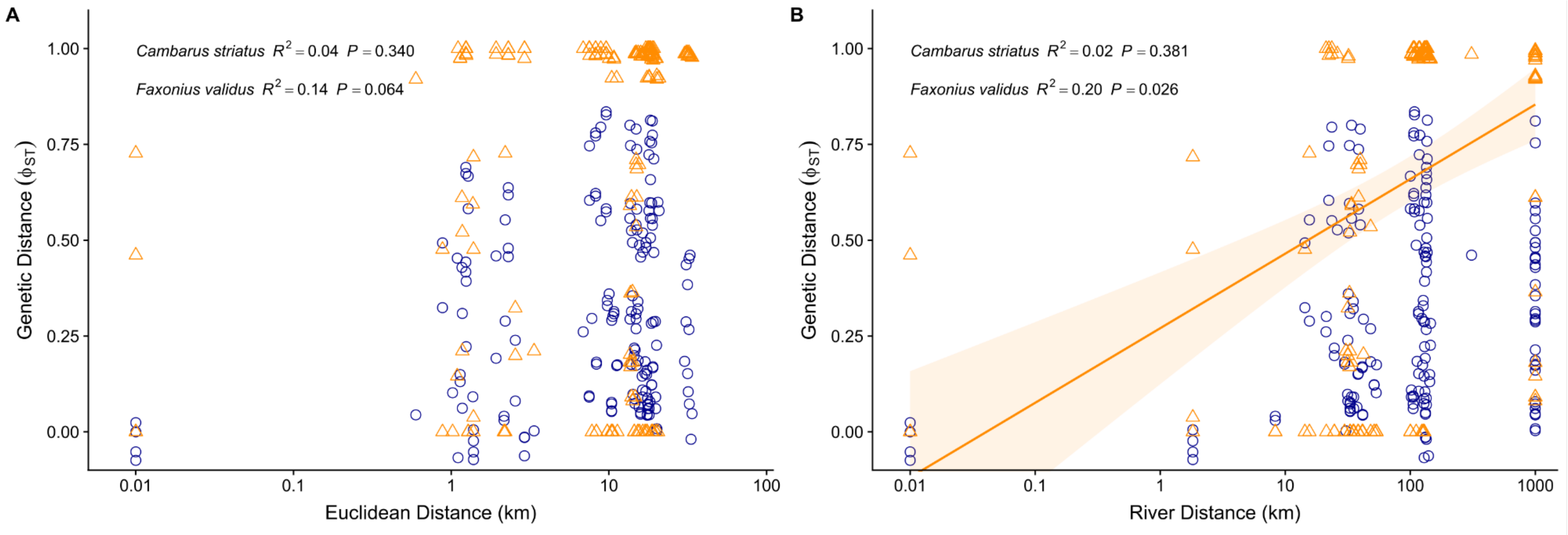

Figure 5.6. Mantel test correlations between genetic distance (φ_{ST}) and A) Euclidean (overland) distance and B) river distance for *Cambarus striatus* (blue circles) and *Faxonius validus* (orange triangles) in headwater streams of the Bankhead National Forest, Alabama.

Chapter 6: Conclusions and future directions

CONCLUSIONS

My dissertation provides important information about the relationships between environmental gradients and community diversity as well as associations between traits and population genetic diversity in streams of an aquatic biodiversity hotspot. I described several stream size – diversity relationships for assemblages of fishes, macroinvertebrates, and fish parasites that might have important implications for their conservation, or in the case of fish parasites implications for host health along longitudinal stream gradients. Additionally, I report contrasting patterns of population connectivity in 2 sympatric crayfishes with distinct burrowing traits. Below, I briefly summarize the primary conclusions of my research but more details on the basis for these conclusions can be found in individual chapters of this dissertation. Following the summary of conclusions for each dissertation chapter, I discuss potential avenues for further research.

In Chapter 2, I have provided rigorous geomorphological (regional curves) and ecological (ecological endpoint curves; EECs) criteria useful for site assessments, restoration design, and evaluating restoration success in streams of the Southwestern Appalachians ecoregion of Alabama. My results confirmed the validity of geomorphological regional curves as restoration design tools because geomorphological stream channel measurements were highly predictable with stream size. Additionally, I showed that previously developed fish-based indices of biotic integrity underperformed in small streams of the Southwestern Appalachians and Piedmont ecoregions of AL, with underperformance occurring as a result of relationships between proportion-based assemblage metrics and stream size creating redundancy among IBI sub-metrics. However, I caution restoration practitioners to consider the amount of natural variability shown in the data on which my assessment tools are based and encourage inclusion of data from additional reference streams whenever possible. Development of EECs for small streams (0.1-100 km^2) in other ecoregions of Alabama could improve assessments, restoration designs, effectiveness of restoration projects, and thus ecological function of restored streams.

