

ABUNDANCE TRENDS AND DRIVERS OF CHANGE IN FRESHWATER FISH
COMMUNITIES OF THE NEW RIVER BASIN

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Thesis submitted to the faculty of the Virginia Polytechnic Institute and State University in
partial fulfillment of the requirements for the degree of

Master of Science

In

Fisheries and Wildlife Sciences

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22 May 2020

Blacksburg, Virginia, United States

Keywords: land use, non-native species, conservation, fishes

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ABSTRACT

Habitat destruction/alteration and non-native species are widely considered the two most serious threats to biodiversity within freshwater ecosystems, which are among the most threatened in the world. I examined the effects of these factors, specifically focusing on land use and non-native species as drivers of abundance patterns of native fishes in the highly invaded and anthropogenically impacted New River basin (NRB) in the Appalachian region of the United States. In chapter 2, I examine current native and non-native species abundance patterns related to the highly variable land-use mosaic present across the NRB, with specific focus on the species-specific effects of intensive land-use practices (agriculture and urbanization) at varying spatial extents (upstream watershed, upstream riparian, and local riparian). In chapter 3, I investigate historical context of basin-wide and site-level abundance spread and decline of natives and non-natives in the upper and middle New River basin (UMNR) over the past 60+ years. Finally, in chapter 4, I partition the variation in native species abundance explained separately by land use and non-native species to determine which factor might be most influential in describing abundance distributions of UMNR native fishes over the past 20+ years. My results indicate widely varying responses of native species to various combinations of intensive land use and non-native species across contributing watersheds and widespread biotic homogenization and native species declines over the past 60+ years. These declines include reductions in unique communities and endemic species provided little consideration or protection under current conservation law. I suggest potential avenues for improvement of conservation actions to help preserve these unique species and communities based on their responses to various land-use and non-native species stressors. My study framework should be broadly applicable to other drainages and should provide opportunities for early identification of potential native species declines and the stressors that may be contributing to them.

ABUNDANCE TRENDS AND DRIVERS OF FRESHWATER FISH COMMUNITY CHANGE IN THE NEW RIVER BASIN

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GENERAL AUDIENCE ABSTRACT

Freshwater fishes are experiencing world-wide declines that have the potential to cause major negative ecological and economic impacts. Two of the biggest contributors to fish declines are habitat destruction and non-native species introductions. I examined populations of numerous fish species in the New River basin (NRB) in the Appalachian region of the United States to identify declining native species and determine how intensive land use (one type of habitat destruction) and non-native species may be contributing to these trends. My results suggest that nearly half of the native species occurring in the NRB may be experiencing widespread reductions in abundance. As a result of these declines and the spread of a few common native and non-native species, fish communities across the NRB are becoming less unique over time. Land-use changes, such as agricultural and road development near streams, which contribute to increased soil erosion and run-off of silt and sand into streams, could be causing broad habitat changes that lead to diminished populations of sensitive species and overall local and regional fish diversity. While no single non-native species may be held responsible for all native fish species declines in the NRB, complex interactions, such as competition and predation, between many natives and non-natives altogether could be contributing to many native fish declines. Farmers and other landowners can help to prevent future fish declines by re-establishing natural vegetation, such as trees, along streambanks and implementing other practices, such as cattle fencing, that reduce the streambank and soil erosion that harms fish habitat. Other stakeholders, such as anglers, can help prevent future native fish declines by limiting introductions of additional non-native species. For example, these stakeholders could avoid releasing aquatic pets and live bait into NRB streams. These practices would help limit future negative impacts caused by non-native species.

DEDICATION

What a journey it has been! Three years ago, I was just a small-town Kansas homebody. Fresh out of college, I wanted a new challenge, but I was fearful of change and apprehensive to venture too far from my comfort zone. Somehow, I worked up the courage to apply to graduate schools across the United States, not so secretly hoping that my search would eventually land me close to home. Never did I actually believe that I would end up at a place like Virginia Tech, over 1,500 miles away. It took much encouragement from many special people to start me on my path to graduate school. Chief among these special people was my undergraduate advisor, David Edds, who opened my eyes to the incredible diversity of life present in freshwaters and helped guide my search for graduate programs. This search led me to about as good of a graduate program and experience as I could have ever hoped for, led by my eventual advisors Paul Angermeier and Emmanuel Frimpong, whose well-earned reputations as top researchers and mentors is surpassed only by their great kindness and hospitality. Thank you both for taking a chance on this shy, small-town kid and opening my eyes to the world of possibilities beyond my Kansas roots.

To my past lab-mates in the Frimpong and Angermeier labs (Gifty Anane-Taabeah, Joe Buckwalter, Zach Martin, and Katie McBaine), thank you for welcoming me and showing me the ropes in the field and on campus. Your guidance and friendship helped me through the rough early times, when I was not sure I belonged. This thesis would not have been possible without your guidance and I wish you all the best in your future endeavors. Likewise, this work would not have been possible without the assistance and encouragement of many other fellow graduate students and undergraduate field workers. I am proud to call many of you my lifelong friends and your value to this thesis and to my quality of life is too vast to be expressed adequately in words. I would like to also give a special thanks to my family and friends back home. The love and support you have shown for me over the past three years is nothing short of remarkable. From expensive in-person visits, to care packages, to long calls that sometimes lasted all night, you were always there when I needed you. Lastly, I cannot go without specially mentioning my father, Richard, who has always pushed me to be the best I can be academically and continues to be a trusted sounding board and source of knowledge and wisdom today. Thank you for all of your patient help, whenever I asked you for it, and thank you for enduring me at my worst, when frustration gets the best of me. Whether it was helping out with writer's block or assistance waging the constant battle with ArcGIS, you were instrumental in providing the momentum I needed to push this thesis to its conclusion.

ACKNOWLEDGMENTS

I thank all funding sources, including the Virginia Tech Department of Fish and Wildlife Conservation, Virginia Cooperative Fish and Wildlife Research Unit, the Edna Bailey Sussman Foundation, the Organization of Fish and Wildlife Information Managers (OFWIM), and the Burd Sheldon McGinnis Fellowship for financial support. Many state agencies contributed data for this project, including VDGIF, NCWRC, NCDEQ, and VADEQ. Additionally, I thank Joe Buckwalter for his work compiling the bulk of the historic fish community data utilized in this study and all of the Virginia Tech students that participated in field fish surveys to supplement this dataset. The Virginia Cooperative Fish and Wildlife Research Unit is jointly sponsored by U.S. Geological Survey, Virginia Tech, VDGIF, and Wildlife Management Institute. Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

ATTRIBUTION

Co-advisors Emmanuel Frimpong (EF) and Paul Angermeier (PA), along with advisory committee member Bryan Brown (BB) each contributed significantly to this thesis. EF acted as the chief statistical analysis and project design supervisor, along with contributing to the writing of the thesis chapters herein. PA acted as the primary editor of each chapter, along with providing guidance in project design, including design of field studies to supplement historical fish collection records for our various analyses. BB acted as a secondary editor of thesis chapters and contributed statistical analysis support. Thesis chapters are written as stand-alone manuscripts with some redundancy in information and slight formatting variations related to guidelines of target scientific journals. Chapter 1 is to be submitted for publication in *Ecography*, Chapter 2 in *Diversity and Distributions*, and Chapter 3 in *Biological Invasions*.

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CHAPTER 1: INTRODUCTION

Freshwater ecosystems are considered some of the most imperiled in the world (Dudgeon et al. 2006). Declines in freshwater fishes are particularly common, as this group experienced the highest extinction rate among all vertebrates in the 20th century (Burkhead 2012). In North America, declines in freshwater fish populations continue to present day, as 336 fish taxa (species, subspecies, and evolutionarily significant units [ESUs]) were downgraded to imperiled status by the American Fisheries Society Endangered Species Committee, based on observed population trends between 1989 and 2008 (Williams et al. 1989; Jelks et al. 2008).

Drivers of freshwater fish imperilment are diverse, including factors such as overharvest, climate change, and disease, but the most commonly cited causes of decline are habitat alteration and non-native species (Miller et al. 1989). Habitat alteration encompasses a number of human activities, including direct in-stream perturbations such as dewatering of streams for municipal and agricultural uses, gravel mining, and channel morphology alterations to increase navigability (Helfman 2007). However, intensive land-use practices (agriculture, urbanization, coal mining, etc.) occurring across watersheds can also have pronounced impacts on in-stream habitat and present fish communities. Increased fine sediment loading, decreased water quality, and alteration of stream temperature through removal of riparian vegetation are all commonly cited impacts of intensive land use that have been linked to declines in fish populations and alteration of freshwater communities (Allan 2004).

Non-native species' effects on fish communities can also be quite diverse. Although some research suggests that most non-native fishes represent fairly harmless introductions (Gozlan 2008), some introductions result in major adverse economic and ecological impacts

(Witte et al. 1992, van der Veer and Nentwig 2015). While much research has gone into generalizing impacts of non-native species based on traits that commonly correlate with invasion and impact, the potential effects of some non-native species are understudied and impacts remain hard to predict. Some of the difficulty in isolating impacts of non-natives stems from the separate or sometimes synergistic impacts of other common drivers of native species declines, including habitat alteration (Didham et al. 2007).

The New River basin (NRB) spans approximately 21,700 km² in the Appalachian region of the United States. Historically, the NRB was almost entirely forested, but is now subject to a complex mosaic of altered forest cover and intensive land use practices. Additionally, the NRB is now home to the highest ratio of non-native to native fishes in the eastern United States, with the number of established non-native species outnumbering natives. Along with these abundant potential threats to native species, the New River is also home to at least nine endemic species, whose restricted ranges and specific habitat requirements may make them more vulnerable to decline and extinction (Burlakova et al. 2011).

This thesis is an attempt to compile information on changing abundance distributions of native and non-native species in the NRB and to describe the relative impacts of habitat alteration (specifically land use) and non-native species on native fish communities to contribute to local fish conservation efforts. Throughout, I utilize a novel fish abundance metric which may serve as a useful alternative to other commonly used metrics for describing fish distributions, as it allows spatiotemporal comparison of fish community data despite variation in historical sampling procedures and may allow earlier detection of species spread and decline. Subsequent chapters are built to describe related, but distinct aspects of the distributions of species and fish communities across the NRB, relative to modern and past drivers of disturbance. In Chapter 2, I

seek to determine the relative importance of land use at different extents on structuring of modern fish communities across the NRB and describe how land use effects differ depending on species. In Chapter 3, I track long-term trends in native and non-native species distribution patterns and related trends in fish community composition in the upper and middle New River Basin (UMNR) in an attempt to identify potential future conservation targets. Finally, in Chapter 4, I seek to identify and compare effects of land use and non-natives on native fish distributions in the UMNR over the last two decades.

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CHAPTER 2: RELATING SPATIAL EXTENT AND PHYSIOGRAPHY TO LAND-USE IMPACTS ON DISTRIBUTION PATTERNS OF FRESHWATER FISHES WITHIN NEW RIVER TRIBUTARY STREAMS

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Abstract

Land use is a major determinant of water and habitat quality in freshwater systems. Intensive land-use practices, such as urban and agricultural development, can have major impacts on freshwater environments, affecting water quality parameters such as temperature, chemical composition, nutrient inputs, and sediment loading. These consequences of intensive land use tend to have negative effects on regional biodiversity, driving populations of sensitive species down, while favoring expansion of habitat generalists. However, generalizing effects of land use on aquatic systems is difficult because impacts of land-use type can vary with spatial scale and the underlying physiographic template within watersheds. In addition, the responses of stream organisms to these impacts vary widely, owing to their diverse life history strategies. Despite this complexity, understanding the varying impacts of land use is essential to conservation of aquatic biodiversity. To contribute to the growing scientific knowledge of land-use impacts on aquatic species, we used a machine-learning species-distribution modeling approach, employing the Least Absolute Shrinkage and Selection Operator (LASSO) and Boosted Regression Trees (BRTs) to disentangle the effects of land use at multiple extents (local riparian to entire watershed) and physiography on abundance patterns of fish species occurring in tributary streams across the New River basin in the eastern United States. Physiographic variables mostly outperformed land-use variables, indicating resilience of most species to broad land-use changes. However, land-use variables quantified at both whole watershed and local riparian extents loaded heavily on some species distribution models, including those for a few endemic species. Road-stream crossings at the watershed extent arose as the most meaningful land-use predictor on average across all species. The importance of land-use variables in describing patterns of abundance in restricted range, potentially vulnerable species and the major effects of watershed road crossings on the abundances of many species are both findings that warrant future study. Such studies could help to inform managers about where and how land-use impacts could be best mitigated to assist in protecting and recovering conservation-relevant species.

Introduction

The impacts of land-use change on riverine biotic communities have been a primary concern of past conservation research due to findings that link intensive agricultural and urban development to alteration of in-stream habitats and associated biotic communities (Larimore and Smith 1963; Allan et al. 1997; Sponseller et al. 2001; Wang et al. 2001; Helfman 2007). Urban and agricultural development often affect adjacent stream systems by increasing fine nutrient and chemical pollution, increasing fine sediment deposition, and altering temperature and flow regimes. However, research suggests that these effects vary given different spatial configurations of intensive land use and the proximity of these practices to potentially affected stream systems (Allan 2004). This realization began a debate among researchers about the influence of proximity on land-use impacts relevant to local freshwater communities. While some authors emphasize local riparian land use as the most relevant to in-stream biotic processes (Richards et al. 1996; Wang et al. 2001; Frimpong et al. 2005), others contend that land use across significant portions of upstream watersheds may be even more predictive of local water quality and biotic community composition (Hunsaker and Levine 1995; Johnson et al. 1997).

Agricultural practices that eliminate forested riparian buffers from stream reaches tend to raise stream temperatures within these reaches and decrease nutrient and sediment uptake efficiency of riparian areas in some cases (Young et al. 1980, Cooper et al. 1987, Hickey and Doran 2004). However, other studies have found that water quality degradation from nutrient pollution in agricultural watersheds depends more on the proportion of the watershed in agricultural production, regardless of local riparian conditions (Omernik et al. 1981). Thus, in-stream biotic communities within agricultural watersheds may be forced to adapt to both riparian reach-scale dynamics of temperature and sediment regulation and nutrient pollutant loading,

which may be more proximate to the prevalence of intensive agriculture at the watershed extent. A similar pattern could be expected in heavily urbanized watersheds, where streams flowing directly through urban areas may be more heavily impacted by floods due to storm water runoff over impermeable surfaces, whereas reaches downstream of urban development may be more heavily impacted by chemical pollution from upstream urban sources.

Allan et al (1997) and May et al (1997) stressed the importance of both watershed-level land use and local or riparian land use, suggesting that land use at these two extents determine different in-stream properties that all combine to influence fish communities. Whereas local riparian conditions may be most predictive of organic inputs and habitat structure within streams, properties such as sediment loading, nutrient supply, and hydrology are influenced by broader, regional land uses occurring throughout upstream watersheds (Allan et al. 1997). Many studies have attempted to determine the single extent at which land-use variables best predict fish community characteristics in ecological models (Richards et al. 1996; Frimpong et al. 2005), but these models fail to capture varied responses of aquatic species to habitat structuring as a result of simultaneous and distinct impacts of basin-wide and local land use patterns. Including variables that describe land usage at multiple extents should thus improve the performance of descriptive aquatic species distribution models (SDMs), yet few studies have accounted for both extents of potential land-use disturbance in the same SDMs (Wang et al. 2001).

Adding an extra layer of complexity, land-use effects on stream ecosystems are often determined, in part, by physiographic context (Poole and Downing 2004, Utz et al. 2009, Utz et al. 2011, Govenor et al. 2018, DeWeber et al. 2019), encompassing the influence of natural features (topography, soil and rock types, etc.) on land-use impacts. For example, Poole and

Downing (2004) noted that agricultural land use and lack of riparian woodlands were two major determinants of freshwater mussel species richness declines in Iowa, but that the presence of Mississippian rock formations across the region mitigated land-use impacts due to the natural groundwater quality and quantity enhancement capacity of these formations. Other physiographic properties, such as soil texture and topographic relief, influence properties such as erosion potential across watersheds. Erosional and depositional processes occurring throughout watersheds determine fine sediment loads (Mukundan et al. 2010) and influence habitat properties within stream channels, including bedrock exposure and particle size of alluvial sediments (Howard et al. 1994). Fishes employ a diverse array of life history strategies relating to substrate size and have differing tolerances of fine sediment pollution and turbidity (Berkman and Rabeni 1987; Frimpong and Angermeier 2009). Thus, physiographic determinants of stream bottom characteristics and sediment loads are critical to understand distributional patterns of stream fish species where direct measures of in-stream habitat are unavailable.

In addition to considerations of the effects of land use at varying extents and the physiographic template, metrics used to describe fish communities can also have an effect on the performance of SDMs. Most previous studies that have examined the effects of land use on freshwater biota at multiple scales have either focused on community metrics such as indices of biotic integrity (IBIs), species richness, evenness, and diversity (Steedman 1988; Richards et al. 1996; Allan et al. 1997; May et al. 1997; Lammert and Allan 1999; Sponseller et al. 2001; Wang et al. 2001), or on presence-absence or abundance records of a single species or a very limited set of species or life history guilds (Richards et al. 1996; Hudy et al. 2008) as the response variables to quantify the effect of land use on aquatic organisms. Community-based metrics, such as IBIs, are useful for making broad statements about how land use affects stream quality for resident

aquatic communities, but fail to capture any variation in response between individual species. Similarly, focusing on abundance or occurrence data for only a single species or limited set of species ignores the effect of land use on some species that may contribute greatly to overall community structure and function. Each species has unique sensitivities and tolerances to all kinds of potential stressors (thermal, chemical, sedimentation, etc.) which are mediated by the location, extent, and type of intensive land-use practices across contributing watersheds. Thus, we argue that community responses to varying land-use disturbance regimes depend upon the species present in these aquatic communities.

The objective of our study was to increase understanding of the influence of land use at multiple extents on fish communities and the potential for these community responses to land use to be mediated by life history characteristics of constituent species. We examine land use as a descriptor of individual fish species abundance using fish community data from tributary streams of the New River basin (NRB). Upstream land use within small watersheds associated with these tributary streams often varies widely, in contrast to larger watersheds which tend to be more homogenous in land-use type. This variation in small watersheds makes ecological signals associated with land use easier to detect. Herein, we modeled abundance of all species occurring within NRB tributaries, for which sufficient data existed, using descriptive land-use variables at multiple extents. Specifically, we hoped to 1) Identify which land-use variables best explained current abundance patterns of each species and 2) Examine how land-use variables, quantified at multiple scales, interacted with each other and with physiographic descriptors to influence abundance patterns of fishes across the NRB.

Methods

Study Area

The NRB spans approximately 21,700 km² in the Appalachian region of the United States. The main-stem New River flows through parts of three states, northward from its headwaters in northwestern North Carolina through southwestern Virginia and culminating at Kanawha Falls in West Virginia. Current land use is quite variable across the historically forested NRB. While some native forest regrowth has been allowed since the mass deforestation of the eastern United States in the early 20th Century and forest is once again the predominant land-cover type (approximately 72.5% of land cover basin-wide) (Figure 2.1), many sub-watersheds remain dominated and heavily influenced by agricultural and urban development (Homer et al. 2015) and riparian vegetation conditions vary widely. In addition to diverse land uses, the NRB supports a unique fish assemblage representing many diverse life history strategies. At least 11 families of fishes occur in the NRB, many containing species with divergent reproductive, feeding, and growth strategies (Jenkins and Burkhead 1994). These characteristics combine to make the NRB an ideal system in which to study multi-scale land-use effects on fish species.

Field Surveys

We used one-pass, double-backpack electrofishing to survey fish communities at 97 randomly selected tributary stream sites across the NRB from 2012 to 2014. Each ‘site’ consisted of two spatially replicated reaches within inter-confluence stream segments of the National Hydrography Dataset (NHD) flowlines (USGS 2016^A), which represented our sampling units (Figure 2.1). Sampled streams ranged in size from second to fifth order (Strahler 1957) and reaches ranged in length from 80 to 140 meters. Reaches within sites were selected as to sample habitats approximately proportionate to their availability to fishes within each stream segment and reaches were bound by natural barriers (riffles and cascades) to reduce fish escapement.

Sites were spread widely across the NRB, with sampled locations within the watersheds of every major tributary (Strahler order five) and some minor tributaries. Land use within contributing watersheds of sampled sites was approximately proportionate to NRB-wide land usage. Sampling data include those collected and analyzed by Peoples and Frimpong (2016) and site selection and fish sampling methods are consistent with their efforts.

All sampling was conducted from late June to early October. Because some sites were sampled repeatedly across years while others were not, fish community data from repeatedly sampled sites were pooled across years. In 2013, stream discharge greatly exceeded median historical values (USGS stream gauge data, 1930-2018) across the NRB during the survey period, whereas 2012 and 2014 represented years of relatively low median flow (USGS 2019). Pooling data across these three years allowed us to examine fish community structure across the basin while also partially accounting for variation in annual streamflow patterns, which can affect abundance of some fish species (Marchetti and Moyle 2001; Bernardo et al. 2003; Benejam et al. 2010). Additionally, the temporal distribution of our repeat samples limited the possibility of biased abundance estimates due to temporal variation in streamflow (Table 2.1).

Data Analysis

To quantify abundance of fish species within sampled streams of the NRB, we applied a novel composite abundance metric (accounting for total numerical catch, proportional abundance, and rank of each species per sampling event) to describe land-use-mediated trends in species distributions. Catch data for each fish species was pooled across repeat samples within sites and divided by the number of visits to compute average catch per species per site. Abundance within sites was measured by manipulating the average catch data in three ways (1. Normalized total count - *TC*, 2. Community proportional abundance - *PA*, and 3. Rank

abundance – RA). For the normalized count metric (equation 1), the average catch (C) for a given species (i) at each site (j) in which that species was detected was divided by average catch of that species within the site where it was most abundant ($C_{i,jmax}$). The normalized total count metric serves as a relative measure of the abundance of each fish species at each site across the NRB. The community proportion metric (equation 2) was calculated by dividing the average catch for a species at a given site by the average total number of fish of all species (T) collected at that site. The community proportion metric was then scaled (PA_{norm}) between 0 and 1 for each species at each site (equation 3). This metric measures the local abundance of each species relative to all fishes at a given site. The rank abundance metric (equation 4) is a scaled score of each species' dominance within each site relative to other species detected there, where a species' abundance rank (R) is scaled by the total species richness (SR) at that site, where ranks are integers that range from 1 (indicating the most abundant species at a site) to SR (indicating the least abundant species at a site). Each of the three abundance metrics gives each species a site-specific abundance score between 0 and 1. A site-specific composite abundance score (CSA) was computed for each species by summing the individual abundance metrics at each site (equation 5).

$$(1) TC_{ij} = \frac{C_{ij}}{C_{i,jmax}}$$

$$(2) PA_{ij} = \frac{C_{ij}}{T_j}$$

$$(3) PA_{norm(i),j} = \frac{PA(i),j}{PA(i),jmax}$$

$$(4) RA_{ij} = \frac{(SR_j) - [(R_{i,j}) - 1]}{SR_j}$$

$$(5) CSA_{ij} = [TC_i + PA_{norm(i)} + RA_i]_{,j}$$

The resulting composite site-abundance scores for each species were used as the response variables in subsequent species-specific analyses. Total numerical abundance, community proportional abundance, and rank abundance are related measures that all provide uniquely descriptive information about overall abundance patterns of species. While numerical abundance helps determine genetic variability and long-term survival probability of populations (Lande and Barrowclough 1987), proportional abundance allows for comparison of populations within streams of different size which are subject to size-associated variation in survey effort, and ranks allow for consideration of species dominance, which increases with the addition of subordinate competitor species (Camargo 1992).