In Chapter 3, my results provided strong support for the River Continuum Concept prediction of a positive stream size – biotic richness (Vannote et al. 1980) relationship in fishes and Ephemeroptera (mayflies) but not in other invertebrates. Contrasting patterns of biodiversity between fishes and macroinvertebrates suggest that these taxa respond differently to environmental factors, which also may act at different spatial scales. Additionally, I found no support for the Mighty Headwater Hypothesis (Finn et al. 2011) prediction that β-diversity is negatively correlated with stream size in either fish or macroinvertebrate assemblages. Observed biodiversity patterns provide the basis for differential recommendations for conservation efforts of these taxa in the region. Fish and mayfly diversity were highest in large streams suggesting that these streams should be prioritized to conserve these taxa. In contrast, diversity of most macroinvertebrates was evenly distributed among different-sized streams and, because there are necessarily more small streams than large streams within a network, this pattern suggests that small streams should be prioritized for maintaining regional diversity of these taxa.

In Chapter 4, I documented parasite metacommunity nestedness and a strong positive relationship between stream size and parasite community richness in *Etheostoma artesiae* populations within a stream network. These patterns are likely to be common in streams because of the natural branching spatial structure of streams, the influence of unidirectional flow on aquatic dispersal stages, and habitat diversity (fish species richness) commonly increases with stream size. Thus, studies of fish parasites in lotic ecosystems should report and account for stream size. The potential mechanism of observed metacommunity patterns need to be systematically tested, and approaches using parasite dispersal traits may offer a path forward. Additionally, the positive relationship between parasite richness and stream size may have important implications for host health and populations in lotic ecosystems, which needs further study.

In Chapter 5, I showed that population genetic structure was stronger in the crayfish *Faxonius validus* than *Cambarus striatus*, a finding consistent with my predictions and hypotheses based on their contrasting burrowing traits and habitat use. Spatial patterns of genetic diversity for *F. validus* and *C. striatus* resemble those predicted by the Stream Hierarchy (Meffe and Vrijenhoek 1988; Hughes et al. 2009, 2013) and Panmixia models (Wright 1943; Hughes et al. 2013), respectively. These results suggest that burrowing traits, and associated habitat use, of stream crayfishes may be a useful heuristic for predicting population structure. However, before this heuristic can be practically applied its usefulness must be tested by examining population structure in many more species of crayfishes including primary burrowers which were not examined in this study. Furthermore, I found that eastern and western populations of *F. validus* in the study region are distinct genetic lineages, each potentially worthy of conservation; this pattern provides additional evidence that crayfishes are a cryptically diverse group (Mathews et al. 2008; Bentley et al. 2010; Taylor et al. 2014), especially in the southeastern United States (Helms et al. 2015).

FUTURE DIRECTIONS

Broadly, I continue to be interested in investigating aquatic biodiversity patterns at the community and population genetic levels, and would like to continue work in this field of study. I also have a fondness for relatively natural systems, such as the streams of the Bankhead National Forest, where anthropogenic impacts are minimal, because I find great value in describing relationships as they exist in the absence of these nearly ubiquitous impacts. Even though I maintain my interest in fish and macroinvertebrate assemblages, I am most attracted to the study of parasite communities in stream fishes because these communities are, in my view, vastly understudied relative to free-living communities in streams and parasite communities in fishes of large rivers and lakes. Thus, basic research needed in this area could seemingly occupy an entire career. Additionally, we still understand little about the potential health impacts of stream fish parasites on their hosts and what ecosystem level effects might occur as a result.

I can envision several ways to extend the research I presented in Chapter 4. First, I will use the existing data I collected on parasite abundance to explore the relationships between stream size and abundance of individual parasites. These relationships are important because parasite abundance can have consequences for host health and populations. For example, abundance of *Uvulifer ambloplitis*, a trematode present in my study system, is negatively associated with body condition and increases mortality in its fish hosts (Lemly and Esch 1984). My preliminary analyses suggest that *U. ambloplitis* abundance in Redspot Darter (*Etheostoma artesiae*) increases with stream size. Additionally, endohelminth parasite loads have also been associated with decreased thermal tolerance in 2 sympatric stream fishes (*Lepomis macrochirus* and *Lepomis megalotis*; Lutterschmidt et al. 2007). It is also noteworthy that maximum water temperature often increases with stream size (Vannote et al. 1980). Thus, concomitant increases in maximum water temperature and abundance of *U. ambloplitis*, or other parasites, with stream size could have additive or synergistic negative effects on individual host health or populations, thereby creating a longitudinal gradient of selection on fish hosts. One avenue of research I would like to pursue is to investigate effects of parasite infections along the stream size gradient on the physiological performance of fish hosts.

I am also interested in combining molecular ecology techniques, such as those used in Chapter 5, with environmental and parasite community composition data to explore mechanisms structuring the spatial distribution of parasite diversity in streams. To do this I would use population genetic structure of stream fish parasite populations as an estimate of dispersal ability and combine this information with environmental data (including host diversity) to examine the relative influence of dispersal and environmental factors in driving spatial patterns of parasite diversity. This approach should allow testing of metacommunity theory (sensu Leibold et al. 2004, Brown and Swan 2010) and potentially identifying mechanisms structuring parasite communities in streams.

The results presented in Chapter 4 also could serve as the basis for investigating stream fish parasite community relationships with other environmental or host characteristics. For example, the host I chose (*Etheostoma artesiae*) was ideal for finding a positive stream size–parasite richness relationship because it does not move long distances, which could dampen this longitudinal relationship by increasing dispersal of parasites among sites. Therefore, it would be interesting to examine this relationship in more wide-ranging host species than *Etheostoma artesiae*. Temporal trends in stream fish parasite community composition also may be fruitful avenues for further research. During my dissertation research I supervised an undergraduate student working with parasites of *Etheostoma artesiae* collected in the spring from the same study reaches sampled for Chapter 4. We found that there were a few parasites present in spring that were not present in the fall, including an adult acanthocephalan. Thus, it would advance knowledge of these stream size–parasite richness relationships to determine their temporal stability through several seasons. That undergraduate's work was also the first time I had used liquid formalin preserved hosts to sample parasites and I was somewhat skeptical that the parasites would be sufficiently preserved for identification purposes. However, to my surprise, the endohelminth specimens were of sufficient quality to be useful for our purposes. This method offers the possibility that fish collections in natural history museums could be used to examine long-term trends in internal parasite communities. This method could greatly expand our knowledge of fish parasite communities because logistically it is difficult to collect long-term data on these communities, and thus few long-term studies of parasite communities exist (Kennedy 2009).

In summary, I think stream fish-parasite interactions offer nearly endless opportunities for study because of the lack of basic research on them and their potential to have important impacts on hosts and potentially ecosystems. As shown in Chapter 4, even longstanding and basic models in stream ecology have yet to be tested in stream fish parasite communities. Tests of these models may result in the description of patterns with important implications for hosts and increase our understanding of ecological processes in stream ecosystems.

LITERATURE CITED

Bentley, A. I., D. J. Schmidt, and J. M. Hughes. 2010. Extensive intraspecific genetic diversity of a freshwater crayfish in a biodiversity hotspot. Freshwater Biology 55: 1861–1873.

Brown, B. L., and C. M. Swan. 2010. Dendritic network structure constrains metacommunity properties in riverine ecosystems. Journal of Animal Ecology 79: 571–580.

Finn, D. S., N. Bonada, C. Múrria, and J. M. Hughes. 2011. Small but mighty: headwaters are vital to stream network biodiversity at two levels of organization. Journal of the North American Benthological Society 30:963–980.

Helms, B. S., R. C. Vaught, S. K. Suciu, and S. R. Santos. 2015. Cryptic diversity within two endemic crayfish species of the Southeastern US revealed by molecular genetics and geometric morphometrics. Hydrobiologia 755: 283–298.

Hughes, J. M., J. A. Huey, and D. J. Schmidt. 2013. Is realised connectivity among populations of aquatic fauna predictable from potential connectivity? Freshwater Biology 58: 951–966.

Hughes, J. M., D. J. Schmidt, and D. S. Finn. 2009. Genes in streams: using DNA to understand the movement of freshwater fauna and their riverine habitat. BioScience 59: 573–583.

Kennedy, C. R. 2009. The ecology of parasites of freshwater fishes: the search for patterns. Parasitology 136:1653–1662.

Leibold, M. A., M. Holyoak, N. Mouquet, P. Amarasekare, J. M. Chase, M. F. Hoopes, R. D. Holt, J. B. Shurin, R. Law, D. Tilman, M. Loreau, and A. Gonzalez. 2004. The metacommunity concept: A framework for multi-scale community ecology. Ecology Letters 7: 601–613.

Lemly, A. D., and G. W. Esch. 1984. Effects of the trematode *Uvulifer ambloplitis* on juvenile Bluegill sunfish , *Lepomis macrochirus*: ecological implications. The Journal of Parasitology 70: 475–492.

Lutterschmidt, W. I., J. F. Schaefer, and R. A. Fiorillo. 2007. The ecological significance of helminth endoparasites on the physiological performance of two sympatric fishes. Comparative Parasitology 74: 194–203.

Mathews, L. M., L. Adams, E. Anderson, M. Basile, E. Gottardi, and M. A. Buckholt. 2008. Genetic and morphological evidence for substantial hidden biodiversity in a freshwater crayfish species complex. Molecular Phylogenetics and Evolution 48: 126–135.

Meffe, G. K., and R. C. Vrijenhoek. 1988. Conservation genetics management of desert fishes. Conservation Biology 2: 157–169.

Taylor, C. A., S. B. Adams, and G. A. Schuster. 2014. Systematics and biogeography of *Orconectes*, subgenus Trisellescens, in the southeastern United States, a test of morphology-based classification. Journal of Crustacean Biology 34: 1–14.

Vannote, R. L., W. G. Minshall, K. W. Cummins, J. R. Sedell, and C. E. Cushing. 1980. The River Continuum Concept. Canadian Journal of Fisheries and Aquatic Sciences 37: 130–137.

Wright, S. 1943. Isolation by distance. Genetics 28: 114–138.