Land use was quantified using the 2011 National Landcover Dataset (NLCD) (Homer et al. 2015), a publicly accessible dataset with the closest temporal proximity to our field surveys. Percentages of area covered by forest, agriculture or grassland, and urban development were calculated at three spatial extents (segment riparian, upstream network riparian, entire upstream watershed) related to each stream segment (section of stream between upstream and downstream confluences with other segments) where fish surveys were conducted. Land-use characteristics within riparian areas (both within a given segment and upstream of the segment) were quantified within a 60-meter buffer zone centered on the corresponding NHD flowlines. Thirty meters represents the spatial resolution of the NLCD data and thus the smallest buffer zone on either side of a given stream that can be used to quantify stream-adjacent land use, which is the optimal riparian dimension for use in aquatic biota-landscape modeling (Frimpong et al. 2005). Entire upstream watershed land use was derived using flow accumulation modeling (using the National Elevation Dataset – NED) in ArcGIS (USGS 2016^B), where land-use characteristics (e.g. number of cells representing urban land use) were determined as the cumulative number of cells of a

specific land use flowing into the cell representing the most downstream point in the stream segment of interest (immediately upstream of its downstream confluence).

The number of road-stream crossings was also quantified at both segment and upstream watershed extents as an extra set of land-use descriptors relating to potential fragmentation of fish habitat by bridges and culverts and potential point-sources of pollutants (e.g. salts). These structures were enumerated within each stream segment by computing the number of intersections between NHD flowlines and roads represented in the 2012 Tiger Line data (US Census Bureau 2012).

Given the potential confounding influence of physiography on the ability to describe the effects of land-use variables on fish abundances, a suite of physiographic descriptors (base elevation, stream gradient, topographic relief [whole watershed], soil texture [fine and coarse], and dominant surficial geology [igneous, metamorphic, or sedimentary]) were also included in our models (Table 2.2). Channel gradient, watershed topographic relief and base elevation were derived for each sampled stream segment using the NED. Soil textural physiographic predictors were derived from the United States Department of Agriculture (USDA) and Natural Resources Conservation Service's (NRCS) Gridded Soil Survey Geographic Database (gSSURGO) (USDA-NRCS 2016) using the convention of Slezzer et al. (1998) (Appendix B: Table B2). Surficial geology data was derived from the State Geologic Map Compilation (SGMC) (Horton et al. 2017).

To account for the possible influence of spatial autocorrelation on patterns of fish species abundance across the NRB, we performed a Euclidean distance-based Moran's eigenvector analysis (Dray et al. 2006) using geographic coordinates of sampled stream segment pour points

(points at stream endpoints representing downstream confluences), resulting in the identification of five significant spatial eigenvectors. These five spatial eigenvectors were included as covariates in our models along with the 11 land-use variables and six physiographic variables previously described (Table 2.3).

Because sensitivity of SDMs, or correctly predicting species presence where they are in fact present, severely declines at low species prevalence (<10% of sampled sites) (van Proosdij et al. 2016), all species with low prevalence values (<10 sites in our dataset) were excluded from analysis. For each species occurring at an adequate number of sites, we used a two-step machine-learning approach to model responses (site-specific composite abundance scores) to the suite of land-use and physiographic predictors. First, the set of candidate land-use and physiographic predictors was narrowed using the Least Absolute Shrinkage and Selection Operator (LASSO) approach (Tibshirani 1996) to eliminate highly correlated variables and those that describe little variation in abundance response from the candidate set. Second, we used Boosted Regression Trees (BRTs) (Elith et al. 2008) to model the response of fish species' abundance to the LASSO-retained set of predictors. BRTs revealed the relative importance of each LASSO-retained variable (variable importance ranks) to each species' abundance pattern and the directional effect of each variable. BRT models explaining less than 45% of a species' abundance pattern (cross validation correlation less than 45%) were excluded from further analysis, as even considering associated standard error, these species' abundance patterns are likely better described by environmental attributes not measured herein.

Together, the LASSO and BRT methods provide answers to three important questions regarding how individual fish species respond to land use. The LASSO approach elucidates 1)

What types (forest, agriculture and grassland, urban) and extents of land-use data compilation (whole upstream watershed, upstream riparian, segment riparian) best explain patterns of abundance for each species? Subsequent BRT models allow assessment of 2) Which individual land-use predictors have the greatest influence on each fish species? and 3) How does each retained land-use variable affect abundance of each species (positively or negatively)? The results also allow judgments regarding a fourth question: 4) How does basin physiography influence impact of land use on fish populations? Our analyses were designed to elucidate the hierarchical effects of land-use configurations on species distributions and abundances and the effect of species identity on the results of land-use studies, in accordance with our study objectives.

Results

We collected 61 fish species, of which 39 were collected at over ten percent of sites and included in our species abundance models (Table 2.4). The subset of species retained for analysis were thus those native and non-native species occurring commonly in tributary streams across the NRB and were not necessarily representative of communities of the mainstem New River. Overall, the retained species list contained representatives of 7 of the 10 major families (families with more than 2 species present in the NRB) of fishes present in the NRB. The only excluded major families (Clupeidae, Esocidae, and Moronidae) are dominated by non-native large-river specialists which are generally uncommon in tributary streams.

Of our land-use variables, those quantified at the segment riparian extent were selected most often as influential variables by the LASSO procedure (Figure 2.2). Road-stream crossings were the most commonly selected land-use variables at both watershed and segment riparian scales, out-pacing agricultural, urban, and forest area at each of these land-use extents. Stream

gradient and base elevation were the most common physiographic variables influencing species abundances, each selected in a majority of LASSO models and more often than any land-use variable.

Of the 39 species included in the LASSO regression analysis, subsequent BRT analysis yielded acceptable results (cross validation correlation greater than 45%) when LASSO-selected variables were used to model species abundance for 21 species. After BRT analysis, variable importance ranks of the LASSO-retained variable sets were summarized across all of these species by dividing the observed variable importance ranks by expected variable importance ranks (assuming all LASSO-selected variables had equal influence on the abundance of a species) to yield normalized variable importance scores across all species (Table 2.5).

Outperformance, or the ability of LASSO-selected variables to exceed expected variable importance ranks in BRT models assuming equal importance of each variable in these models, was used as a measure of each variable's predictive capability. Only 16% of LASSO-selected, land-use variables outperformed expected contributions to species abundance patterns (gray highlighted values in Table 2.5) while physiographic and spatial variables did so 42% and 55% of the time, respectively. The high outperformance value for the spatial predictor variables indicates a high degree of spatial autocorrelation in species abundance patterns across the study area.

Outperformance values for individual land-use variables varied with variable type and quantification extent. The number of road-stream crossings variables outperformed expected contributions to species abundance patterns 46% of the time while land-use type percentage variables (Agriculture = 9% outperformed, Forest = 9%, Urban = 12%) were much less predictive of these patterns. The predictive capability of land-use type percentage variables

varied with extent, with watershed-level variables (15% outperformance) performing slightly better than such variables at the segment riparian (13%) and watershed riparian (5%) extents.

Among variables of interest within our study (the physiographic and land-use descriptors), physiographic variables (except dominant surficial geology), on average, were all ranked as more important than land-use percentage variables in determining species abundances. In fact, the only traditional land-use variable that outperformed average expected effects on species abundance patterns was the watershed road-stream crossings variable, while two physiographic variables (stream gradient and base elevation) did so (Figure 2.3).

The watershed road-stream crossings variable also provided the most readily interpretable results among the land-use variables in terms of strength and consistency of response by species with similar taxonomies and/or life histories. For example, centrarchid species were consistently and strongly positively correlated with watershed crossings, while trends for small-bodied leuciscids and percids were more mixed, but tended to be more negative (Table 2.5). Stream gradient was the strongest predictor of all physiographic variables in terms of average variable importance in BRT models and tended to have a positive or neutral influence on species groups adapted for life in swift currents and/or cold water environments (e.g. small percids, cottids, and salmonids), while abundance of species adapted to warmer, slower waters (e.g. centrarchids and many leuciscids) tended to correlate negatively with stream gradient.

Although not as heavily weighted (lower average variable importance) as other variables in our SDMs, LASSO-selected percent-forest variables at both the segment and watershed scales were more commonly positively correlated with species abundance than any other land-use variables (Figure 2.4A) according to interpreted BRT partial dependence plots (PDPs, Figure

2.4B). Among the species correlating positively with these percent-forest variables were NRB endemics, the Kanawha Darter (*Etheostoma kanawhae*) and Bigmouth Chub (*Nocomis platyrhynchus*).

Discussion

Effects of land use on fish species abundance

By far, the land-use variable most highly correlated with fish species abundance patterns in our study was the number of road-stream crossings within upstream watersheds (Figure 2.3). Generally, the correlation of upstream crossings with fish population sizes may be associated with four distinct but related impacts of these structures: 1. downstream habitat alteration, 2. fragmentation of populations due to crossing structures as barriers to movement, 3. chemical pollution from road run-off, and 4. alteration of biotic interactions resulting from the previous three factors.

Downstream habitat alterations associated with upstream crossings are quite diverse. Density of upstream crossings has been linked to changes in basin hydrology, including increases in frequency and intensity of flood peaks and debris flows associated with floods, pool creation and associated sediment mobilization by river-bottom scour downstream of bridges and culverts (Jones et al. 2000, Wellman et al. 2000). Pool creation tends to aggregate fishes, especially during summer low-flow periods, during which most of our fish surveys took place. Additionally, Leitão et al (2018) found upstream road-stream crossings reduced depth of the water column, bed stability and stream-bottom complexity, negatively affecting species adapted for life among coarse benthic substrates and structured microhabitats. Leitão et al (2018) also indicated that species occupying the water column were negatively affected by upstream road-stream crossings due to associated decreases in water depth. Our results are consistent with these

patterns, with both hard-bottom, benthic-dwelling species like Fantail Darter (*Etheostoma flabellare*) and water-column dwellers such as Rosyside Dace (*Clinostomus funduloides*) and Mountain Redbelly Dace (*Chrosomus oreas*) all exhibiting abundance attenuation in response to increasing upstream road-stream crossings.

The role of road-stream crossings as potential barriers to fish dispersal often depends on crossing type. While bridges do not often act as barriers to dispersal of fishes, culverts, which are much more commonly used as road-crossing structures in smaller streams (typical of streams sampled in our study), can act in this capacity. Culverts can restrict the flow of the stream current, causing increases in flow velocity which can slow or prevent passage by small, benthic species and others that lack strong swimming abilities, especially during high stream flows (Warren and Pardew 1998). Culverts that are perched above the water table can also prevent movement of fishes and the probability of fish movement across culverts varies with culvert type (Norman et al. 2009).

Road-stream crossings also act as points of introduction of foreign substances into streams, including road salts and other chemical applicants, which can alter water chemistry downstream of crossing sites, having varying effects on resident aquatic species (Findlay and Kelly 2011, Kotalik et al. 2017). Most studies on the toxicity of road salts on the biotic components of stream communities have thus far focused on invertebrate communities, as aquatic macroinvertebrates are relatively immobile and thus less able to avoid periodic increases in road pollutant concentrations associated with high-runoff events and are more firmly entrenched as indicators of water quality than other aquatic organisms (Kenney et al. 2009). However, chronic effects of road salts on larval aquatic vertebrates have been documented, including reduced survival, growth, and activity along with increases in physical abnormalities in

larval frogs exposed to varying concentrations of road salts (Sanzo and Hecnar 2006). In fishes, documented sub-lethal effects of various pollutants include alteration of reproductive morphology, behavior, and output that could have population-level effects (Jones and Reynolds 1997), so the potential impacts of road applicants on the abundance patterns observed in our study should not be discounted.

Whereas increases in upstream road-stream crossings correlated negatively with many species abundance patterns in our study, some larger-bodied fishes (most notably *Ambloplites rupestris* and *Lepomis auritus*) showed positive abundance trends with increasing road-stream crossings. These cover-oriented species likely use bridge structures for refuge, in contrast to smaller-bodied potamodromous species that often access small streams from larger contiguous streams (Hitt and Angermeier 2006) but may be absent in small streams when barriers to dispersal are present (Perkin and Gido 2012). However, another possible explanation for decreased abundance of small-bodied fishes is increased predatory interactions with the large-bodied centrarchids that seem to respond positively to increasing numbers of basin-wide road-stream crossings.

Investigating specific mechanisms underlying correlations between road-stream crossings and fish species abundance patterns was beyond the scope of our study. However, given the strong performance of road-stream crossings as descriptors of abundance distribution patterns of many species within our study, future studies focused on quantifying specific effects of crossings on habitat availability, fish movement, pollution, and biotic interactions between fishes seem appropriate. Elucidating specific effects of these structures could further help to explain prevalent fish abundance patterns relating to crossings and give managers a better chance to mitigate these impacts.

Relationships between broader patterns of urban and agricultural land use and fish abundance patterns were relatively weak in our study. However, the positive relationship between abundance of Bluehead Chub (*Nocomis leptcephalus*) and increasing percent coverage of agricultural land within upstream riparian areas (Table 2.5) is consistent with previous studies. *N. leptcephalus* and other *Nocomis* species are often characterized as sediment-tolerant because the gravel mounds they build for spawning enable them to persist and thrive in habitats where pervasive agriculture has increased fine sediment loading (Jenkins and Burkhead 1994; Scott and Helfman 2001; Peoples et al. 2011).

In addition to the reproductive benefits mound-building provides to *Nocomis* chubs, many other species (nest associates) utilize chub nests for spawning. These nest associates may benefit from spawning on chub mounds, experiencing increased brood survival as a result of egg protection provided by the silt-free nest itself, nest-guarding behavior of the resident male chubs, and the dilution of predation by egg predators due to sheer numbers of eggs of other species present within the nests (Johnston 1991; Wallin 1992; Johnston 1994; Peoples and Frimpong 2013). Despite these potential benefits to nest associates, we found little evidence in our BRT models that abundances of common nest associates such as *C. funduloides*, *C. oreas*, *L. albeolus*, Rosefin Shiner (*Lythrurus ardens*), Saffron Shiner (*Notropis rubricroceus*), and Central Stoneroller (*Campostoma anomalum*) were linked to abundance of *N. leptcephalus*.

Another finding was the common occurrence of positive trends between forest coverage and species abundance. Particularly noteworthy were the positive responses of two endemic species (*E. kanawhae* and *N. playrhynchus*) to increasing forest coverage. These NRB endemics, and many other highly localized species across the eastern United States, evolved in highly forested environments. Thus, the link between forest coverage and endemic abundance is not

surprising. These trends suggest the importance of preserving forested landscapes to conserve unique fish assemblages.

Effects of spatial extent on predictive capability of land-use variables

Results of LASSO and BRT models suggest mixed responses of species to land use at varying extents. While segment riparian land-use percentage variables tended to be selected more often by the LASSO procedure, the level of outperformance and average contributions to species-specific BRT models were similar to values for the same variables quantified at the watershed extent. As no single land-use extent arose as the most proximate to broad species abundance patterns, these results suggest that future studies featuring SDMs should include land-use variables at multiple extents to maximize insights into potential population responses.

Effective conservation of species and/or communities relies on identifying extents of relevant stressors and appropriate actions. For example, our finding that NRB endemics respond positively to increases in forest coverage at the segment riparian scale suggests that actions to restore or maintain healthy riparian areas, if applied to streams within the historic range of these species, may be a successful conservation tactic. Currently, reported application of agricultural best management practices (BMPs), including lands registered in the Conservation Reserve Enhancement Program (CREP) in the Virginia portion of the NRB, include over 4,600 acres of established, forested riparian buffers (DCR 2019). However, if not applied in specific areas, application of riparian forest conservation procedures could have little overall effect on resident fish populations, as our analyses suggest that abundance of many fish species are not responsive to variation in riparian forest cover. If fish conservation is a goal of programs such as CREP, priority should be placed on identifying areas with populations of conservation-relevant species, such as restricted-range endemics, that are likely to benefit from these restoration procedures and

incentives for landowner enrollment in such programs should be commensurate with expected benefits to fish communities.

Impacts of physiography and spatial autocorrelation on fish species abundance patterns

Physiographic and spatial variables generally outperformed land-use variables as significant predictors of fish species abundance in our analysis. This pattern indicates at least some level of resilience by most fish species to widespread anthropogenic changes to the landscape. Our focus on mostly small streams may have contributed to this result, as past studies have suggested that temperate headwater fishes may be resilient to human disturbance due to their evolution in naturally harsh, unstable environments (Walser and Bart 1999). Harding et al (1998), in a study including watersheds near ours, found that past land use had a greater effect on current freshwater diversity than did modern-day land use. We did not consider past land use in our analysis, which could have contributed to our overall lack of detection of land-use related effects and overall prominence of physiographic predictors over land use as important predictors of fish abundances in our study.

Spatial autocorrelation was common in our study; the LASSO procedure identified at least one significant spatial predictor of abundance for every species we modeled and such spatial predictors outperformed expected contributions to subsequent BRT models in most cases. Some of the strongest correlations we observed between species abundance and spatial predictors were for non-native species and native endemics with highly restricted ranges. For example, abundance of non-native Brown Trout (*Salmo trutta*) was strongly correlated with a spatial variable (dbMEM1), generally indicating greater *S. trutta* abundance in the southern portion of the NRB. *S. trutta* were first stocked in the North Carolina portion of the NRB shortly after 1905 (Mickey Jr. 1984) and heavy stocking continues in these streams today (NCWRC 2020). The

endemic Candy Darter (*Etheostoma osburni*), known only from Virginia and West Virginia, is considered extirpated from many streams within the southern portion of its historical range (USFWS 2018), including a few of our sites (Jenkins and Burkhead 1994). Therefore, the negative correlation we observed between *E. osburni* abundance and dbMEM1 is not surprising.

The strong spatial correlates of species abundance in our study, however unsurprising, illustrate the importance of accounting for spatial autocorrelation when attempting to identify environmental stressors proximate to population trends in stream fishes. Failing to account for spatial effects could lead to erroneous conclusions about potential causes of apparent population responses to environmental gradients.

The dominance of physiographic and spatial variables as correlates of species abundances in our study indicates some degree of resistance by most fish species to stressors related to land use. However, we did not examine biotic interactions, which can vary across space, land-use disturbance regime, and physiographic template. Such biotic interactions are widespread and range from differing outcomes of competition among co-occurring species along environmental gradients (Echelle et al. 1972) to exclusion by non-native predators (Shelton et al. 2015) and facilitated persistence of fishes in degraded habitats via mutualisms with tolerant species (Helms et al. 2005). Therefore, we suggest that future studies of effects of land use on fish distribution also consider biotic interactions.

Conclusions and suggestions for future research

Number of road-stream crossings was the most important land-use variable structuring species abundance patterns in our analysis. This suggests the need for more detailed research on the specific effects of crossing structures (bridges, culverts, etc.) on differential movement, habitat use, biotic interactions, and survival of stream fishes.

Land-use trends at both segment riparian and whole watershed extents appeared to contribute to some species abundance patterns. As land use at these different extents affect stream habitat in different ways, it is crucial to establish which extent is most relevant to population trends of potentially imperiled species when designing conservation strategies to ameliorate negative impacts linked to land-use patterns. Studies like ours, applied in different regions, could thus provide managers useful information about where mitigation of land-use impacts may be most effective in reaching conservation goals.

The dominance of physiographic and spatial variables as correlates of species abundances in our study indicates some degree of resistance by most fish species to stressors related to land use. However, we did not explicitly examine biotic interactions, which can vary across both space and physiographic template, and in cases of mutualism, could help limit land-use impacts on some species. Therefore, we suggest that future studies of the effects of land use on fish distribution and abundance also consider the role of biotic interactions in mitigating or exacerbating land-use impacts.

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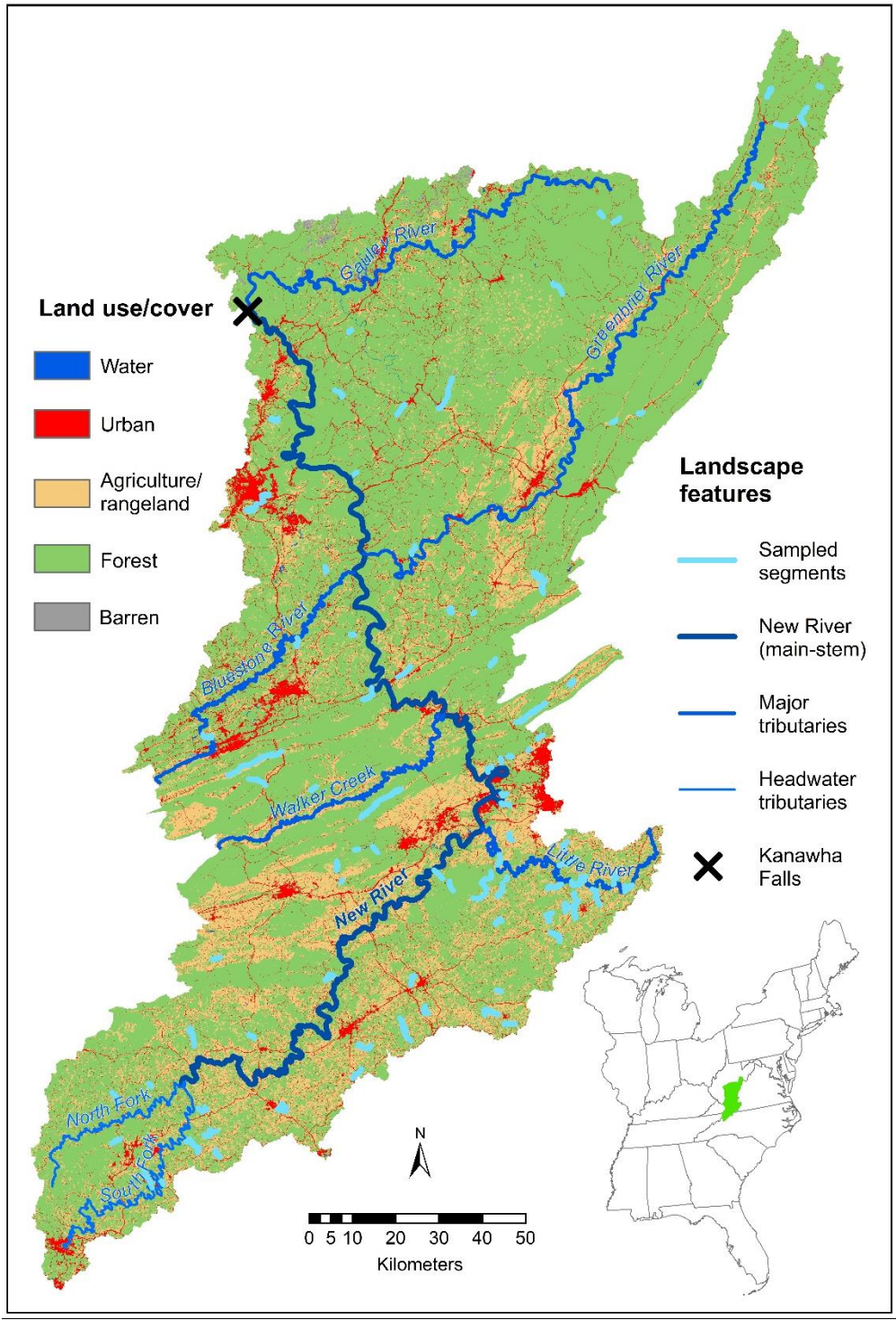


Figure 2.1: Study Area: New River basin (NRB) land use/cover, sampling locations, and other pertinent landmarks.

Table 2.1: Frequency of sites sampled across wet and dry (high versus low stream discharge) survey seasons. The wet and dry year scores were calculated as the proportional contribution of wet and dry year samples to the total number of sites, where a site sampled once in a wet year and once in a dry year receives both dry and wet scores of 0.5. Total wet and dry year scores are complimentary to the total number of sites (n = 97) and indicate negligible differences in sample density between wet and dry survey seasons.

	Wet year only (2013)	Dry year only (2012 or 2014)	Repeat samples: 1 wet, 1 dry year	Repeat samples: all years (1 wet, 2 dry)	Totals
Number of sites	27	13	11	46	97
Wet year score	27	0	5.5	15.3	47.8
Dry year score	0	13	5.5	30.7	49.2

Table 2.2: Physiographic variables, derivations, and descriptions.

Physiographic variables	Units	Source	Derivation	Purpose
Stream gradient	Meters/kilometer	NED*	Subtraction of downstream from upstream endpoint elevation values of each given NHD* stream segment divided by the length of the segment	Partial proxy for erosion potential and average flow velocity
Relief	Meters	NED*	Subtraction of the elevation at the most downstream endpoint of a stream segment from the highest elevation in its contributing watershed	Partial proxy for basin-wide erosion potential
Base elevation	Meters	NED*	Raw elevation of the most downstream endpoint of a stream segment	Partial proxy for stream temperature
Fine	-	gSSURGO*	Weighted average of soil texture descriptions in gSSURGO, given ordinal rank values in Table B2 (columns 1-2), derived across contributing watersheds of sampled stream segments	Partial proxy for erosion and fine sediment deposition potential and type (size) of fine sediment inputs to sampled streams
Coarse	-	gSSURGO*	Weighted average of soil “textural modifier” descriptions in gSSURGO database, given ordinal rank values in Table B2 (columns 3-4), derived across upstream riparian areas (defined by a 60-m buffer) of sampled stream segments	Partial proxy for larger substrate particles occurring on stream bottoms
Surficial geology (Sedi)	-	SGMC*	Dominant rock type within the contributing watersheds to sampled stream segments, where watersheds with predominantly sedimentary bedrock are scored a 1 and igneous (uncommon in the New River basin) and metamorphic-dominated watersheds scored a 0	Proxy for bed habitat and stream network characteristics given differing erosion patterns of these different rock types and water chemistry properties affected by parent material

* NED – National Elevation Dataset (USGS 2016^B)

*NHD – National Hydrography Dataset (USGS 2016^A)

* gSSURGO – gridded Soil Survey Geographic Database (USDA-NRCS 2016)

*SGMC – State Geologic Map Compilation (Horton et al. 2017)

Table 2.3: Summary statistics for the 17 land-use and physiographic descriptor variables in our analysis. Key – Variables: Ag = Agriculture, For = Forest, Urb = Urban, X = Road-stream Crossings, SEG = Stream segment riparian extent, UPS = Upstream riparian extent, WAT = Entire watershed extent; Type (variable type): BIN = Binary, CON = Continuous, INT = Integer (ordinal or count).

	St_Order	SEG_Ag%	SEG_X	SEG_For%	SEG_Urb%	UPS_Ag%	UPS_For%	UPS_Urb%	WAT_Ag%	WAT_X	WAT_For%	WAT_Urb%	Coarse	Fine	Sedi	Base_Elev (m)	St_Gradient (m/km)	Relief (m)
Mean	2.8	20.7	3.2	62.5	13.3	22.5	64.3	10.0	20.5	53.6	69.6	7.1	1.9	12.3	1	674.0	8.4	438.8
Median	3	15.0	2	64.9	10.7	23.3	64.2	6.8	21.1	33	67.0	5.3	1.7	9.4	1	663.6	5.9	409.4
Minimum	1	0	0	2.5	0	0	21.5	0.4	0	1	22.6	0	0.7	7.0	0	412.3	0.6	161.9
Maximum	5	86.6	20	100	54.3	68.7	99.5	48.3	65.6	541	99.9	46.9	4.8	21.9	1	993.7	58.6	951.0
Range	4	86.6	20	97.5	54.3	68.7	77.9	47.9	65.6	540	77.3	46.9	4.1	14.9	1	581.4	58.0	789.0
Type	INT	CON	INT	CON	CON	CON	CON	CON	CON	INT	CON	CON	CON	CON	BIN	CON	CON	CON

Table 2.4: Fish species captured within at least 10 out of 97 sites during 2012-2014 surveys. Species are listed by family and in order of their prevalence (percentage of sites in which they were detected [% columns]). Superscripts indicate species not included in the final analysis due to poor model fit.

<u>Cyprinidae</u>	%	<u>Catostomidae</u>	%	<u>Centrarchidae</u>	%
<i>Camptostoma anomalum</i>	95.9	<i>Hypentelium nigricans</i>	74.2	<i>Ambloplites rupestris</i>	64.9
<i>Nocomis leptocephalus</i>	84.5	<i>Catostomus commersoni</i> ^B	71.1	<i>Micropterus dolomieu</i> ^B	43.3
<i>Rhinichthys atratulus</i> ^B	81.4			<i>Lepomis auritus</i>	40.2
<i>Clinostomus funduloides</i>	71.1	<u>Ictaluridae</u>		<i>Lepomis cyanellus</i> ^B	32.0
<i>Chrosomus oreas</i>	64.9	<i>Noturus insignis</i>	37.1	<i>Lepomis macrochirus</i> ^L	20.6
<i>Semotilus atromaculatus</i>	52.6			<i>Micropterus salmoides</i> ^L	10.3
<i>Luxilus albeolus</i>	48.5	<u>Salmonidae</u>			
<i>Rhinichthys cataractae</i> ^B	46.4	<i>Oncorhynchus mykiss</i> ^B	33.0	<u>Percidae</u>	
<i>Nocomis platyrhynchus</i>	37.1	<i>Salmo trutta</i>	28.9	<i>Etheostoma flabellare</i>	89.7
<i>Pimephales notatus</i> ^B	33.0	<i>Salvelinus fontinalis</i>	18.6	<i>Etheostoma blennioides</i> ^B	34.0
<i>Luxilus cerasinus</i> ^B	32.0			<i>Etheostoma kanawhae</i>	28.9
<i>Lythrurus ardens</i>	25.8	<u>Cottidae</u>		<i>Percina gymnocephala</i> ^B	24.7
<i>Notropis telescopus</i>	24.7	<i>Cottus bairdii</i>	63.9	<i>Etheostoma caeruleum</i> ^B	15.5
<i>Notropis rubellus</i>	21.6	<i>Cottus kanawhae</i> ^B	21.6	<i>Etheostoma osburni</i>	10.3
<i>Phenacobius teretulus</i> ^L	20.6				
<i>Notropis rubricroceus</i> ^B	20.6				
<i>Notropis scabriceps</i> ^B	16.5				
<i>Cyprinella galactura</i> ^B	16.5				
<i>Exoglossum laurae</i>	13.4				

^L - Lasso not successful in variable selection, excluded from BRT analysis

^B - Poor BRT model results (cv correlation < 45%), models excluded from further analysis

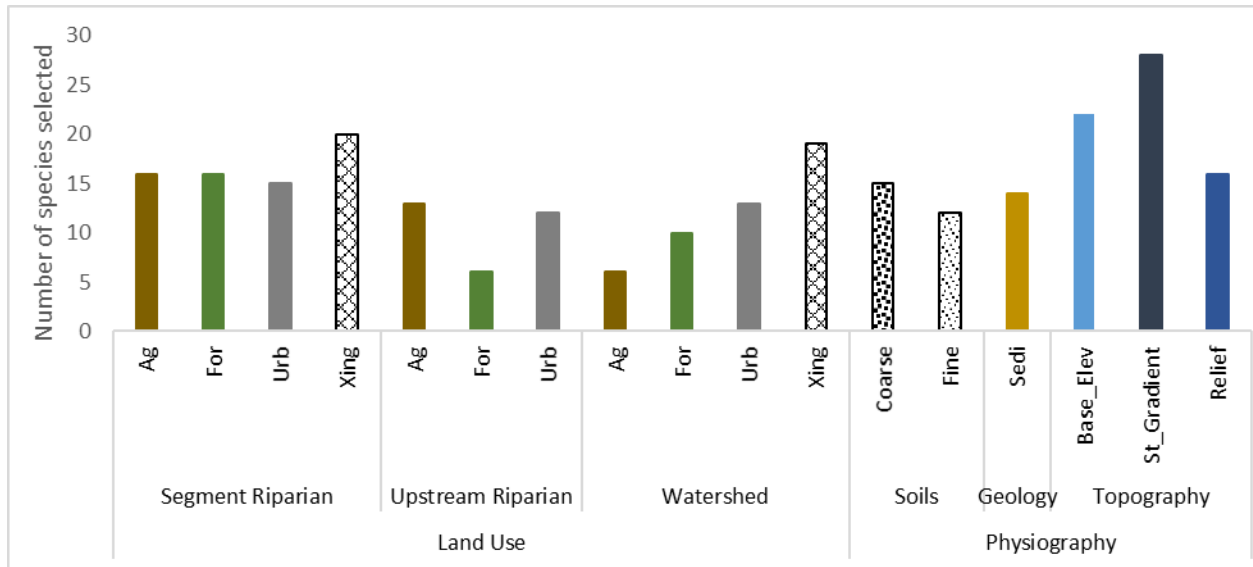


Figure 2.2: LASSO variable selection results for 39 fish species within the New River basin (NRB), where the height of bars represents the number of species for which each variable was retained (selected) by the LASSO selection procedure and passed on to the boosted regression tree (BRT) final modeling step. Key – *Ag* = % Agriculture, *For* = % Forest, *Urb* = % Urban, *Xing* = number of road-stream crossings, *Coarse* = Coarse substrate score of upstream riparian area, *Fine* = Fine substrate score of contributing watershed, *Sedi* = Binary variable (1 if sedimentary bedrock is dominant across contributing watershed, 0 if otherwise), *Base_Elev* = Elevation at downstream confluence of sampled stream segment with another river or stream, *St_Gradient* = Stream Gradient, *Relief* = Watershed topographic relief.

Table 2.5: Normalized variable importance scores from BRT models for 21 fish species across the study area. Variables with “-” entries were not selected by the LASSO procedure for the given species and thus were not included in the BRT modeling step for that species. All scores > 1 or < -1 (highlighted in gray) indicate variables outperforming expected effects on the abundance of a species assuming equal importance of all selected variables in these models.

		Leuciscidae											
		Nocomis		Campostoma	Semotilus	Clinostomus	Chrosomus	Notropis		Lythrurus	Luxilus	Exoglossum	
		<i>N. platyrhynchus</i>	<i>N. leptocephalus</i>	<i>C. anomalum</i>	<i>S. atromaculatus</i>	<i>C. funduloides</i>	<i>C. oreas</i>	<i>N. rubricroceus</i>	<i>N. telescopus</i>	<i>L. ardens</i>	<i>L. albeolus</i>	<i>E. laurae</i>	
		Family	Genus		Species								
		Stream order	0.22	0.40	0.05	-	0.01	-	0.20	-0.01	-	0.52	-
Land use (LU)	Segment riparian	% Agriculture	-	-	0.09	-	-	-	-0.05	-	0.37	-	-0.20
		Number of road-stream crossings	-2.11	-	0.13	-0.13	-	-	0.32	0.50	-	0.03	0.10
		% Forest	1.38	-	-0.25	-	0.96	-	0.34	0.26	-	-	0.20
		% Urban	-	-	-0.13	0.18	0.79	1.04	-	-	-	-	1.85
	Upstream riparian	% Agriculture	-	0.97	0.27	-	-0.43	-	-	0.41	-	1.27	-0.38
		% Forest	-	-	-0.24	-	-	-	-	-	-	-	-
		% Urban	-	-	0.03	-	0.10	-	-	0.44	-	-0.50	-
		Watershed	% Agriculture	-	0.16	-	-1.22	-0.25	-	-	-	0.35	-
		Number of road-stream crossings	1.16	-	1.62	-1.18	-1.82	-5.25	0.03	-	1.41	-	-
		% Forest	-	-0.81	-0.14	0.11	-	-	0.50	-	-0.63	-0.14	-
		% Urban	-	-	0.4	-	-0.54	-	-	0.19	-	0.86	-
	Physiography (Phys)	Upstream riparian coarse substrate score		-0.24	-	0.50	-	-	-	0.24	-	0.64	-
Watershed fine substrate score		-	-	0.94	1.32	-0.13	-	-	-	-	-	-	
Sedimentary bedrock		-	-0.19	0.02	0	-0.07	-0.23	-	0.26	-	-	0	
Base elevation		-	-	-0.95	-	0.43	-	-0.14	-0.83	-1.8	-3.22	-	
Stream gradient		-5.11	-2.41	-2.00	-0.23	1.52	0.93	-0.10	-1.33	-2.53	-4.45	-1.01	
Relief		1.04	-	0.75	-0.43	-	-	-	2.75	-	-	0.08	
Spatial		dbMEM1		-	4.41	-0.07	-1.76	2.03	1.72	10.16	-1.41	-	-0.25
	dbMEM2		-	-2.66	0.06	0.99	-1.11	-1.18	-	-	-3.57	-1.29	-
	dbMEM3		2.32	-	-1.2	-1.82	-	1.92	-0.08	-	-	-	7.2
	dbMEM4		-0.21	-0.90	-0.24	-	-0.41	1.20	0.94	1.46	-	0.07	-
	dbMEM5		-	-	-0.91	0.40	1.73	-	0.05	-1.74	-1.05	-	-
CV correlation		0.47	0.67	0.65	0.67	0.66	0.51	0.63	0.6	0.59	0.53	0.71	
CV correlation standard error		0.07	0.07	0.11	0.04	0.04	0.10	0.11	0.09	0.07	0.07	0.07	
% Variation explained (Phys+LU)		79.2	43.1	80.3	61.9	61.6	54.7	19.7	66	66.4	88.7	33.6	

	Family	Catostomidae	Ictaluridae	Salmonidae		Centrarchidae		Percidae			Cottidae	
	Genus	Hypentelium	Noturus	Salvelinus	Salmo	Lepomis	Ambloplites	Etheostoma			Cottus	
	Species	<i>H. nigricans</i>	<i>N. insignis</i>	<i>S. fontinalis</i>	<i>S. trutta</i>	<i>L. auratus</i>	<i>A. rupestris</i>	<i>E. osburni</i>	<i>E. flabellare</i>	<i>E. kanawhae</i>	<i>C. bairdii</i>	
	Stream order	0.52	0.95	-0.80	-	-	0	0.05	-	-	-	
Land use (LU)	Segment riparian	% Agriculture	-0.37	0.11	0.28	-	0.64	-	-	-	0.76	
		Number of road-stream crossings	-	-0.45	0.18	0.04	-	0.03	-0.21	0.01	-0.36	-
		% Forest	1.55	-	-0.14	-	-	-	0.14	-0.80	0.83	-
	Upstream riparian	% Agriculture	-	0.65	-	-0.73	-	0.20	-	-	-	-
		% Forest	-	-	0.28	-	-	-	-	0.33	0.008	-
		% Urban	-0.30	-0.13	-	-	-	-	-	-	-0.58	-
	Watershed	% Agriculture	-	-	-	-0.07	-	-	-	-	-	-
		Number of road-stream crossings	-	1.47	-	0.56	2.52	2.72	0.08	-2.03	-0.07	-
		% Forest	-	-0.14	-	-	-	-	-	-	-	-
		% Urban	-	0.44	-	-	-	0.14	-	-1.06	-	-0.50
	Physiography (Phys)	Upstream riparian coarse substrate score	-1.55	-	-3.70	-	-	-	-0.22	-	0.02	-
		Watershed fine substrate score	-	-	-	-	-0.62	-	0.79	-0.15	-0.39	-
		Sedimentary bedrock	-0.26	-	0	-	-	0	-	-	-1.21	0
Base elevation		-	-	3.02	-	-2.98	-3.41	3.22	-0.49	-	2.38	
Stream gradient		-5.45	-2.05	-	1.08	-4.29	-6.18	0.29	-	-0.19	1.77	
Relief		1.19	-0.36	-	-	-	0.14	0.36	-	-	-	
Spatial	dbMEM1	-	1.51	-	7.50	-	-0.22	-5.63	-	7.67	-	
	dbMEM2	-	-3.51	-	1.26	-1.43	-	-0.28	-0.85	-0.28	-0.64	
	dbMEM3	0.37	-0.17	-	1.68	-	-	0.91	2.80	-	3.54	
	dbMEM4	0.46	1.08	-	-	-	-0.46	-	0.95	0.80	-2.23	
	dbMEM5	-0.43	-	1.04	0.23	-1.09	-1.16	-	-1.29	0.17	-	
CV correlation		0.49	0.63	0.48	0.69	0.49	0.74	0.83	0.52	0.66	0.50	
CV correlation standard error		0.06	0.06	0.13	0.08	0.10	0.05	0.04	0.10	0.07	0.11	
% Variation explained (Phys+LU)		90.3	55.1	92.1	21.5	81.4	86	50.1	53.8	36.1	49.9	

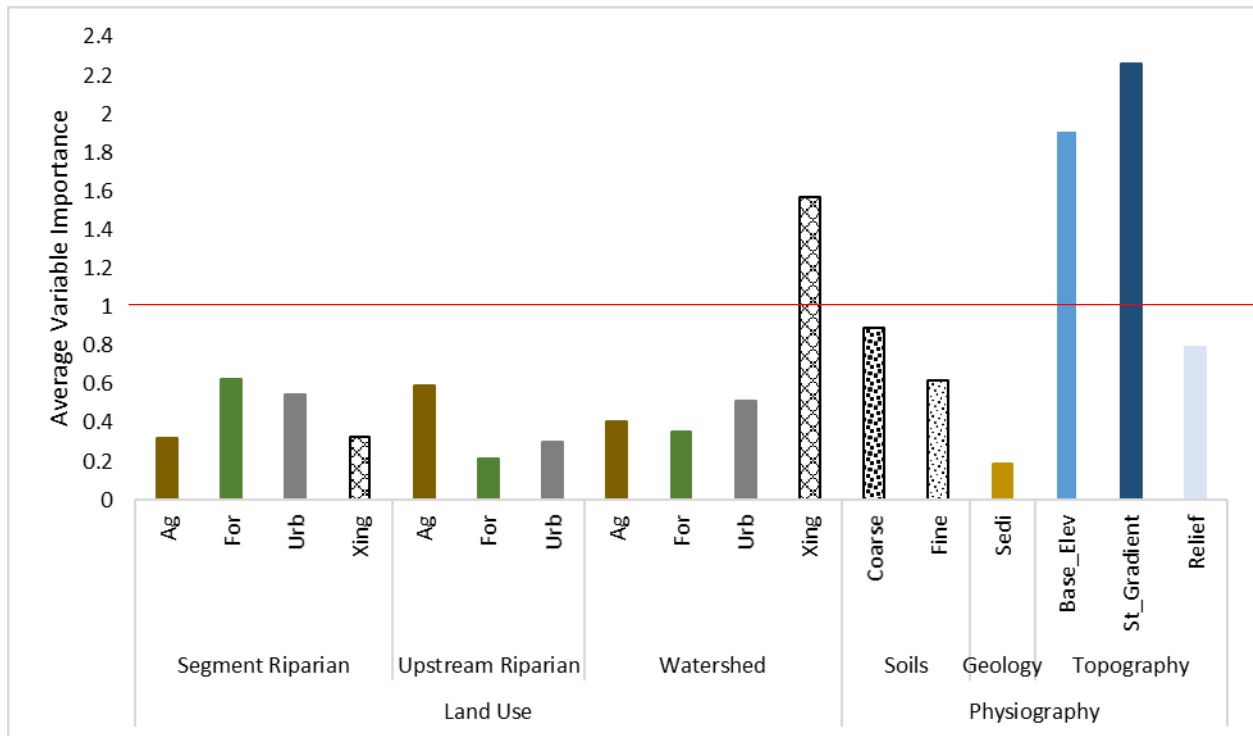


Figure 2.3: Mean BRT land-use and physiographic variable importance results for modeling abundance of 21 fish species within the New River basin (NRB). For each variable, average variable importance scores were only computed across those species for which the variable was selected by the LASSO procedure as an important predictor. The red horizontal line represents the threshold for outperformance. *Key – Ag = % Agriculture, For = % Forest, Urb = % Urban, Xing = number of road-stream crossings, Coarse = Coarse substrate score of upstream riparian area, Fine = Fine substrate score of contributing watershed, Sedi = Binary variable (1 if sedimentary bedrock is dominant across contributing watershed, 0 if otherwise), Base_Elev = Elevation at downstream confluence of sampled stream segment with another river or stream, St_Gradient = Stream Gradient, Relief = Watershed topographic relief.*

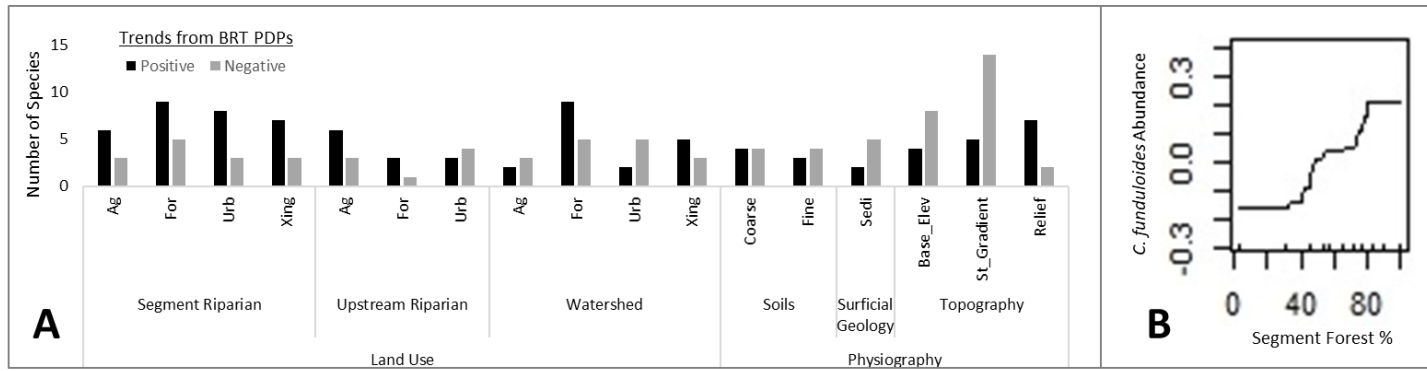


Figure 2.4: A – Counts of species displaying positive (black) and negative (gray) abundance trends in response to their corresponding LASSO-selected land-use and physiographic variables, derived from boosted regression tree (BRT) partial dependence plots (PDP) for each species-variable combination. *Key* – Ag = % Agriculture, For = % Forest, Urb = % Urban, Xing = number of road-stream crossings, Coarse = Coarse substrate score of upstream riparian area, Fine = Fine substrate score of contributing watershed, Sedi = Binary variable (1 if sedimentary bedrock is dominant across contributing watershed, 0 if otherwise), Base_Elev = Elevation at downstream confluence of sampled stream segment with another river or stream, St_Gradient = Stream gradient, Relief = Watershed topographic relief. B – Example PDP, showing a positive relationship between Rosyside Dace (*C. funduloides*) abundance and percent forest coverage within segment riparian areas. PDPs for 21 species were interpreted (positive or negative) and enumerated to create plot 4A.

CHAPTER 3: BIOTIC HOMOGENIZATION AND LONG-TERM SPECIES ABUNDANCE TRENDS IN FISH COMMUNITIES OF A HIGHLY INVADED WATERSHED

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Abstract

Aim: We tested for biotic homogenization and assessed the consequences of this phenomenon through analysis of resident fish population abundances at both basin-wide and site-level extents. Furthermore, we prioritized potential future conservation targets based on the presence of diverse and unique fish communities as often-overlooked components of regional biodiversity.

Location: Upper and middle New River (UMNR) basin, Appalachian Mountains, USA.

Methods: We compiled catch data from fish community surveys throughout the study area over 61 years (1958-2019) and used a novel fish abundance metric (scaled composite abundance scores; SCSAs) to describe spatiotemporal fish community trends. We used dispersion analyses to test for biotic homogenization over time across sites sampled repeatedly in the 1900s and 2000s and compared between these centuries. We then used non-metric multi-dimensional scaling (NMDS) to visualize potential trends in homogenization. In addition, we ranked all sites by community uniqueness (site contributions to beta diversity) and Shannon diversity to identify hotspots of these biodiversity measures across the basin, then used regression analyses and Wilcoxon signed-rank tests to examine species-specific basin-wide and local abundance trends to identify species of potential conservation concern.

Results: Dispersion of sites in species-abundance space was significantly greater in the 1900s compared to the 2000s, indicating homogenization had occurred. Of 36 native species analyzed, 44.4% (16) showed basin-wide declines. Non-native species results were mixed; site-level abundance increased in 2 of 15 non-natives analyzed (13%).

Main Conclusions: Our results indicate an overall trend of biotic homogenization within the UMNR, but also many persisting unique and diverse communities that, if preserved, could help maintain regional fish diversity. In addition, our study suggests basin-wide declines in four endemic species and spread patterns of some non-native and native species that may warrant increased study. We expect our methods to be broadly applicable to other regions for which similar historical fish abundance data exist, with results informing conservation strategies. Our study illustrates the value of long-term monitoring of fish communities, including abundance and occurrence patterns of resident species across entire regions, as a means of informing conservation planning.

Key words: freshwater fish conservation, invasion biology, native species declines, non-native species, community uniqueness

Introduction

Due to myriad human-induced threats, freshwater fishes are in decline worldwide (Moyle & Leidy, 1992). Freshwater fishes experienced the highest extinction rate of any vertebrate group in the 20th century (Burkhead, 2012) and new extinctions, such as that of Chinese Paddlefish (*Psephurus gladius*) (Zhang et al., 2020) continue to occur with alarming frequency. In North America, modern extinction rates of freshwater fishes are 877 to 1000 times the background extinction rate (Ricciardi & Rasmussen, 1999; Burkhead, 2012). Imperilment of North American freshwater fishes is also on the rise; 700 fish taxa (species, subspecies, and evolutionarily significant units [ESUs]) were listed as imperiled by the American Fisheries Society Endangered Species Committee in 2008, representing a 92% increase since 1989 (Williams et al., 1989; Jelks et al., 2008).

Broad drivers of native freshwater fish imperilment are well-established, with habitat degradation, pollution, stream-flow alteration, overexploitation, non-native species, hybridization, and climate change all playing roles (Miller et al., 1989; Allan & Flecker, 1993; Dudgeon et al., 2006; Helfman, 2007). Because many fish species are exposed to several threats at once and the effects of potential threats vary widely depending on species life history traits (Mouillot et al., 2013), it is often difficult to assess the relative influence of each factor on decline of individual species. However, the cumulative effects of these threats often manifest in ecological signals across biotic communities at the landscape level (Wilcove et al., 1998; Milardi et al., 2018).

One landscape-level process often associated with increases in anthropogenic threats is community homogenization, wherein regional disturbance patterns tend to favor populations of non-native and native generalist species over species intolerant of encroaching threats (Scott &

Helfman, 2001). Over time, communities across the landscape become dominated by common, generalist species and communities become less distinct from one another (Rahel, 2000; Olden & Poff, 2004). Homogenization thus leads to loss of biotic communities with uniquely evolved combinations of native species. These unique communities represent an important, yet often overlooked component of biodiversity (Angermeier & Winston, 1999).

Past conceptions of the term “biodiversity” have conflated natural diversity with non-native (artificial) diversity (Angermeier, 1994). While non-native species may contribute to larger regional species pools, they can be erosive to other components of diversity, ranging from the uniqueness of communities to the genetic integrity of local populations. So, while much past conservation effort has been narrowly focused on species-level components of diversity, overall biodiversity is a much broader, hierarchical conservation target that includes taxonomic, genetic, and ecological diversity components (Angermeier & Karr, 1994; Angermeier & Schlosser, 1995) and the physical, evolutionary, and ecological processes that preserve these components at multiple spatiotemporal scales (Noss, 1990).

While assessing all components of biodiversity across all hierarchical levels of organization (taxonomic, genetic, and ecological) is not tractable for a single study, addressing multiple components at multiple hierarchical levels can help prioritize conservation targets (Noss, 1990). We integrated local patterns in species abundance to summarize regional spatiotemporal trends in fish community uniqueness in an attempt to aid in identification of conservation targets at landscape, community, and population levels. Unique assemblages are the most likely to be lost and are thus more reliant on immediate conservation efforts (Angermeier & Schlosser, 1995). In addition, conserving unique communities has both economic and ecological advantages over single-species approaches (Williams et al., 1989; Angermeier & Winston, 1999;

Williams et al., 2011). Conservation based on community uniqueness, in contrast to the focus on individual species, may allow for easier prioritization of conservation targets and necessitates formulation of balanced conservation strategies that account for the conflicting needs of the many component species at once (Franklin, 1993; Angermeier & Schlosser, 1995). In addition, unique communities encompass communities that contain endangered species, species with utilitarian value, and/or species with unique ecological attributes, all of which may warrant consideration for conservation (Franklin, 1993; Angermeier & Winston, 1999).

To test the efficacy of a community uniqueness-based approach to targeting areas of conservation need, we chose to focus on the upper and middle New River basin (UMNR) in the Appalachian region of the eastern United States. The UMNR possesses four qualities that make it particularly amenable to such a study. First, this basin has been subject to much ecological exploration, resulting in a relative wealth of available fish community data, with fish species abundance records spanning much of the basin and historical abundance records going back to the 1950s. Second, the potential for spatial heterogeneity in community uniqueness is particularly high in the UMNR, as it contains at least eight species endemic to the New River basin writ large, resulting in the second-highest rate of endemism for any drainage in the eastern or central United States (Jenkins & Burkhead, 1994). Endemic species, especially those with poor dispersal abilities, are generally at high risk of extinction and often contribute disproportionately to community uniqueness (Burlakova et al., 2011). Third, the UMNR has an extensive history of changing and diverse anthropogenic threats, commonly implicated in declines of freshwater biodiversity. Although most flow diversion and major dam building in the UMNR were finished before the earliest of our fish abundance records, smaller dam projects have occurred over the study timeframe (NID, 2020). Agricultural and urban expansion has

occurred across the study area, contributing to chemical and sediment pollution gradients. Lastly, perhaps the most visible and ever-growing threat to native fishes of the UMNR comes from continued introductions of non-native species, which now outnumber native species basin-wide (Jenkins & Burkhead, 1994; Buckwalter et al., 2018).

At least two types of spatiotemporal data are needed to prioritize unique assemblages for conservation in the face of biotic homogenization: patterns of species occurrence and abundance. Buckwalter et al. (2018) documented the geographic expansion of non-native fishes in the UMNR and associated declines in range size of native species, focusing on basin-wide occurrence patterns of these two groups. However, broad-scale species occurrence trends alone are not descriptive of population-level processes, wherein species undergo within-site shifts in abundance in response to various stressors. Declining abundance of individuals within populations is a precursor to localized extirpations (Jackson & Harvey, 1995) that are essential for identifying range retractions. Thus, abundance trend analyses may allow quicker identification of declines in potentially conservation-relevant species.

We expand on Buckwalter et al.'s (2018) work by analyzing local and basin-wide trends in native and non-native fish abundance, thereby describing a related but distinct aspect of species spread and decline. Studies providing information on both geographic and population-level aspects of spread and decline of most resident species in a given drainage are relatively rare, despite the fact that both analyses may be especially important for understanding the conservation status of native species, overall changes in regional fish assemblages, and the potential for impacts of non-native species (Lyons et al., 1998; Senecal et al., 2015). During successful invasions, non-natives usually undergo predictable periods of establishment within the initial area of introduction, signified by increases in local abundance, followed by geographic

spread to nearby locations and research suggests that different characteristics of invaders may be favorable during each of these invasion phases (Marchetti et al., 2004). As such, abundance and occurrence analyses, when paired, provide greater insight into the progress of ongoing invasions from the establishment to the geographic spread phase.

Given the litany of threats and historic presence of unique species and communities within the UMNR, we expected to find evidence of landscape-level (hereby referred to as “basin-wide”) biotic homogenization. Thus, our study objectives were to 1. Track spatiotemporal fish community uniqueness and diversity trends across the UMNR to determine whether biotic homogenization has occurred, 2. Identify species-level abundance trends (spread and decline) that may have contributed to regional biotic homogenization over time, and 3. Suggest priorities for new potential conservation targets, given population trends of native and non-native fishes and the distribution of unique communities across the basin.

Methods

Study Area

The New River flows north through the approximately 7620-km² UMNR study area (Figure 3.1) from North Carolina through southwest Virginia to the West Virginia border. The West Virginia portion of the New River basin was not included in our study due to inaccessibility of long-term fish community records comparable to the rest of our dataset. While the West Virginia border is not a barrier to fish movement, Bluestone Dam, occurring approximately 37 river-kilometers north of the border, effectively isolates fish communities within the UMNR from most West Virginia aquatic communities.

Data acquisition and analysis

Fish community data were compiled from multiple sources, including public data clearinghouses, such as FishNet2 and MARIS, and databases from the Virginia Department of Game and Inland Fisheries (VDGIF), the Virginia Department of Environmental Quality (VADEQ), the North Carolina Wildlife Resources Commission (NCWRC), and the North Carolina Department of Environmental Quality (NCDEQ). Duplicate records, or those present in more than one of our compiled databases, were excluded. As our focus was to describe abundance trends in fish populations and community structure, only data containing catch totals of all species captured during sampling events were retained for our analysis. Other standards for data inclusion in our study (including field collection methodology, taxonomic considerations, spatial coordinate accuracy, total fish collected, etc.) are detailed in Appendix A.

Fish community data were categorized by year of collection, inter-confluence stream segment (determined from proximity of spatial coordinates of each sample to nearest National Hydrography Dataset [NHDPlus; USEPA, 2019], version 2 flowline), stream order, and 10-digit Hydrologic Unit Codes (HUC10s), representing standardized drainage-defined subdivisions of the UMNR (USGS, 2013). Data from sampling events that occurred on the same day within the same stream segment (hereafter a “site”) were summed to represent a single sample and sampling events occurring within the same segment in the same year were averaged and treated as a single event.

Because sampling techniques and sampled areas were quite variable across fish survey events retained for our study, ranging from multi-pass backpack electrofishing surveys to simple seining, a composite abundance metric was utilized to minimize discrepancies in sampling intensity and efficiency across samples. Composite species abundance scores (CSAs) within each sample were computed as the sum of three abundance scores: a scaled total-catch score

(*TC*) computed as raw catch (*C*) of individuals of a species (*i*) at one site (*j*) over the maximum catch for that species across all sites and sampling events (equation 1); a scaled rank-abundance score (*RA*) where the most abundant species at a site received a 1 and all others were assigned smaller values proportionate to their abundance ranks (*R*) in the sample in relation to the total species richness (*SR*) or total number of species captured (equation 2); and a proportional abundance (*PA*) score calculated as a species' raw catch within a site divided by the total number of individuals captured at that site (equation 3). One example of the efficacy of such an approach could be the comparison of the abundance of a locally rare species (species R) within 2 samples, one involving the sampling of a 100-meter section of a small, second order stream (sample A) and one of a 200-meter section of a much larger, fourth order stream (sample B). Imagine that 10 individuals of species R were recorded within each survey, but that due to vastly different sample areas, 100 total fish were captured in sample A versus 1000 in sample B. While the *TC* metric alone would identify the sites as having equal abundances of species R, the *RA* and *PA* metrics allow consideration of numeric dominance of species R in comparison to other species within each sample and its abundance as a function of overall fish abundance within each sample, resulting in a higher abundance score in sample A, where species R was more densely populated.

$$(1) TC_{ij} = \frac{C_{ij}}{C_{i,jmax}}$$

$$(2) RA_{ij} = \frac{(SR_j) - [(R_{i,j}) - 1]}{SR_j}$$

$$(3) PA_{ij} = \frac{C_{ij}}{T_j}$$

Species-specific *CSAs* were thus assigned at each site as the sum of *TC*, *RA*, and *PA* (equation 4) with a maximum theoretic value of 3 if the species was the only fish present in the sample and minimum of 0 if the species was absent from the sample. Because single-species

samples were excluded from analysis due to concern that these records may represent targeted sampling instead of the holistic community-level data required for our study, the maximum value of 3 was never reached for any species-site combination. Finally, to make CSA values directly comparable across species despite large variations in abundance patterns, CSAs were scaled (SCSAs) between 1 and 0 to yield the final response variable, based on the maximum CSA value for each species (equation 5). This final scaling procedure was necessary because species that tend to be more common on the site level have naturally higher *RA* and *PA* scores than species that tend to be locally rare when present, increasing local maxima in common species CSAs unattainable by most uncommon species, even at the sites where they were most abundant. By scaling the CSAs, we eliminated these discrepancies in local maxima and allowed the distribution patterns of each species to have equal weight in the analysis.

$$(4) \text{CSA}_{i,j} = [TC_i + RA_i + PA_i]_j$$

$$(5) \text{SCSA}_{i,j} = \frac{\text{CSA}_{i,j}}{\text{CSA}_{i,j\max}}$$

To test for biotic homogenization across the UMNRR, we first identified sites that had been sampled during two time periods (1:1958-1999 and 2:2000-2019). We then ordinated these samples in SCSA space with Non-Metric Multi-Dimensional Scaling (NMDS) (Kruskal, 1964), using the *vegan* package in program R (Dixon, 2003). We compared dispersion of point clouds between time periods using function *betadisper* (Anderson, 2006), where a significant retraction in point cloud dispersion from time period 1 to 2 represented more restrictive community membership in the later time period, indicating biotic homogenization. Dispersion was compared between 1900s and 2000s fish community samples within sites that were sampled at least once in both time periods. Dispersion comparisons were conducted across all of these repeatedly sampled sites with and without non-native species included in the analysis and between sub-

samples representing sites of similar stream size (stream order) to determine the potential effects of non-native species and stream size on identification of biotic homogenization trends.

To identify native species as potential conservation targets, we conducted species-specific abundance trend analyses at both the basin-wide and site levels of organization. For basin-wide analyses, we regressed all non-zero species SCSAs (y) (representing all known occurrences of the species within the retained dataset) against year of survey (x), fitting null, linear, and exponential response curves (equations 6-8) as candidate models of spread and/or decline, where β_0 = y -intercept or scale and β_1 = slope or growth rate. We identified the best-fitting models using the second-order Akaike's information criterion (AICc) (Mazerolle, 2006). Species for which either the exponential or the linear models best fit the data were considered those exhibiting directional trends in abundance over the survey time period. We classified these directional trends for each species based on the direction of the slope or rate term in the chosen model, where species with a positive slope or rate term were classified as basin-wide abundance "spreaders" and those with negative slope or rate terms as "decliners". Species for which the null model performed best were considered "stable" across the UMNR. Basin-wide abundance trends were analyzed for all species that occurred within at least 10 samples within our dataset.

(6) Intercept-only (null) model: $y = \beta_0$

(7) Linear model: $y = \beta_0 + \beta_1x$

(8) Exponential model: $y = \beta_0e^{\beta_1x}$

In addition to basin-wide abundance trends, we examined site-level species abundance trends within sites with repeat sample histories including at least one sampling event per centennial time period (1900s and 2000s) by comparing mean SCSA values between time periods for species occurring within at least five repeatedly sampled sites. Non-parametric, paired

Wilcoxon signed-rank tests were used to identify significant differences ($\alpha = 0.05$) in site-level mean abundance between time periods as species SCSAs were not necessarily normally distributed. Species that displayed significantly higher mean abundance in the 2000s were considered site-level “spreaders” and those more abundant in the 1900s, “decliners”. Species displaying non-significant differences in mean SCSAs between time periods were considered stable. Non-native species trends were also examined at both levels of organization to quantify spread and infer potential threat level.

To pinpoint locations worthy of conservation consideration, we identified sampled sites that contributed most to basin-wide uniqueness over time within the UMNR. We used a beta diversity partitioning approach to measure fish community uniqueness based on a matrix of species abundances (SCSAs) within sites. Beta diversity is a measure of species turnover across sites and beta diversity partitioning allows the calculation of local contributions (LCBDs) to regional species diversity, calculated as the site-level variance in community composition (Legendre et al., 2013).

Because of the nature of the SCSA metric, within-site abundance of common species tended to vary more around their mean basin-wide SCSA values than did abundances of rare species with mean basin-wide SCSAs near zero. Since the calculation of LCBDs relied on comparison of site-level SCSAs with mean basin-wide SCSAs in this case, these metrics were useful for identifying unique communities, where the abundance of various common species differed greatly from their basin-wide mean, but tended to underemphasize rarer species which often occurred at site-level SCSA abundances near basin-wide mean values. Therefore, in addition to analyzing LCBD patterns across the UMNR, we also examined site-level alpha diversity (Shannon-Wiener Diversity Index [hereafter, Shannon]), based on SCSAs, to quantify

diversity of native communities in isolation from others across the landscape and decouple common and rare species from their mean basin-wide SCSA values. To identify locations containing both locally diverse (high Shannon score) and highly unique (high LCBD) fish communities, we also created a composite metric of uniqueness and diversity (U-D composite; equation 9) as the summation of scaled LCBDs and scaled Shannon scores for each fish community sample. LCBDs, Shannon scores, and U-D scores were then ranked across samples and averaged across HUC10 drainages and mapped across the UMNR study area to determine what areas might be important for future biodiversity conservation. Non-native species were excluded from all diversity calculations (LCBDs, Shannon scores, and U-D scores), but cumulative site-level and average HUC10-level non-native species SCSAs were also mapped across the basin to identify areas relatively un-impacted by introductions, another potential criterion for prioritization of conservation targets.

$$(9) U-D_j = \frac{LCBD_j}{LCBD_{jmax}} + \frac{Shannon_j}{Shannon_{jmax}}$$

Results

The final database included 638 unique fish community samples from 357 inter-confluence stream segments (sites) across the UMNR. Of these sites, 145 were sampled repeatedly (2 to 8 times) during the survey timeframe and 56 were sampled during both the 1900s and the 2000s. Repeat sampled sites were dominated by small to medium-size streams (Strahler stream orders 1-4) and were widely spread across the UMNR. Analysis of variance (ANOVA) tests of multivariate dispersion were conducted to compare mean dispersion of fish samples in species SCSA space between the 1900s and 2000s. Results of these dispersion tests (Table 3.1) and inspection of ordination plots (Figure 3.2) indicated significant overall reductions in community uniqueness across sites in SCSA space between the 1900s and the 2000s. These

reductions indicated more restrictive fish community membership in modern samples, providing evidence for community homogenization across the UMNR. Evidence for homogenization was present in all comparisons of native assemblages between the 1900s and 2000s, regardless of stream size. The inclusion of non-native species in the data affected detection of a significant homogenization trend only in small streams (1st and 2nd order).

Given our restrictive inclusion criteria, we were able to model species-specific basin-wide abundance trends for 64 taxa (36 natives; 28 non-natives) and site-level abundance trends for a subset of these taxa (42: 27 natives; 15 non-natives). Of the 42 taxa modeled at both extents, 47.6% (20: 8 natives; 12 non-natives) tested as stable at both basin-wide and site-level extents (Tables 3.2 and 3.3), with only two of these species displaying even marginal patterns ($0.1 > p > 0.05$) of spread or decline at the site-level extent. Significant directional abundance patterns (spread or decline) were detected much more commonly at the basin-wide extent (46.9%) than at the site level (16.7%). Of the significant trends at the basin-wide extent, 17 best fit a linear model of spread or decline, while the exponential model best fit 13 others. We observed basin-wide declines ($p < 0.05$) for 16 native species (44.4%), including four of five endemic species analyzed (Candy Darter [*Etheostoma osburni*], New River Shiner [*Notropis scabriceps*], Appalachia Darter [*Percina gymnocephala*], and Kanawha Minnow [*Phenacobius teretulus*]). Basin-wide abundance spread was identified in six native species, representing mostly high-prevalence species across sites.

Non-native species displayed a wide range of spread and decline patterns at basin-wide and site levels, including some seemingly contradictory patterns. Eight non-native species displayed significant basin-wide directional trends (four spreaders, four decliners), while two displayed significant trends of numerical within-site spread (Redbreast Sunfish [*Lepomis auritus*]

and Warpaint Shiner [*Luxilus coccogenis*]). Notably, Redbreast Sunfish displayed a contradictory trend at the basin-wide extent, where this species was classified as a decliner.

To prioritize sites for potential conservation action across the UMNR, we separately mapped sites with communities ranked in the top 10 percent (63 total) of LCBD (uniqueness) scores, Shannon (diversity) scores, and sites within the top 10 percent of both metrics (Figure 3.3A, B, and C). We also mapped sites within the bottom 10 percent of cumulative SCSA abundance of non-native species (Figure 3.3D). LCBD, Shannon, and U-D rankings, along with cumulative non-native SCSA scores, were also averaged across HUC10 drainages for a broader view of each of these conservation-relevant criteria across the basin.

Of the 638 samples included in the analysis, only 180 were taken during the 1900s (28.2%). However, 1900s sites were disproportionately represented within the top 10 percent of LCBD rankings (63.5%) and in sites ranking within the top 10 percent of both measures of uniqueness and diversity (52.9%). The HUC10 drainages with the highest average uniqueness and diversity rankings tended to cluster in the northeastern portion of the basin, specifically in the Little River and Reed Island Creek drainages. In addition, these drainages both contained several sites where few non-native species occur, even in samples collected since 2000, and had low average non-native species SCSA scores in comparison to most other HUC10 drainages. A pair of recently sampled sites in the North Fork New River drainage also stand out as having potential conservation value in the southern portion of the UMNR, ranking high in uniqueness and diversity but low in non-native species abundance.

Discussion

Overall, results suggested widespread biotic homogenization of UMNR fish communities over the past 60+ years. Coincident with this trend were basin-wide declining abundances of 16

native fishes, including four endemic species, and 10 instances of basin-wide abundance spread, mostly in non-native species and in common, generalist natives. Locations that appear to have persisting unique and diverse communities, relatively un-impacted by non-native species, include the Little River, Big Reed Island Creek, and North Fork New River drainages. These drainages, perhaps important for future conservation efforts, will be henceforth referred to as “hotspot drainages”.

Whereas biotic homogenization is a widely recognized form of biodiversity loss in freshwater fish communities, past studies have generally taken two paths to describe these trends: 1. Compiling historical occurrence data for fishes across very broad (continental and global) areas (Rahel, 2000; Villéger et al., 2011) and 2. Collecting contemporary data on fish communities to identify regional changes in species richness, proportional abundance or occurrence of resident species in response to known stressors (dams, land use, etc.) (Scott & Helfman, 2001; Clavero & Hermoso, 2011). The first method suffers from a lack of data on individual populations and limited application to conservation problems at scales relevant to regionally-focused conservation managers. The second suffers from lack of historical information key for quantifying the extent and rate of regional species spread and decline that are contributing to homogenization. Our methods allowed for an analysis of biotic homogenization that was conducted at a scale relevant to regional managers, allowed assessment of the state of current fish populations and communities, and provided historical reference points for these populations and communities, key for quantifying the severity of biodiversity losses over time.

Past studies within the UMNRR have emphasized declines of a few resident species (Buckwalter et al., 2018; Dunn & Angermeier, 2019), but none have indicated the mass community- and species-level losses in fish biodiversity suggested by the current study. This

new insight stems from our innovative analytical approach focused on species abundance rather than species occurrence as the measure of spread and decline. Buckwalter et al. (2018) similarly classified native and non-native species as spreaders and decliners (as a function of occurrence-based range size within HUC12 drainages), but many of their results differ from ours. Comparing basin-wide models of fish distribution between our study and Buckwalter et al's (2018), we found 57.1% agreement with our species classified as spreaders, but only 10.5% agreement in decliners. Only two native species were classified as decliners in both studies (Rosyface Shiner [*Notropis rubellus*] and Sharpnose Darter [*Percina oxyrhynchus*]).

These results illustrate the importance of considering multiple aspects of the distributional dynamics of species. Our abundance-based analyses are more sensitive to trends in well-established populations, including declining abundance of native species, but may be less reliable indicators of the status of on-going non-native invasions. Because our basin-wide abundance trends are based on abundance comparisons across all occupied sites, for rapidly spreading non-natives, this includes sites that have only recently been colonized, which can lead to an erroneous signal of basin-wide decline. This phenomenon is best exemplified by the contradictory patterns of local abundance increase and basin-wide decline suggested for non-native Redbreast Sunfish in our study. Buckwalter et al's (2018) occurrence-based study identified Redbreast Sunfish as a strong geographic spreader and this provides important perspective when interpreting our results. In fact, Redbreast Sunfish is likely spreading so fast as to outpace our ability to track this species adequately with abundance analyses alone. Thus, integration of abundance and occurrence methods are needed to adequately summarize distribution trends of all types of conservation-relevant species. These species include declining

natives, spreading non-natives, and expanding native generalist populations that may signal degradation in habitat or water quality.

Examples of each of these three groups of species are present within the UMNR. Endemic species, such as the New River Shiner, are inherently more vulnerable to extinction than other species due to their naturally restricted ranges and their evolution within unique and often remote habitats. While past range size analyses have identified no prevailing trend in distribution patterns of New River Shiner (Buckwalter et al., 2018), we found a significant negative trend in basin-wide abundance of this species across the UMNR (Figure 3.4B). Non-native Warpaint Shiner (Figure 3.4D) and Redbreast Sunfish both exhibited site-level abundance increases in our analysis and occurrence analyses indicate modest to severe increases in range size for these species (Buckwalter et al., 2018) that may be precursors of widespread ecological impacts. Basin-wide native spreaders identified by the current analysis include Central Stoneroller (*Campostoma anomalum*) (Figure 3.4A), whose distribution may be limited by riparian canopy that limits sunlight penetration and thus the algal crops on which this species feeds (Matthews et al., 1987). As such, Central Stoneroller increases may indicate widespread loss of riparian forest, including altered stream temperatures and food webs. However, the actual causes of most species' abundance trends remain unknown and may warrant future research to inform potential management actions.

In addition to species-level trends, our study also revealed several unique and diverse communities that may warrant conservation. While sampling during the 1900s suggested more uniqueness and diversity existed in UNMR fish communities of the past, several highly unique and diverse communities have persisted into the 2000s. Present-day uniqueness and diversity of fish communities is perhaps best preserved within those drainages we have identified as potential

conservation hotspots. Six native species could be considered highest priority conservation targets in the UMNR given declining trends identified by our study and other potential risk factors, such as endemism (Candy Darter, New River Shiner, Appalachia Darter, and Kanawha Minnow) and concordant declines in UMNR range size (Rosyface Shiner and Sharpnose Darter; Buckwalter et al., 2018). The previously-named hotspot drainages each contain populations of at least four of these six species, which have persisted into the current century.

Recognizing hotspot drainages may be a first step toward developing effective basin-wide conservation plans. In the UMNR, only one (Candy Darter) of the six high-priority species mentioned above does not occur within any hotspot drainage. However, because the Candy Darter was recently listed as federally Endangered, it already is the subject of many conservation actions, ranging from captive propagation to prohibitions on using live bait and stocking non-native species within the waters it occupies (USFWS, 2018). In contrast, the other five high-priority species appear sporadically on current state lists of species of greatest conservation need (SGCN) and, in some cases, lack clearly articulated conservation actions or research needs (NCWRC, 2015; VDGIF, 2015).

More deliberate conservation planning, aimed at hotspot drainages rather than at individual species, may help fill gaps in the conservation of SGCN-listed and still-unlisted fishes in the UMNR. More focus on ecosystem and community-level conservation has been suggested by many researchers in the past (Rohlf, 1991; Angermeier & Schlosser, 1995; Angermeier & Winston, 1999; Collares-Pereira & Cowx, 2004), yet application of community conservation approaches is hampered by a number of factors. Perhaps the most influential factor is the fact that, unlike species, community conservation has no direct legal mandate in the United States. As such, species-specific approaches, mandated under the Endangered Species Act, currently rule

most fish conservation in the United States. In the absence of legal mandates, recommendations for community-level conservation typically need to be based on the underlying species-level trends driving erosion of unique and diverse communities, as well as knowledge of how managing ecosystem-level processes can mitigate species declines (Collares-Pereira & Cowx, 2004; Burlakova et al., 2011).

The state agencies that preside over conservation of fishes in the UMNR already apply some ecosystem-level strategies that could be effectual within hotspot drainages, such as riparian revegetation to reverse potential effects of intensive land-use practices on adjacent stream communities, ranging from increased sediment loading to altered stream temperatures, and live-bait restrictions that may limit the spread of non-native species (NCWRC, 2015; VDGIF, 2015). In addition, partnering with stakeholders within hotspot drainages to establish freshwater protected areas (FPAs) could benefit many SGCN species at once, as the establishment of FPAs in other regions has enhanced rehabilitation of declining species (Suski & Cooke, 2007). Elsewhere, the application of methods similar to ours could enhance the ability of conservation managers to understand both emerging conservation needs of fishes within their jurisdictions and the best locations to address these needs.

Transferability of our methods to other drainages should be possible wherever similar caches of historical fish community data are available. Past research suggests that many eastern and mid-western states share similar densities of historic fish sampling records at appropriate spatial resolutions (Frimpong et al., 2016). Each state's historical data likely shares obstacles to use similar to those we faced, including many disparate procedures for sampling fish communities and recording data.

Although sampling efficiency and intensity may vary among common methods (e.g. single- versus multi-pass electrofishing), sampling gears (e.g. traditional seines, electric seines, boat and barge electrofishers), and capture modes (e.g. AC versus DC electrical current), past research suggests similar relative capture rates among common fish families across methods. Angermeier et al. (1991), using an AC-current electric seine, reported consistently higher sampling efficiency across all common fish families than Mahon (1980) reported from the use of a pulsed DC-current method. However, both studies saw variation in efficiency across a range of taxa, wherein percids were least efficiently sampled and catostomids were most efficiently sampled. Similarly, Texeira-de Mello et al. (2014) documented consistent proportional abundances of species making up samples, despite increases in total fishes captured when using multi- versus single-pass backpack electrofishing. Thus, assuming similar representativeness of fish communities across methods as suggested by these studies, our usage of novel SCSAs allowed for reduction of inherent differences in sampling efficiency and intensity by modifying count abundance estimates using community proportion and abundance ranks. As such, SCSAs offer future researchers a relatively easily calculated and promising supplement to simple occurrence-based analyses for examining shifts in species distributions, which are more sensitive to early signatures of species declines.

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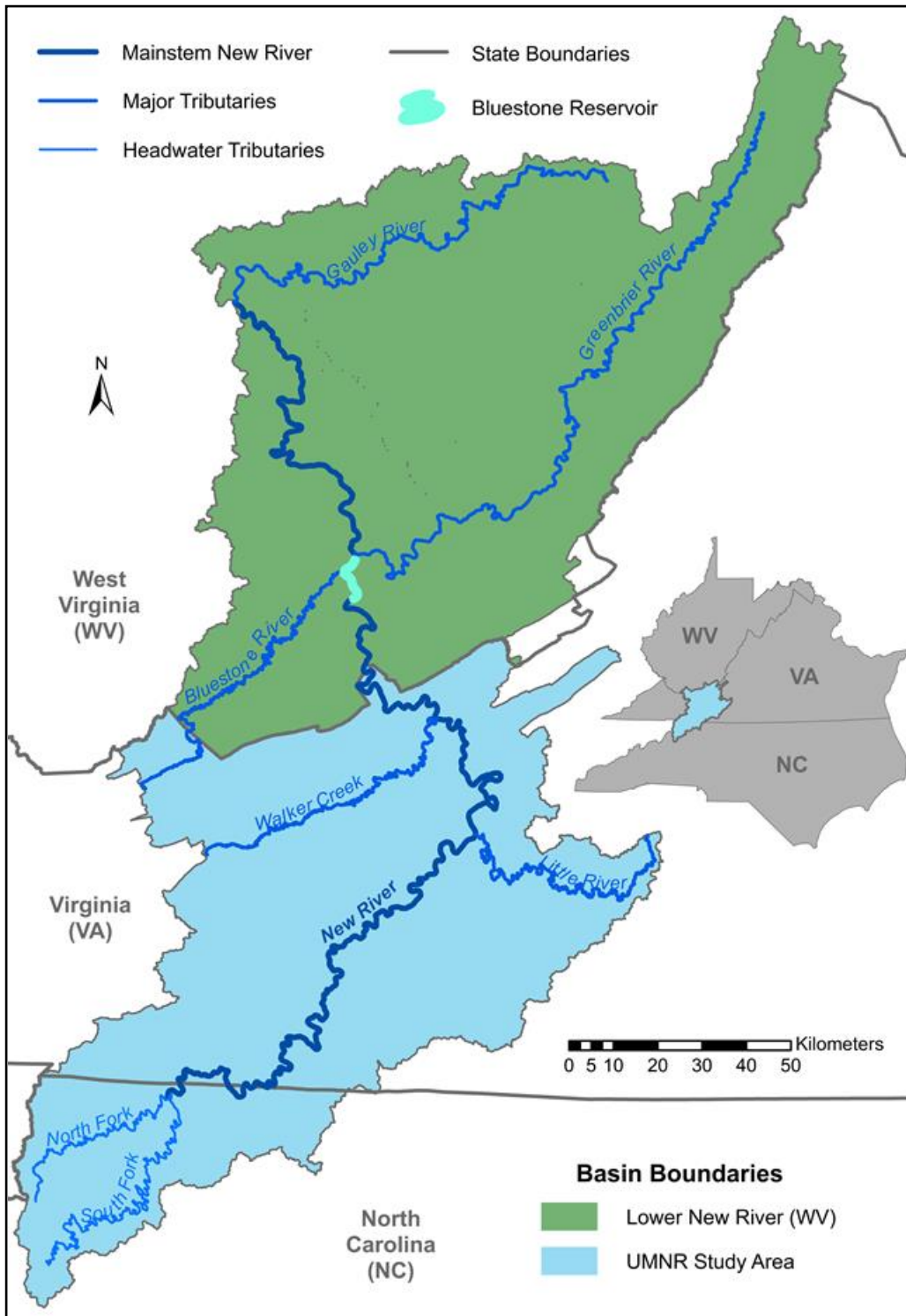


Figure 3.1: Major landmarks within and nearby the upper and middle New River basin (blue). Fish collections from the lower New River basin (green) were not included in our analysis.

Table 3.1: Results from analysis of variance tests of multivariate dispersion among fish communities between the 1900s and 2000s across repeatedly sampled sites in the upper and middle New River basin. Tests were applied first across all sites sampled during both centuries and then within sub-samples of these sites representing streams of different sizes, determined by Strahler stream order (Strahler 1957). Significant p-values ($p < 0.05$) indicate significant differences between mean distances to point-cloud centroids between 1900s and 2000s samples.

	Number of sites	Mean distance to centroid		p-value
		1900s	2000s	
<u>Natives only</u>				
All sites	56	0.475	0.376	0.000
Strahler 1 and 2	16	0.386	0.315	0.022
Strahler 3 and 4	35	0.478	0.377	0.000
Strahler 5 and 6	5	0.490	0.380	0.049
<u>All species</u>				
All sites	56	0.502	0.426	0.000
Strahler 1 and 2	16	0.432	0.386	0.096
Strahler 3 and 4	35	0.505	0.420	0.000
Strahler 5 and 6	5	0.485	0.373	0.028

Significant p-values in bold

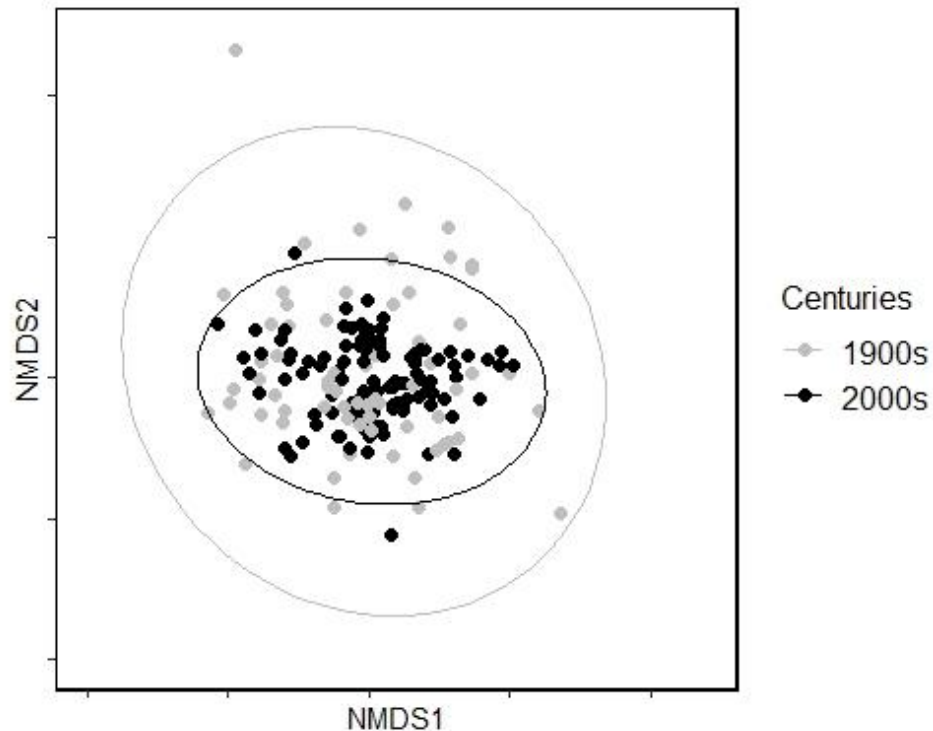


Figure 3.2: Non-metric multidimensional scaling (NMDS) ordination showing fish samples from repeatedly surveyed sites in scaled composite species abundance (SCSA) space. Ellipses represent 95% confidence limits around the centroid of the abundance data for 1900s samples (gray) and 2000s samples (black).

Table 3.2: Abundance trends for native fishes of the upper and middle New River basin, quantified at the basin-wide extent (with all non-zero scaled composite species abundance scores [SCSAs] for each species included within regression analyses) and the site-level extent (including only SCSAs from repeat sample sites where each species occurred). Sample size (n) is the number of occurrences of each species within samples included in the basin-wide analysis (638 total samples) and the number of occurrences within sites repeatedly sampled in the 1900s and 2000s (56 total sites) for the site-level analysis. Blank values for sample size under site-level trends indicate that trends were not investigated because of limited sample size ($n < 5$). Significant basin-wide trends were suggested by better fit of either linear (lin) or exponential models (exp) compared to the intercept-only (null) model in regression analyses, where positive slope or rate indicated spread and negative slope or rate indicated decline. Significant trends in site-level abundance were determined via paired Wilcoxon test p-values ($\alpha = 0.05$), comparing species SCSAs between 1900s and 2000s samples. Black arrows indicate significant spread (\uparrow) and decline (\downarrow). Site-level trends suggested by marginal p-values ($0.05 < p < 0.10$) are also shown (gray arrows).

Native Species	n	Basin-wide trends (Regression results)		Site-level trends (Paired Wilcoxon results)	
		Best model	Trend	n	Trend
<i>Campostoma anomalum</i>	576	lin	\uparrow	52	\uparrow
<i>Catostomus commersonii</i>	353	lin	\downarrow	42	
<i>Chrosomus oreae</i>	334	lin	\uparrow	31	
<i>Clinostomus funduloides</i>	388	null		39	
<i>Cottus sp.</i>	424	lin	\uparrow	45	\uparrow
<i>Cyprinella spiloptera</i>	63	lin	\downarrow	6	\downarrow
<i>Etheostoma blennioides</i>	191	lin	\downarrow	26	
<i>Etheostoma caeruleum</i>	15	exp	\uparrow		
<i>Etheostoma flabellare</i>	561	lin	\uparrow	53	\uparrow
<i>Etheostoma kanawhae</i> ^x	223	null		25	
<i>Etheostoma nigrum</i>	24	null			
<i>Etheostoma osburni</i> ^x	15	lin	\downarrow		
<i>Etheostoma simoterum</i>	19	null			
<i>Exoglossum laurae</i>	102	null		21	
<i>Hypentelium nigricans</i>	470	exp	\downarrow	52	
<i>Ictalurus punctatus</i>	12	null			
<i>Lepomis cyanellus</i>	122	null		20	\uparrow
<i>Luxilus albeolus/cornutus</i>	234	null		27	\downarrow
<i>Luxilus chrysocephalus</i>	13	null			
<i>Lythrurus ardens</i>	119	exp	\downarrow	12	
<i>Nocomis sp.</i>	551	null		53	\uparrow
<i>Notropis rubellus/micropteryx</i>	167	exp	\downarrow	21	
<i>Notropis photogenis</i>	112	null		16	
<i>Notropis scabriceps</i> ^x	148	exp	\downarrow	20	
<i>Notropis volucellus</i>	68	exp	\downarrow	8	
<i>Noturus insignis</i>	190	null		21	
<i>Percina caprodes</i>	14	lin	\uparrow		
<i>Percina gymnocephala</i> ^x	133	lin	\downarrow	21	
<i>Percina oxyrhynchus</i>	28	lin	\downarrow		
<i>Phenacobius teretulus</i> ^x	167	lin	\downarrow	22	
<i>Pimephales notatus</i>	182	exp	\downarrow	25	
<i>Pylodictis olivaris</i>	30	exp	\downarrow		
<i>Rhinichthys cataractae</i>	300	null		42	
<i>Rhinichthys atratulus/obtusum</i>	394	lin	\downarrow	42	
<i>Salvelinus fontinalis</i>	54	lin	\downarrow	9	
<i>Semotilus atromaculatus</i>	207	null		21	

\uparrow - Spread, \downarrow - Decline, \uparrow - Marginal spread, \downarrow - Marginal decline

^x - Endemic species

Table 3.3: Abundance trends for non-native fishes of the upper and middle New River basin, quantified at the basin-wide extent (with all non-zero scaled composite species abundance scores [SCSAs] for each species included within regression analyses) and the site-level extent (including only SCSAs from repeat sample sites where each species occurred). Sample size (n) is equal to the number of occurrences of each species within samples included in the basin-wide analysis (638 total samples) and the number of occurrences within sites repeatedly sampled in the 1900s and 2000s (56 total sites) for the site-level analysis. Blank values for sample size under site-level trends indicate that trends were not investigated because of limited sample size ($n < 5$). Significant basin-wide trends were suggested by better fit of either linear (lin) or exponential models (exp) compared to the intercept-only (null) model in regression analyses, where positive slope or rate indicated spread and negative slope or rate indicated decline. Significant trends in site-level abundance were determined via paired Wilcoxon test p-values ($\alpha = 0.05$), comparing species SCSAs between 1900s and 2000s samples. Black arrows indicate significant spread (\uparrow) and decline (\downarrow). Site-level trends suggested by marginal p-values ($0.05 < p < 0.10$) are also shown (gray arrows).

Non-native Species	n	Basin-wide trends (Regression results)		Site-level trends (Paired Wilcoxon results)	
		Best model	Trend	n	Trend
<i>Ambloplites rupestris</i>	421	null		47	
<i>Ameiurus natalis</i>	12	null			
<i>Ameiurus nebulosus</i>	15	null			
<i>Cyprinella galactura</i>	89	null		13	
<i>Cyprinus carpio</i>	10	exp	\uparrow		
<i>Etheostoma olmstedi</i>	26	null			
<i>Etheostoma rufilineatum</i>	10	exp	\uparrow		
<i>Exoglossum maxillingua</i>	32	null			
<i>Hybopsis hypsinotus</i>	10	exp	\uparrow		
<i>Lepomis auritus</i>	233	lin	\downarrow	26	\uparrow
<i>Lepomis gibbosus</i>	40	null		6	\downarrow
<i>Lepomis macrochirus</i>	131	null		18	
<i>Luxilus cerasinus</i>	140	null		16	
<i>Luxilus coccogenis</i>	74	null		13	\uparrow
<i>Micropterus dolomieu</i>	260	lin	\downarrow	33	
<i>Micropterus punctulatus</i>	28	lin	\downarrow		
<i>Micropterus salmoides</i>	54	null		10	
<i>Moxostoma cervinum</i>	26	null			
<i>Notropis chiliticus</i>	50	null		8	
<i>Notropis hudsonius</i>	41	null		7	
<i>Notropis leuciodus</i>	15	null			
<i>Notropis procne</i>	13	null			
<i>Notropis rubricroceus</i>	152	null		17	
<i>Notropis telescopus</i>	116	null		18	\uparrow
<i>Oncorhynchus mykiss</i>	147	null		26	
<i>Percina roanoka</i>	32	exp	\downarrow		
<i>Pimephales promelas</i>	19	exp	\uparrow		
<i>Salmo trutta</i>	199	null		20	

\uparrow - Spread, \downarrow - Decline, \uparrow - Marginal spread, \downarrow - Marginal decline

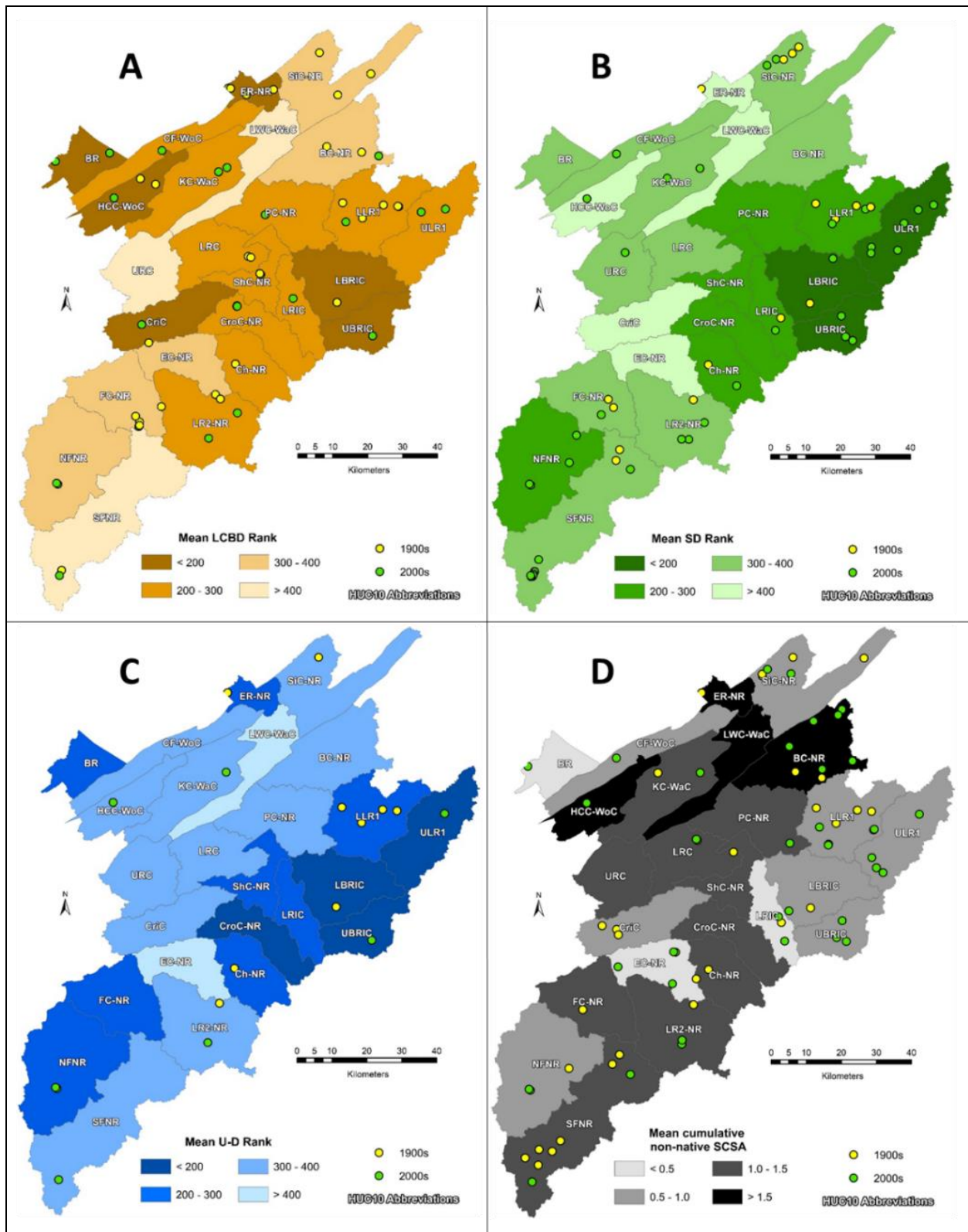


Figure 3.3: Distribution of unique and diverse native fish communities across the upper and middle New River basin (UMNR), as shown by local contributions to regional beta diversity (LCBDs) (A), Shannon diversity index (B), and a composite metric combining both Shannon scores and LCBDs (U-D rank) (C).

Average rank is shown for each of these metrics across 10-digit hydrologic unit code (HUC10) drainages of the UMNR, where lower ranks indicate higher average community uniqueness and diversity. Cumulative average non-native scaled composite abundance scores (SCSAs) are also shown across the same HUC10 drainages (D), where larger values indicate increases in average non-native abundance. Yellow (1900s) and green (2000s) dots represent sites that rank high in terms of potential conservation value (upper 10% of all sites) inferred by each of these four criteria. HUC10 Abbreviation Key: BC-NR = Back Creek-New River, BR = Bluestone River, Ch-NR = Chestnut Creek-New River, CF-WoC = Clear Fork-Wolf Creek, CriC = Cripple Creek, CroC-NR = Crooked Creek-New River, ER-NR = East River-New River, EC-NR = Elk Creek-New River, FC-NR = Fox Creek-New River, HCC-WoC = Hunting Camp Creek-Wolf Creek, KC-WaC = Kimberling Creek-Walker Creek, LRIC = Little Reed Island Creek, LR2-NR = Little River-New River, LWC-WaC = Little Walker Creek-Walker Creek, LBRIC = Lower Big Reed Island Creek, LLR1 = Lower Little River, LRC = Lower Reed Creek, NFNR = North Fork New River, PC-NR = Peak Creek-New River, ShC-NR = Shorts Creek-New River, SiC-NR = Sinking Creek-New River, SFNR = South Fork New River, UBRIC = Upper Big Reed Island Creek, ULR1 = Upper Little River, URC = Upper Reed Creek.

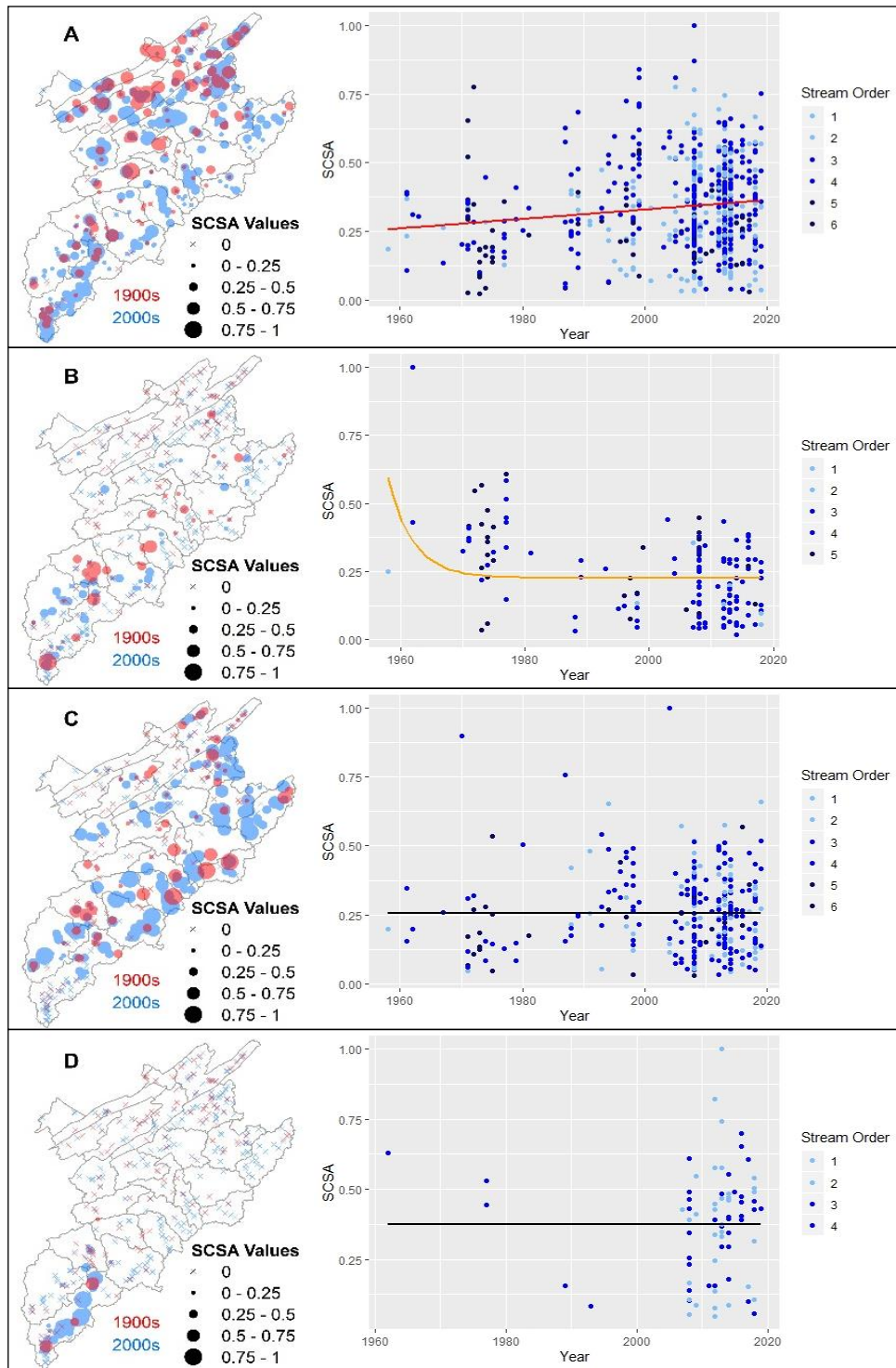


Figure 3.4: Basin-wide spatiotemporal abundance trends, based on scaled composite species abundance scores (SCSAs), for four fish species in the upper and middle New River basin - A. *Campostoma anomalum* (Central Stoneroller): Native spreader, B. *Notropis scabriceps* (New River Shiner): Native decliner (basin-wide only), C. *Rhinichthys cataractae* (Longnose Dace): Native stable, D. *Luxilus coccogenis* (Warpaint Shiner): Non-native spreader (site-level only). The best fitting basin-wide model

for each species is shown as a scatter plot, color-coded by model type – linear (red), exponential (orange), null (black).

CHAPTER 4: DETERMINANTS OF LANDSCAPE-LEVEL NATIVE FISH ABUNDANCE PATTERNS IN AN EASTERN US WATERSHED: NON-NATIVE SPECIES VERSUS LAND USE

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Abstract

Ecologists and conservation biologists continue to debate the relative roles of non-native species versus habitat degradation in widespread declines of native species and the concomitant structural shifts in biotic communities. We utilized redundancy analysis for variation partitioning and conditional inference trees to examine the relative effects of non-native invasions and land use on fish populations in streams of the upper and middle New River basin (UMNR) in the eastern US. The UMNR features a high proportion of non-native fish species (>50%) and wide variation in land use, which strongly influences instream habitat conditions. Whereas non-native species abundance accounted for a greater proportion of variation (36%) in native species abundance than did land use (19%), individual land-use descriptors were more commonly linked to negative responses in abundance by individual native species and life history guilds. Specifically, results suggested negative impacts of riparian agriculture on native species with limited parental care (open-substrate spawners and brood hidiers) and declines of benthic-feeding specialist species over the past two decades. These patterns suggest conservation of native fishes in the UMNR would be advanced more effectively by focusing on land use remedies such as restoration of riparian areas than on direct measures to eliminate or prevent further spread of established non-native fishes. Our novel methods for describing fish abundance accommodated usage of fish collection records resulting from several sampling methodologies over a broad timeframe and, as such, represent an advance over previous methods for describing species distributions. This approach enhanced our ability to detect large-scale spatiotemporal trends in fish abundance. Adequate data are available to support analogous analyses in many other watersheds and such studies are likely to uncover different results owing to differing physiographic templates, land-use disturbance regimes, non-native species pools, and structure of native fish communities. Future application of our methods could help to inform conservation strategies in other areas, especially where the ecological impacts of ongoing non-native invasions and shifts in land use are unassessed.

Introduction

Biological invasions by non-native taxa have often been implicated as drivers of diverse ecological impacts, including native species extinctions and losses of ecosystem function (Miller et al. 1989; Charles and Dukes 2008). Invasive species can be especially problematic in freshwaters, perhaps the most threatened of all ecosystems (Dudgeon et al. 2006). Introductions of non-native fishes have been implicated in the declines of at least 44 native fish species listed as threatened or endangered under the U.S. Endangered Species Act (Wilcove and Bean 1994). While invasions are rarely the only cause of extinction (Gurevitch and Padilla 2004), invasive species have been cited as factors in 68% of the 40 documented extinctions of North American native fishes in a recent 100-year period (Miller et al. 1989). In rare cases, invasive species can even eliminate large portions of local fish communities. For example, the introduction of non-native predatory Nile Perch (*Lates niloticus*) caused the extinction of around two-thirds of the endemic haplochromine cichlids in Lake Victoria of East Africa (Witte et al. 1992).

The only factor cited more often as a cause of fish extinctions in North America than invasive species is habitat alteration (Miller et al. 1989). Destruction of riparian vegetation, instream flow alterations due to damming and channelization (Etnier 1972; Jungwirth et al. 1995; Helfman 2007), in- and near-stream substrate mining (Brown et al. 1998), removal of instream habitat for boat traffic, and increases in siltation due to agricultural activity (Berkman and Rabeni 1987) have all been implicated in declines of native fishes. In addition, some of these disturbances may increase the likelihood of invasion by non-native species (Lee et al. 2017, Raab et al. 2018).

As freshwaters are often subject to both anthropogenic habitat modifications and non-native species invasions simultaneously, it is often difficult to disentangle root causes for change

in native fish communities, including population declines. MacDougall and Turkington (2005) suggested that non-native species may act either as drivers of change (acting as the only direct cause of change) in native ecosystems or as “passengers” (successfully invading but having little to no effect on ecosystems already heavily influenced by other factors). Other effect pathways suggest a complex mix of possible effects, including independent effects of habitat alteration and non-native species or even synergy between them (Didham et al. 2007, Hermoso et al. 2011). While some studies suggest that most non-native fishes play a passenger role (Gozlan 2008), others point to non-native fishes as the most influential drivers of native fish declines (Light and Marchetti 2007, Hermoso et al. 2011).

Analyses based on species traits provide some capacity to predict which species are likely to become invasive (likely to establish, spread, and cause harmful ecological and/or economic outcomes; García-Berthou 2007). For example, large predatory fishes impose some of the most drastic impacts on native fish communities (Helfman 2007), but even the effects of these introductions vary with region, habitat, and original community composition. In addition, some of the most impactful invasive fishes in North America, such as the parasitic Sea Lamprey (*Petromyzon marinus*) and the planktivorous Silver Carp (*Hypophthalmichthys molitrix*) are not large predators (Miller et al. 1989; Chick et al. 2020).

Outcomes of species invasions are, therefore, difficult to predict and vary widely among regions with different combinations of native species, habitat properties, and disturbance regimes. As such, those tasked with conserving regional fish diversity often lack adequate information to make confident decisions about prioritizing potential management actions. Managers currently employ a diverse array of tactics to mitigate fish diversity losses, including riparian and in-stream habitat restoration, dam removals, freshwater protected areas, imperiled

species rearing and reintroduction, and non-native species removal (Helfman 2007). The efficacy of these tactics relies on sound region- and species-specific information on specific drivers of native species declines. The lack of such information has rendered many management actions ineffective or led to unintended consequences (Salant et al. 2012; McLaughlin et al. 2013), wasting limited conservation funds and potentially undermining public trust. Thus, more regionally-focused studies are necessary to quantify the relative effects of species invasions and habitat modifications at conservation-relevant spatial extents to assist managers with development of effective conservation strategies.

To assist conservation managers and enhance knowledge of the role of non-native species in driving fish-community change, we focus on the New River basin in the Appalachian region of the eastern United States. This basin is a model system in which to study the consequences of biological invasions, as it features the highest ratio of non-native to native fish species of any drainage in the eastern United States (Jenkins and Burkhead 1994). Due in part to its isolation and geologic history, the basin's native species pool is small in relation to other eastern United States river systems of similar size (Cincotta et al. 1999). However, the New River basin is home to several endemic fish species (Jenkins and Burkhead 1994), which may be especially vulnerable to extinction (Burlakova et al. 2011). In addition, the once-forested basin has a recent history of intensive and varied land use, including agriculture and urbanization. Finally, the New River basin has six large hydroelectric dams and hundreds of smaller impoundments, which often act as hotspots for new fish introductions (Jenkins and Burkhead 1994).

We compiled long-term fish community data from a variety of sources to analyze abundance patterns of native fish species in the upper and middle New River basin (UMNR) to describe how these patterns relate to changes in non-native fish abundance and land use over

time. Our objectives were to (1) Document recent changes in community composition that may represent biodiversity loss, including community homogenization and individual native species declines, (2) Determine whether changes in land use versus non-native species abundance better account for native species abundance patterns across the UMNR and (3) Identify potential mechanistic pathways that may explain apparent effects of specific land uses and non-native species on native species abundances.

Methods

We compiled fish community survey data from many sources, including Virginia Department of Game and Inland Fisheries' (VDGIF) Fish & Wildlife Information Service (VAFWIS 2013), North Carolina Museum of Natural Sciences (NCSM 2014), North Carolina Stream Fish Community Assessment Program (SFCAP 2014), Multistate Aquatic Resources Information System (MARIS 2013), FishNet2 (2014), Virginia Department of Environmental Quality (VADEQ), and North Carolina Department of Environmental Quality (NCDEQ). Together, these data provide the most complete set of contemporary fish community data available across the UMNR.

We filtered the compiled data through a set of criteria to maximize compatibility among collection records based on different survey techniques and sampling intensities. Since we intended to model species abundances, we excluded all records of simply presence-absence data, as well as surveys that indicated sampling bias towards specific habitats, game species, or single species, as we suspected these records did not represent entire fish communities. We used geographic locations of all retained records to assign each survey to its corresponding stream segment in the NHDPlus version 2 flowlines dataset (USEPA 2019), where segments spanned the stream distance between two stream confluences and acted as the sampling units in our study.

We further defined sampling units by the year in which each fish survey occurred, where surveys occurring within the same stream segment during different years were treated as separate samples. We computed average species-specific catch within segments that were surveyed multiple times per year to yield annual estimates of species abundance within each segment. Abundance data were compiled by species when possible, but because of uncertain field identification of some species and taxonomic revisions during the study timeframe, we occasionally grouped multiple species into a single taxon. More information on data inclusion standards, geographic location corrections, and taxonomic groupings is included in Appendix A.

To quantify species abundances within each sampled stream segment, we derived scaled composite species abundance scores (SCSAs) from raw catch data using the convention of Sleezer (Chapter 3). SCSAs combine total catch, rank abundance, and proportional abundance into a single measure of species abundance during each survey event, allowing spatiotemporal comparisons of species abundance based on fish community data collected via disparate survey techniques and sampling intensities.

After calculating SCSAs for each species within each sample, we trimmed the dataset to include only surveys that occurred between 1998 and 2019 to match the timeframe of readily available land-use data (Homer et al. 2020). To test for temporal changes in native fish community composition, we grouped the remaining records into four bins (1:1998-2003, 2:2004-2008, 3:2009-2013, 4:2014-2019), centered on the 2001, 2006, 2011, and 2016 National Landcover Dataset (NLCD) layers, respectively, and further trimmed the dataset to include only those stream segments in which sampling occurred in at least two temporal bins (Figure 3.1).

Excluding non-native SCSAs, we then applied the *betadisper* function within the *vegan* package in program R (Dixon 2003) to a Bray-Curtis transformed distance matrix of native species SCSAs, to test for temporal differences in dispersion (Anderson et al. 2006) in species abundances that may indicate biotic homogenization across the UMNR. The *betadisper* function used principal coordinates analysis to plot fish samples in SCSA space, then used analysis of variance to test for inter-bin differences in mean deviation of plotted points from their respective centers of bin-specific point clouds. We assigned fish samples to the four temporal bins described above and interpreted declines in dispersion between sequential bins as indicators of homogenization in UMNR fish communities. We also plotted temporally stratified samples in SCSA space using non-metric multidimensional scaling (NMDS; Kruskal 1964) to visualize any potential homogenization trends.

To determine whether land use or non-native species correlated more strongly with native species abundances, we utilized a variation partitioning approach (Borcard et al. 1992). We used redundancy analysis (RDA) to determine how four explanatory variable matrices (non-native species abundance, land use, spatial, and physiography) differ in their ability to account for variation in the native SCSA response matrix. The non-native species matrix included SCSA scores for 29 species and a variable enumerating non-native richness within each survey. The land-use matrix included 15 variables quantifying urban and agricultural development, along with bridges and dams, within entire watersheds upstream of the lower terminus of sampled stream segments. We also quantified these common land uses at a more local extent, within 60-meter riparian buffers centered on these segments, representing the narrowest buffer distance allowed by the input land-use data, which has been suggested as the optimal riparian dimension for use in aquatic biota-landscape modeling (Frimpong et al. 2005). The spatial matrix was

constructed to account for spatial autocorrelation in the native SCSA matrix and included four significant distance-based Moran's spatial eigenvectors (dbMEMs). Finally, the physiography matrix included a suite of 10 variables quantifying properties such as stream and watershed size, geology and soils, channel slope, and elevation. These properties often correlate with land use and simultaneously may affect instream properties relevant to fish populations, such as sediment loading, water quality, and hydrology. More specific information on the variables in the land-use and physiographic explanatory matrices is provided in Appendix B.

Finally, we constructed conditional inference trees (CITs) to uncover potential effect pathways between specific explanatory variables and abundance of individual native species. We performed recursive binary partitioning using the *ctree* function within the *party* package in program R (Hothorn et al. 2006). A CIT was constructed for each of 33 native species; explanatory variables representing CIT nodes were catalogued by number of species, number of nodes, importance (levels) of nodes, direction of putative effects on native species, and connectivity with other explanatory variables. In addition to species-specific CITs, we also built CITs for trait-based life history guilds of native species representing selected spawning modes (open-substrate spawners, brood hiders, and nest guarders), water-column feeding positions (benthic and non-benthic), and breadth of benthic substrate use (generalists and coarse-particle specialists). We assigned species to guilds based on the FishTraits database (Frimpong and Angermeier 2009).

Results

The final SCSA dataset contained 281 distinct fish community samples from 91 stream segments across the UMNR. Multi-sample segments represented 334.6 km of stream length, 4% of the total UMNR stream length (3,595 segments). Sample density was greatest in the southern

portion of the basin (North Carolina), but sites were spread across the UMNR. The number of samples generally increased in subsequent temporal bins. We compiled 19 samples during 1998-2003, 57 samples during 2004-2008, 111 samples during 2009-2013, and 94 samples during 2014-2019.

We detected no significant difference in mean dispersion between any pair of temporal bins (all pairwise p-values > 0.31, $\alpha = 0.05$). In addition, visual inspection of the NMDS plot suggested no directional changes in fish communities over the study timeframe, as evidenced by extensive overlap of 95% confidence ellipses of bin-specific point clouds (Figure 3.2). These results justified our exclusion of a time variable from the follow-up RDA conducted to identify the relative influences of explanatory matrices (land use, non-native species, space, and physiography) on native fish abundances.

Cumulatively, explanatory variable matrices explained 54% of the variation in the native SCSA matrix (Figure 3.3). The non-native SCSA matrix explained the most exclusive variation (12%) in native species abundance, while 5% of the variation was explained exclusively by the land-use matrix. Interactions between matrices also explained a substantial portion of the variation. For example, interactions between non-native species abundances and physiographic variables described 7% of the variation. Summing all of the exclusive and shared variation of each explanatory matrix, non-native SCSAs helped explain 36%, followed by the physiography (23%), land use (19%), and spatial (18%) matrices.

Impacts of individual covariates on SCSAs for individual species were quite variable. The most commonly occurring covariates within native species-specific CITs were Brown Trout (*Salmo trutta*) abundance and non-native species richness, each appearing in 33% (11 of the 33)

of the CITs (Table 3.1). Despite the expectation that abundances of most non-native species would be negatively correlated with native abundances, most CIT nodes involving *S. trutta* abundance or non-native species richness indicated positive relationships with abundance of native species. Overall, such positive relationships greatly outnumbered negative relationships in CITs. In contrast, while land-use covariates accounted for fewer nodes in total than non-native covariates in CITs, land use (especially barren land and number of upstream dams) was associated with more negative relationships with native species abundance (Figure 3.4) than were non-natives. The most common effect pathway (identified by consecutive nodes between two covariates) indicated by the species-specific CITs was between Rock Bass (*Ambloplites rupestris*) abundance and proportion of upstream watershed as wetland (av_Wetland), but this pathway occurred only five times within the 33 CITs. An example CIT, showing some of the most common species-specific patterns is shown in Figure 3.5.

Trait-based CITs revealed the strong influence of physiographic variables (such as stream size and topography) on fish abundance (Table 3.2). Upstream watershed area appeared in all seven trait-based trees and stream order and stream slope appeared in six. The most common non-native species in the trait-based trees was Rainbow Trout (*Oncorhynchus mykiss*), which correlated negatively with abundance of native open-substrate spawners, brood hiders, non-benthic feeders, and benthic substrate generalists. Land-use variables were less common in trait-based trees, but the proportional coverage of agriculture in riparian buffers was negatively correlated with abundances of open-substrate spawners and brood-hiders. Once again, common connections between explanatory covariates indicating generalizable synergistic effects on natives were scarce, as no variable pair occurred more than twice in the seven CITs. Figure 3.6 shows an example trait-based CIT depicting some of the common patterns.

Discussion

Results were mixed with respect to their support of study predictions. We detected little evidence for major biodiversity losses across UMNR fish communities and found only a few instances of native species decline in abundance over the past 20+ years. Non-native species described a greater portion of the variability in native species abundances than did land use, but most individual linkages between native and non-native abundance patterns suggested positive associations. Negative relationships were more common between land-use covariates and native species abundances than between abundances of non-natives and natives. Land uses ranging from agriculture to barren land to upstream dams appeared to be commonly associated with negative abundance trends in several species and life history guilds. We were unable to identify any specific non-native species and land-use driven effect pathways that would suggest widespread synergies between specific stressors affecting abundance across many individual native species or guilds. These findings suggest that effects of these common stressors on native species are generally unique to the native species in question and that stressors have mostly additive rather than synergistic effects.

Temporal shifts in community composition and fish abundances

Despite pervasive invasions by non-native fishes across the UMNR, we documented little change in native fish abundance over the last two decades. Dispersion and NMDS results indicated no significant community-level homogenization and year of survey was not particularly common or strongly effectual as a covariate in structuring most species-specific and trait-based CITs. However, in the few CIT models in which time did play a role, a vast majority of these relationships were negative, indicating declines in abundance of individual species and functional groups. Declining species included the endemics, New River Shiner (*Notropis*

scabriceps) and Kanawha Darter (*Etheostoma kanawhae*), which may be especially vulnerable to extinction owing to their small ranges (Burlakova et al. 2011). Year of survey was also negatively related to abundance of benthic-feeding specialists. Benthic specialization, like endemism, has often been linked to declines and increased imperilment in freshwater fishes, particularly in darters (Angermeier 1995, Warren et al. 1997, Freeman et al. 2005, Angermeier and Pinder 2015).

Several factors may obscure declines in native species abundance and the drivers associated with these declines. For example, closer analysis of the decline suggested by the *N. scabriceps* CIT indicates that this trend is better classified as a series of widely-separated trends within a few 3rd-order streams with relatively high non-native species richness (>3 species) (Figure 3.7). Toms Creek (one of these streams) contains a slew of non-native minnows (*Notropis hudsonius*, *Notropis procne*, *Notropis telescopus*, *Luxilus cerasinus*, etc.) that overlap ecologically with *N. scabriceps* (Frimpong and Angermeier 2009). Many of these non-native minnows occur in Toms Creek sporadically and biotic interactions between these species and *N. scabriceps* have not been studied.

Our limited detection of negative relations between abundances of native and non-native species may be an artifact of the study timeframe. Many non-native species within the UMNRR have extensive invasion histories dating back more than a century (Jenkins and Burkhead 1994) and past studies have documented temporal shifts in distributions of native and non-native species, in terms of both number of occupied sites and abundance within those sites (Buckwalter et al., 2018; Sleezer, Chapter 3). Thus, relative stability of native fish abundance over the last two decades may not reflect long-term trends. Nevertheless, despite these limitations imposed on our analysis, the *N. scabriceps* example above illustrates how CITs could be used in other

systems to identify negative trends in conservation-relevant species to inform more focused studies to pinpoint factors and processes driving such declines.

Relative influences of non-native species versus land use

Our variation partitioning analysis showed that non-native species helped explain a much larger portion of the total variability in native species abundances (36%) than land use (19%), but many of the apparent effects of non-natives were unexpected. In particular, our CITs suggested that positive relationships between individual native and non-native species abundances were much more common than negative relationships. This pattern suggests that negative effects of non-natives on natives are currently uncommon in the UMNR despite large numbers of introduced species. In contrast, land-use covariates, while accounting for less variation in native species abundances collectively, were represented in more negative associations with individual native species than were non-native species covariates (Figure 3.6).

Positive relationships between native and non-native abundances were more common than we expected. While some of these trends could represent real mutualistic interactions between native and non-native species pairs, many of these positive associations may represent situations where unmeasured temporal variation in habitat is simultaneously driving trends in native and non-native species with similar habitat preferences. Because our data sources lacked information about instream habitat conditions associated with fish surveys, we could not include potentially important habitat variables (such as mesohabitat type, water depth, and flow velocity) in our analysis. Streamflow, in particular, can strongly influence temporal variation in local fish abundance and habitat conditions in Appalachian streams (Roberts et al. 2016). Nevertheless, regardless of the underlying causes of positive correlations between native and non-native

species abundances in our analysis, the existence of these patterns suggests co-occurrence and limited negative interaction between natives and non-natives.

Rock Bass (*Ambloplites rupestris*) and *O. mykiss* were prominent in the few negative associations we found between native and non-native abundances. *A. rupestris* abundance was negatively associated with a host of headwater species, whereas *O. mykiss* abundance was negatively associated with various functional groups, including open-substrate spawners, brood hiders, and non-benthic feeders. While these trends do not necessarily prove direct negative interactions between these non-natives and native species, *O. mykiss* in particular has been linked to negative ecological impacts in many cases in which this species has been introduced for sport-fishing purposes, including drastic alteration of native food webs (Konishi et al. 2001, Shelton et al. 2015).

Land-use variables most commonly associated with negative species abundance trends included percentage of barren land and number of dams within upstream watersheds. Barren land in the UMNR often indicates coal mining operations. Altered water quality, including increases in salinity, specific conductance, and various dissolved ions, as well as significant impacts on macroinvertebrate communities, are common in mining-influenced streams in Appalachia (Timpano et al. 2015, Boehme et al. 2016). Mining covers a minute portion of the watersheds we analyzed (averaging 0.1%), but our results suggest that even sparse mining activity may significantly affect fish abundance. Upstream dams have been linked to numerous effects on stream-obligate fishes, including reductions in suitable habitat, interference with feeding and reproduction, and introduction of large predatory fishes escaping from impoundments created by dams (Mammoliti 2002). Native fishes exhibiting negative responses to increases in upstream

dams included Mountain Redbelly Dace (*Chrosomus oreas*) and Northern Hog Sucker (*Hypentelium nigricans*), two stream-obligate species.

In addition to impacts on individual species, land use influenced abundance of some life history guilds. We found negative relationships between the collective abundance of species exhibiting little parental care (open-substrate spawners and brood hidiers) and the extent of agriculture and pasture land within adjacent riparian areas. Deforested riparian areas are more susceptible to streambank erosion, leading to increases in fine sediment deposition in streams (Abernethy and Rutherford 2000). Livestock, if allowed access to streams, can exacerbate this process (Trimble 1994). Increases in fine sediment deposition can limit fish egg survival through suffocation of eggs (Kemp et al. 2011) and lead to decreased abundance of benthic specialists (Jones III et al. 1999). The negative effects of fine sediments can also be pronounced in fishes that do not tend their eggs, such as open-substrate (simple lithophilic) spawners (Berkman and Rabeni 1987).

The UMNR is distinct from most drainages in the United States due to its combination of low native fish species richness, high level of endemism, unique geologic history and isolation from adjacent drainages, and high richness of non-native fish species. As such, similar studies in different drainages are likely to uncover different patterns of the relative effects of non-native species and land use on native fishes. The data to conduct such studies are readily available, as many drainages, especially in the eastern and mid-western United States, share similar temporal and spatial coverage of community-level fish survey data (Frimpong et al. 2016). Insights from such studies could inform local and regional freshwater conservation strategies in the future.

Management implications

Current state and federal regulatory frameworks in place to conserve native species (including the United States Endangered Species Act and the Virginia Wildlife Action Plan) tend to focus resources on species and populations already considered threatened or endangered (Angermeier and Schlosser 1995). As such, conservation agencies have no mandate to monitor populations of unlisted native species whose declines and their underlying causes may go unnoticed if not for the efforts of external researchers. Such is the case for *N. scabriceps*, whose apparent recent decline may be of conservation concern due to the restricted range of this species. Understanding potentially complex biotic interactions between *N. scabriceps* and many co-occurring non-natives may be crucial to conserving this endemic species, currently listed as a species of greatest conservation need (SGCN) in the state of Virginia (VDGIF 2015). However, limited sampling, especially within large rivers, may have hindered our ability to identify potential mechanisms underlying this decline. Increased sampling effort within large rivers may be crucial to confirm and adequately address potential causes of decline in species with large river affinities within the UMR.

Studying temporal distribution dynamics of local communities, including common species and functional groups, increases the likelihood of early detection of potential threats and can inform conservation strategies that consider the needs of all species, as well as the poorly-known biotic interactions that help to keep these communities intact (Frimpong 2018). For example, our finding that riparian agriculture may be hindering reproductive success of fishes that exhibit little parental care may be an early warning signal of species-specific declines within this common functional group, which includes *N. scabriceps*. Future management tactics to mitigate *N. scabriceps* declines and guard against future declines of similar species could include riparian revegetation aimed at decreasing fine sediment loads.

State regulatory agencies already employ several freshwater conservation strategies that seem likely to be effective within the UMNR (NCWRC 2015; VDGIF 2015). Our results suggest that agriculture commonly has negative effects on native fish abundance. As such, the application of best management practices (BMPs), such as riparian revegetation and livestock fencing, may be particularly effective for conserving native aquatic communities. Currently, approximately 63,000 hectares are reportedly benefitting from agricultural BMPs across the Virginia portion of the UMNR (DCR 2019); this area is just over 26% of all agricultural and range land. However, effects of these BMPs on fish communities within the UMNR are unknown. More research is needed to determine the benefits of these BMPs to stream fish communities.

Although we were unable to identify many specific non-native species as having broad negative influences on native fishes, *O. mykiss* may be one species that has broad ecological impacts in the UMNR. Many watersheds across the United States have *O. mykiss* stocking programs that continue today without much oversight as to the effects of these introductions on native ecosystems. Better understanding of the effects of this species may be important for ongoing native fish recovery efforts in many geographically distant habitats in the future. Management would benefit from monitoring to account for unintended impacts of *O. mykiss* on native ecosystems when considering new and ongoing sportfish enhancement programs.

Aside from the effects of specific non-native species, CITs indicated that increases in non-native species richness may have greater effects on native species and specific life history guilds. Thus, our results suggest that if widespread negative impacts of non-natives are occurring in the UMNR, complex interplay between non-natives collectively and native communities may be more important drivers of decline. With this in mind, we should strive to understand the

conservation value of local communities in terms of their regional contributions to biodiversity and the vulnerability of constituent species to potential invasions when considering future intentional introductions of foreign fishes and when deciding on appropriate measures to prevent unintentional introductions. Researchers generally agree that preventing introductions is the best way to prevent negative impacts from non-native species (Vander Zanden and Olden 2008). Therefore, observing cautionary measures against species introductions while research continues to better understand the drivers of native species declines, may be critical to long-term conservation goals.

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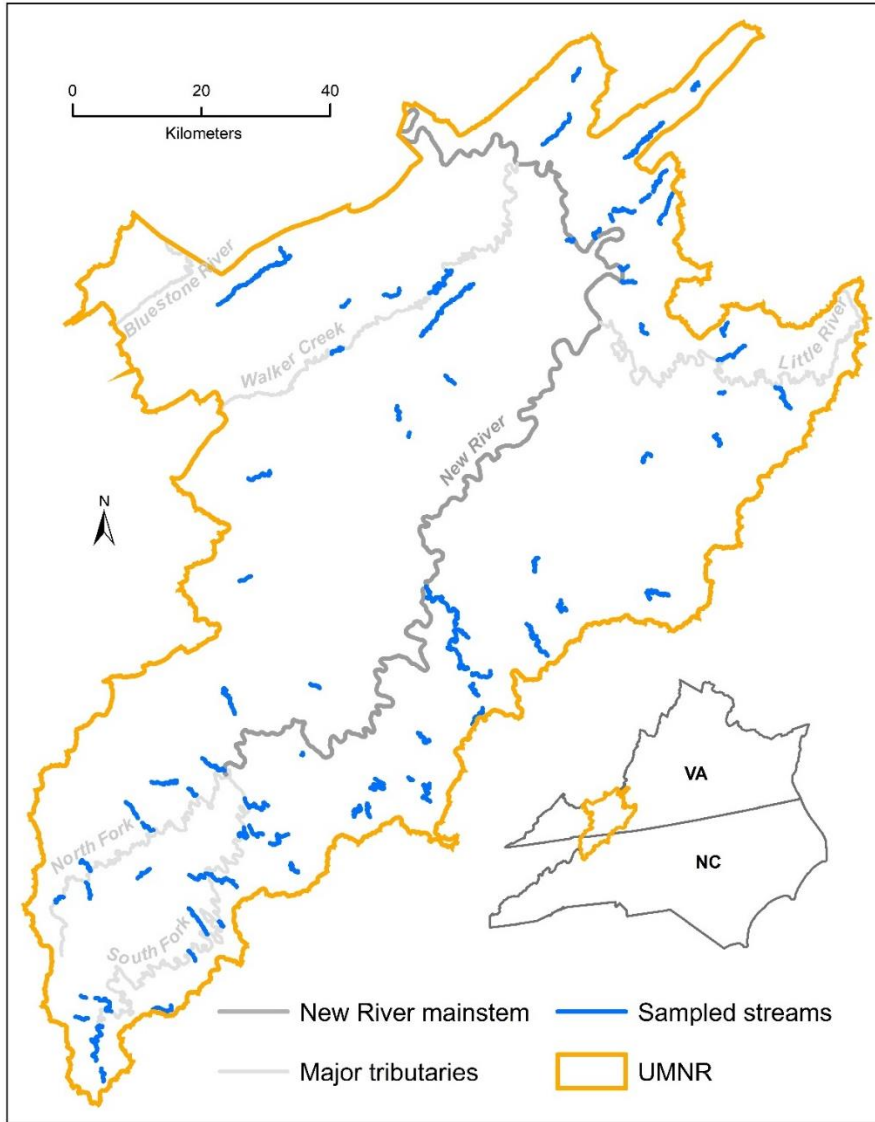


Figure 4.1: The upper and middle New River basin, showing the New River mainstem, major tributaries, and the final set of repeatedly sampled stream segments retained for analysis.

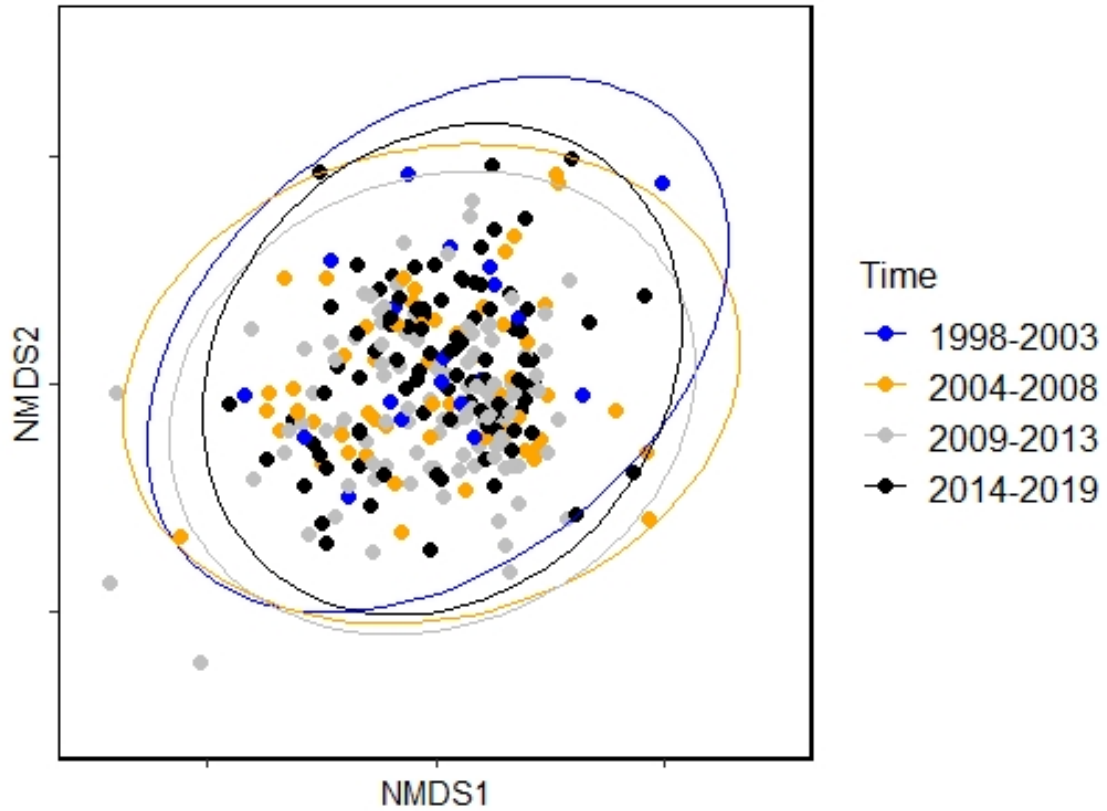


Figure 4.2: Non-metric multidimensional scaling (NMDS) ordination of sampled stream segments in scaled composite species abundance (SCSA) space. Each point represents a single sample and ellipses represent 95% confidence intervals around the centroid of each temporal bin. Ellipses all overlap each other's centroids, indicating no directional differences in fish communities over time.

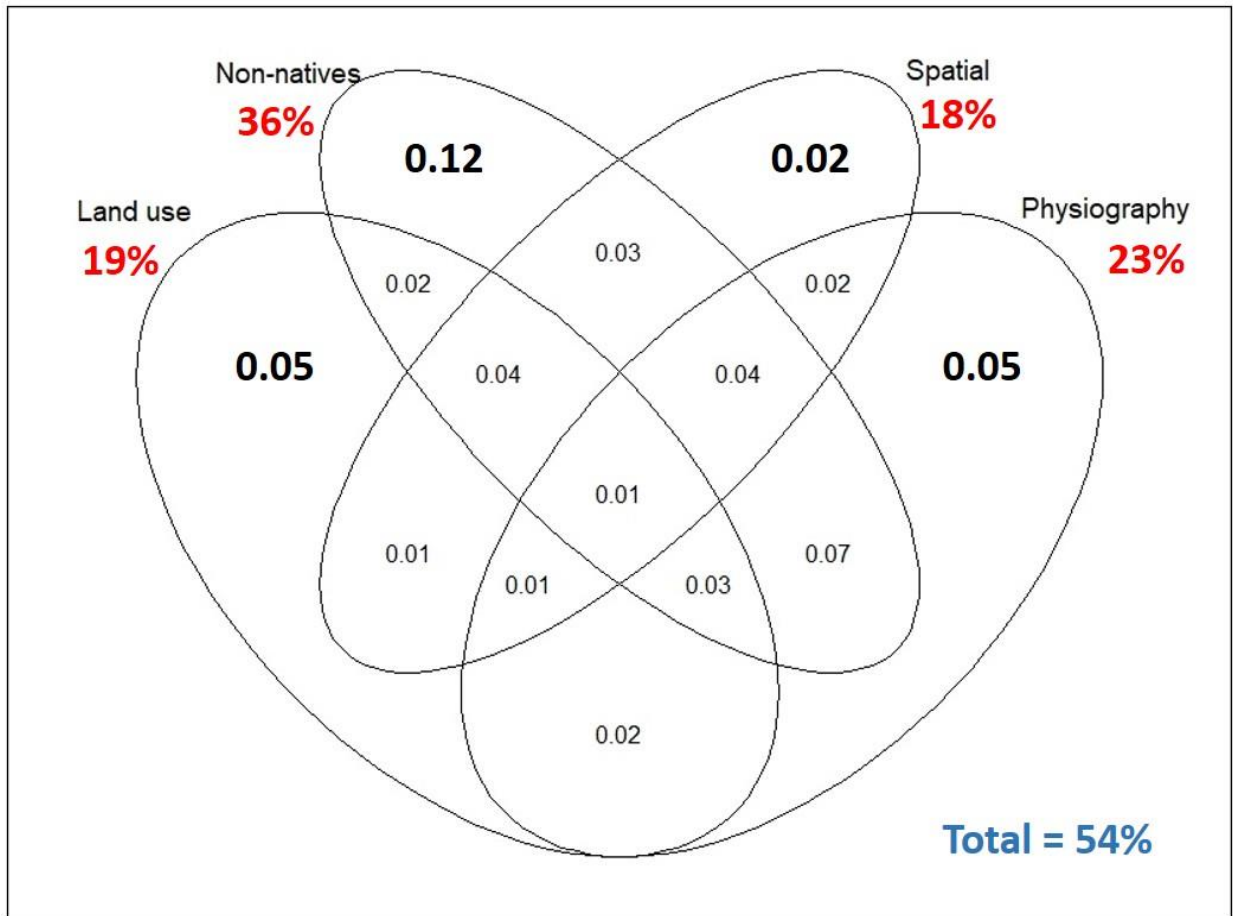


Figure 4.3: Venn diagram, showing proportions of exclusive variation (bold) and percentage of total shared variation (red) in the native scaled composite species abundance (SCSA) response matrix explained by each of the explanatory matrices. In total, explanatory matrices accounted for 54% of the total variability in the SCSA response matrix.

Table 4.1: Top ten covariates, ranked by number of native species (Sps_chosen) for which each covariate made up at least one significant ($\alpha = 0.05$) node within species-specific conditional inference trees. Some covariates appeared in trees for some species more than once, allowing total “Tree appearances” to exceed Sps_chosen in some cases. Covariates chosen for the same number of species are sorted by average node, or the average level within the set of trees where the covariate appears, where lower numbers indicate greater importance (nodes earlier in tree formation). Effect direction indicates counts of the number of times the covariate positively (plus) or negatively (minus) correlated with species abundance within the set of trees and the difference between these counts (differential). These “Top covariates” could be interpreted as those having the most widespread indicated impacts across various species-level components of native fish communities. “Exotic_Rich” stands for non-native species richness, italic binomials represent individual non-native species, and dbMEMs represent spatial variables derived from Moran’s eigenvector analyses. Descriptions of land-use and physiographic covariate abbreviations can be found in Appendix B (Table B1).

Top covariates	Effect prevalence and importance			Effect direction		
	Sps_chosen	Tree appearances	Average node	Plus	Minus	Differential
Exotic_Rich	11	15	3.2	10	5	5
<i>Salmo trutta</i>	11	11	3.7	10	1	9
dbMEM2	10	13	3.3	8	5	3
av_Barren	10	11	3.8	6	5	1
dbMEM1	9	10	2.9	7	3	4
<i>Ambloplites rupestris</i>	9	13	3.5	6	7	-1
Wat_Area_km2	9	10	3.5	8	2	6
av_Wetland	9	10	3.7	9	1	8
dbMEM3	8	9	3.0	5	4	1
MEAN_Fine_Wshed	8	10	3.7	7	3	4

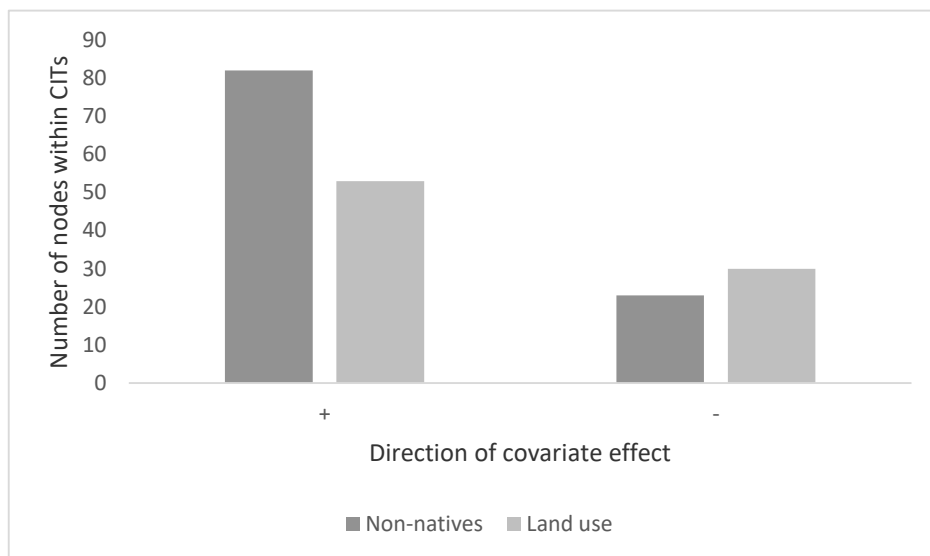


Figure 4.4: Cumulative appearances of non-native species abundance and land-use variables as significant positive (+) and negative (-) explanatory nodes in 33 native species abundance-based conditional inference trees (CITs).

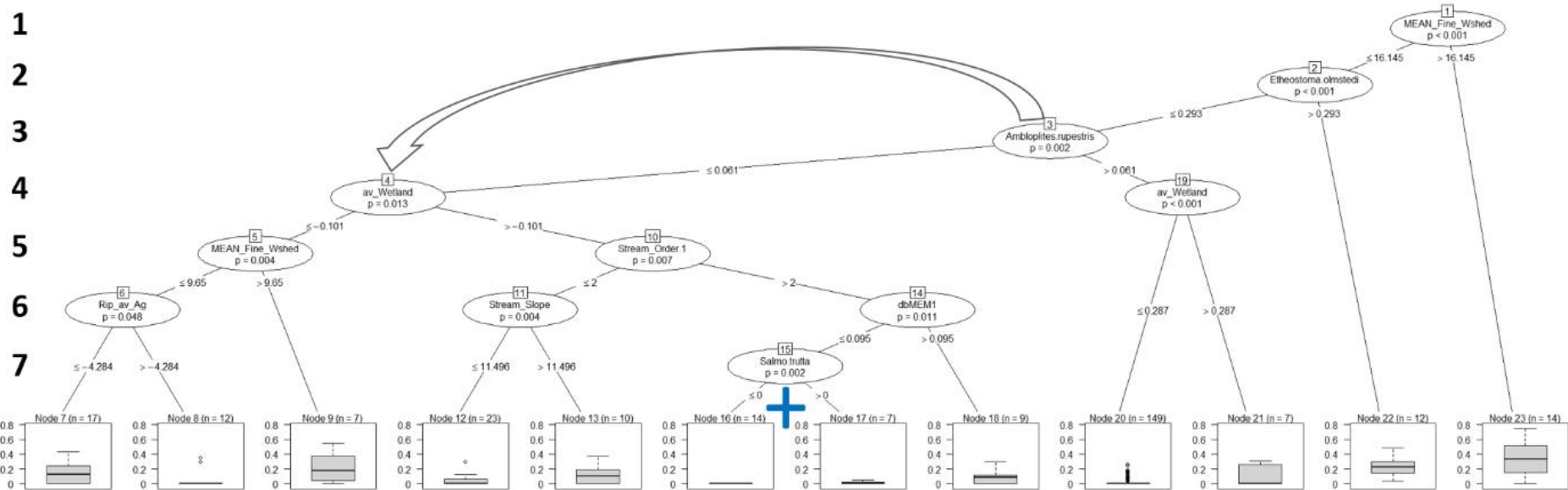


Figure 4.5: Conditional inference tree (CIT) partitioning variation in Creek Chub (*Semotilus atromaculatus*) abundance across four time periods in the UMR. The ovals represent nodes of the tree where average SCSA abundance is significantly different ($\alpha = 0.05$) on either side of a specific value for the covariate representing the node (shown below each splitting node), indicating an ecological signal (greater or lesser species abundance) associated with the covariate representing the node. Node 1 (upper right) represents the first major node in the tree and subsequent nodes can be followed in numerical order based on lined connections between nodes. The roots of the tree display SCSA abundance of *S. atromaculatus* across samples grouped at terminal nodes, displayed by box plots. Cumulative numbers of sampled sites involved in each node (displayed above terminal nodes) decline with tree level (from 1 to 7, shown on the left side of the plot), where the node at level 1 could be interpreted as the most important node (involving all of the data) and subsequent nodes involve smaller portions of total sampled stream segments. The blue “+” represents a slight positive relationship at node level 7 between Brown Trout (*S. trutta*) abundance (commonly involved in minor positive nodes across many native species) and *S. atromaculatus* abundance, as the box plot at the right side of this node (associated with higher *S. trutta* abundances) indicates a significantly higher mean *S. atromaculatus* abundance than the box plot at the left side of the node. The gray block arrow at the top of the plot shows a pathway link between non-native Rock Bass (*Ambloplites rupestris*) abundance and percentage of the

upstream watershed as wetland (av_Wetland), the most common pathway link (5 occurrences) across all 33 species-specific CITs. See Appendix B (Table B1) for descriptions of abbreviations for other covariates involved in CIT construction.

Table 4.2: Top ten covariates, ranked by number of trait-based life history guilds for which each covariate made up at least one significant ($\alpha = 0.05$) node (Trts_chosen) within trait-based guild conditional inference trees. Some covariates appeared in trees for some guilds more than once, allowing total “Tree appearances” to exceed Trts_chosen in some cases. Covariates chosen for the same number of guilds are sorted by average node, or the average level within the set of trees where the covariate appears, where lower numbers indicate greater importance (nodes earlier in tree formation). Effect direction indicates counts of the number of times the covariate positively (plus) or negatively (minus) correlated with abundance of native species within a guild and the difference between these counts (differential). These “Top covariates” could be interpreted as those having the most widespread indicated impacts across various functional group-level components of native fish communities. “Exotic_Rich” stands for non-native species richness, italic binomials represent individual non-native species, and dbMEMs represent spatial variables derived from Moran’s eigenvector analyses. Descriptions of land-use and physiographic covariate abbreviations can be found in Appendix B (Table B1).

Top covariates	<u>Effect prevalence and importance</u>			<u>Effect direction</u>		
	Trts_chosen	Tree appearances	Average node	Plus	Minus	Differential
Wat_Area_km2	7	10	4.8	5	5	0
Stream_Slope	6	10	3.0	3	7	-4
Stream_Order	6	8	3.6	7	1	6
dbMEM4	5	5	4.8	1	4	-3
<i>Oncorhynchus mykiss</i>	4	4	3.5	0	4	-4
Exotic_Rich	4	6	4.0	6	0	6
dbMEM2	4	4	4.0	4	0	4
dbMEM1	3	4	2.5	3	1	2
<i>Micropterus dolomieu</i>	3	3	3.7	1	2	-1
<i>Notropis rubricroceus</i>	3	3	4.7	2	1	1

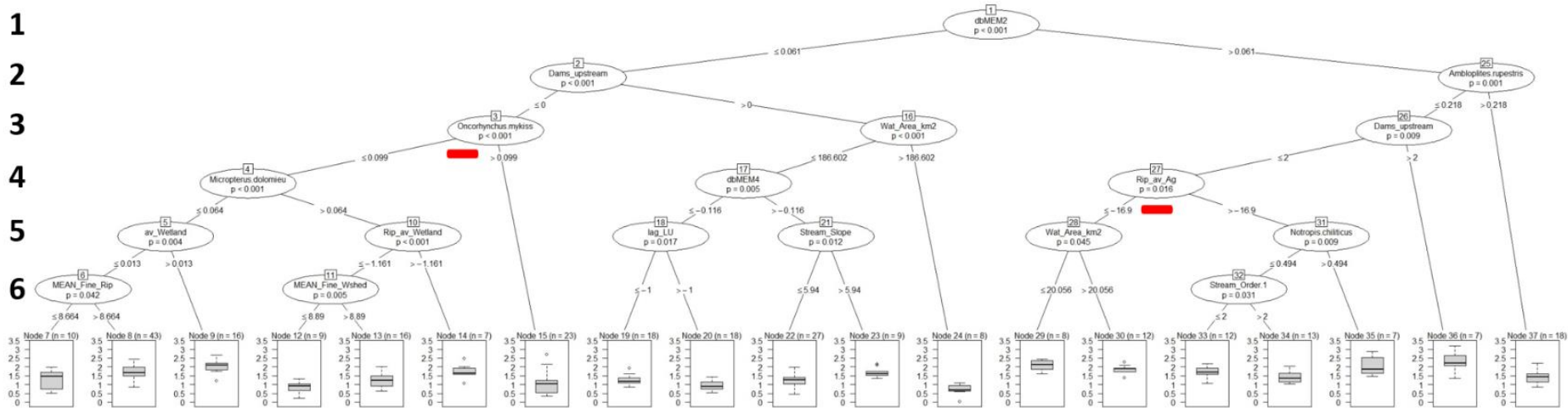


Figure 4.6: Conditional inference tree (CIT) partitioning variation in cumulative brood hider SCSAs across the UMNR. The ovals represent nodes of the tree where average SCSA abundance is significantly different ($\alpha = 0.05$) on either side of a specific value for the covariate representing the node (shown below each splitting node), indicating an ecological signal (greater or lesser brood hider cumulative SCSA abundance) associated with the covariate representing the node. Node 1 (upper right) represents the first major node in the tree and subsequent nodes can be followed in numerical order based on lined connections between nodes. The roots of the tree display cumulative SCSA abundance of brood hidiers across samples grouped at terminal nodes, displayed by box plots. Cumulative numbers of sampled sites involved in each node (displayed above terminal nodes) decline with tree level (from 1 to 7, shown on the left side of the plot), where the node at level 1 could be interpreted as the most important (involving all of the data) and subsequent nodes involve smaller portions of total sampled stream segments. The red symbols indicate negative tree nodes involving Rainbow Trout (*Oncorhynchus mykiss*) (commonly detected as having negative interactions with various life history guilds in our analysis) and percentage of agricultural land within the segment riparian area (Rip_av_ag), which also exhibited a negative node in another spawning mode guild (open-substrate spawners). See Appendix B (Table B1) for descriptions of abbreviations for other covariates involved in CIT construction.

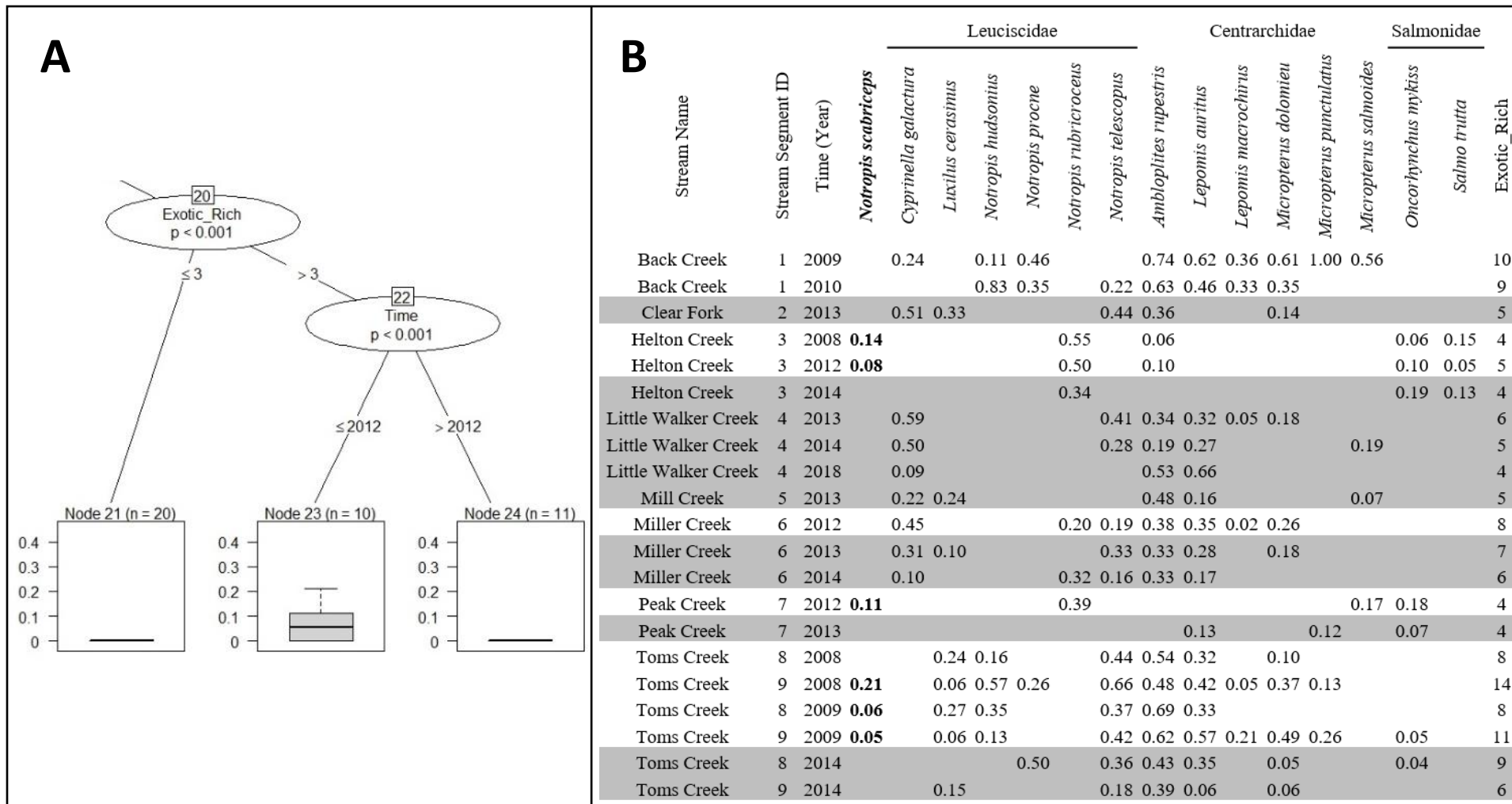


Figure 4.7: Recent declines in New River Shiner (*Notropis scabriceps*), as indicated by conditional inference tree (CIT) analysis. Panel A shows the section of the constructed CIT model for *N. scabriceps* indicating declining abundance, post-2012 (with scaled composite abundance scores [SCSAs] plotted on the y-axis of the terminal box plots), in streams with relatively high numbers of non-native species (Exotic_Rich). Panel B shows the species-specific abundance data underlying node 22 in the CIT model. SCSA abundance data for *N. scabriceps* (bold) and commonly co-occurring non-native species representing potential competitors (Leuciscidae) and predators (Centrarchidae and Salmonidae) are shown across all sites represented in the terminal CIT node. In these recent samples, post-2012 (gray-shaded), *N. scabriceps* were not detected, even within

streams in which they were present at varying abundances in previous samples (Helton Creek, Peak Creek, and Toms Creek), less than a decade earlier. The complex mosaic of changing populations of various non-natives and the potential changes in interactions between these species and *N. scabriceps* could be a contributing factor to recent declines.

CHAPTER 5: CONCLUSIONS

Generally, concluding remarks can be summarized as answers to three main questions relevant to the objectives of this thesis: 1. What do long- and short-term trends in native species abundance patterns and community uniqueness and diversity tell us about the current conservation status of the fishes of the UMNR?, 2. How do land use and non-native species affect stream fish abundance patterns? and 3. How can future researchers and managers use the results herein to improve fish conservation measures and fill current gaps in data and overall knowledge about freshwater fishes that limit current understanding of mechanisms driving species abundance trends?

Long-term trends in fish communities suggest widespread biotic homogenization across the UMNR over the past 60+ years. Underlying this overall trend in decreasing uniqueness among communities across the UMNR are common declines in abundance of native fish species, including a host of endemic species that may be particularly vulnerable to future extinction. More recent fish community trends suggest that the overall trend of biotic homogenization over time has slowed over the past two decades, but some signals of native species declines for endemic species such as the New River Shiner (*Notropis scabriceps*) persist to present day. While basin-wide declines in native species and community uniqueness paint a bleak picture for fish conservation in the UMNR, my research suggests that some unique and diverse communities are still relatively common in certain areas, providing opportunities for effective future conservation efforts to prevent further erosion of native communities. Specifically, the Little River and Big Reed Island Creek drainages in the northwest portion of the UMNR and the North

Fork New River drainage in the southern portion of the UMNR appear to be reservoirs of remaining unique and diverse native fish communities that could be important areas for future native fish conservation.

Effects of land use on native fishes of the UMNR are quite diverse and appear to vary depending on species identity and life history. Fishes also appear to respond differently to land use occurring at multiple extents relevant to sampled streams, ranging from entire contributing watersheds to local riparian areas. Of the land-use variables tested, number of upstream watershed road-stream crossings appear to be the strongest structurers of present fish communities, favoring increased abundances of non-native centrarchids and decreases in abundances of several smaller-bodied native species. Results also suggest that forested riparian areas may be particularly important for maintenance of populations of some endemic species and that riparian agriculture may be detrimental to vulnerable species groups, including species with low levels of parental care. Despite outperforming land-use variables as correlates of native community composition in my variation partitioning analysis, individual non-native species accounted for fewer suggested negative interactions with native species than did land-use variables. However, the common occurrence of non-native species richness as a significant structuring force in models of native species and functional group abundance suggest that complex interactions between many invaders could be more proximate to potential declines in native species than the effects of any single invader.

Management strategies that seem particularly likely to be effective at conserving remaining fish biodiversity and halting ongoing declines of native species in the UMNR include ecosystem-based strategies such as restoration of vegetated riparian buffers and prevention of any further non-native introductions, especially in drainages such as Little River, Big Reed

Island Creek and North Fork New River, which currently contribute disproportionately to UMNR-wide fish community diversity and uniqueness. Research gaps relevant to future conservation of fishes in the UMNR include an overall lack of information on populations of non-game fishes in the main-stem New River, limited understanding of specific mechanisms underlying the effects of road-stream crossings on fishes, and specific interactions between declining natives and numerous non-native species whose combined effects may be contributing to decline. My methods for describing abundance distributions of species within the UMNR, including the application of novel species SCSAs, allowed incorporation of a wide variety of fish community data that were otherwise not comparable and may allow for earlier identification of declines in native species than other commonly-used methods for describing species distributions. As such, these methods should be broadly applicable to other drainages with similar densities of historic fish community sampling and have the potential to help inform fish conservation procedures in other areas.

More broadly, my results emphasize the importance of recognizing effects of temporal scale on identification of native biodiversity losses (including community homogenization and native species declines) driven by a variety of factors (land use, non-native species, etc.). Broad ecological patterns are best described when reference conditions, ideally representing pristine conditions, are known. However, data representing pristine conditions of stream communities are exceedingly rare, so shifting baselines of reference conditions should always be a concern for any temporally explicit study of species distributions. Our analytical approach, involving the application of novel SCSAs, allowed extraction of the most possible historical and contemporary data on species abundances. SCSAs are more sensitive to population-level changes in native species distributions than commonly applied occurrence-based metrics. Without this approach

and consideration of multiple temporal scales, widespread declines in community uniqueness and populations of native species could have gone unnoticed. Our understanding of ecological phenomena will always be limited by data availability but, in many cases, the data we do have is underutilized. In the face of continued biotic degradation, utilization of untapped historical data resources may be key to successful biodiversity conservation in the future.

APPENDICES

Appendix A: Data inclusion standards, GPS location corrections, and taxonomic resolution and correction decisions (Table A1).

- Data inclusion standards
 - Collections must include over 100 individual fish.
 - Collections must include more than one species.
 - Collections must contain non-game species (species within the Catostomidae and Cyprinidae families and/or species within the *Etheostoma*, *Percina*, or *Noturus* genera.
 - Collections where metadata suggest sampling only occurred in 1 habitat type (riffle, run, or pool) were excluded.
 - Collections with numerous repeating numbers were excluded due to concern that many of these sampling events were targeting a certain number of specimens from a few different species and not true community samples.
 - Duplicate collections, reported from 2 or more sources from which we downloaded data, were deleted if records were identical. If small discrepancies existed in duplicate collections, we retained the data from the source closest to the original data entry (least opportunity for data entry errors) or the one containing the most plausible collection of species given the collection location.
- Correction of inaccurate GPS locations: 2 types of errors (less than 10 percent of records changed)
 - Mismatches between “GNIS_name” field for nearest stream segment in the NHD+ V2 flowlines shapefile (USEPA 2019) and the stream name in the metadata for the collection record.
 - Corrections to these records were made by manually changing the stream order, HUC12, HUC10, and stream segment fields to match the nearest stream segment with a GNIS_name matching the reported stream name from the collection record.
 - If no stream name was available in our database of collections, the reported community was checked against community expectations given stream order of the stream segment to which each collection was related. Presences of species commonly found in headwater streams were checked against presences of species occurring more commonly in large to medium-sized rivers. Sample segments were flagged, rechecked, and in many cases re-assigned to a stream segment more likely to be the collection origin if...
 - For 1st- and 2nd-order streams: large-water species outnumber headwater species
 - For 3rd- and 4th-order streams: suspicious imbalances between headwater and large-water species (e.g., 3 or more headwater species and 1 or 0 large-water species or vice-versa)

- For 5th- and 6th-order streams: headwater species outnumber large-water species
- Species considered headwater species: *Chrosomus oreas*, *Clinostomus funduloides*, *Cottus spp.*, *Nocomis leptocephalus*, *Rhinichthys atratulus/obtusus*, *Salvelinus fontinalis*, *Semotilus atromaculatus*
- Species considered large-water species: *Cyprinella analostana*, *Cyprinella galactura*, *Cyprinella spiloptera*, *Cyprinus carpio*, *Esox masquinongy*, *Micropterus salmoides*, *Notemigonus crysoleucas*, *Notropis photogenis*, *Notropis scabriceps*, *Notropis telescopis*, *Percina caprodes*, *Phenacobius teretulus*, *Pomoxis annularis*, *Pomoxis nigromaculatus*, *Pylodictis olivaris*, *Sander vitreus*

Table A1: Taxonomic resolution edits and correction decisions for final New River species abundance database.

Taxonomic group	New group name	*Reason(s) for revision	Species affected and number of records for each
<i>Cottus spp.</i> (5 species)	<i>Cottus</i>	1, 2	<i>C. baileyi</i> = 1, <i>C. bairdii</i> = 368, <i>C. carolinae</i> = 6, <i>C. cognatus</i> = 1, <i>C. kanawhae</i> = 78, <i>C. sp.</i> = 10
<i>Luxilus albeolus</i> and <i>Luxilus cornutus</i>	<i>Luxilus albeolus/cornutus</i>	1, 3	<i>L. albeolus</i> = 232, <i>L. cornutus</i> = 2
<i>Nocomis spp.</i> (3 species)	<i>Nocomis</i>	1, 2	<i>N. leptocephalus</i> = 509, <i>N. micropogon</i> = 5, <i>N. platyrhynchus</i> = 172, <i>N. raneyi</i> = 1
<i>Notropis micropteryx</i> and <i>Notropis rubellus</i>	<i>Notropis rubellus</i> comp.	1, 2	<i>N. rubellus</i> = 162, <i>N. micropteryx</i> = 3
<i>Percina gymnocephala</i> , <i>Percina maculata</i> , and <i>Percina peltata</i>	<i>Percina gymnocephala</i>	4	<i>P. gymnocephala</i> = 130, <i>P. maculata</i> = 2, <i>P. peltata</i> = 1
<i>Phenacobius mirabilis</i> and <i>Phenacobius teretulus</i>	<i>Phenacobius teretulus</i>	4	<i>P. mirabilis</i> = 1, <i>P. teretulus</i> = 166
<i>Rhinichthys atratulus</i> and <i>Rhinichthys obtusus</i>	<i>Rhinichthys atratulus/obtusus</i>	1, 2	<i>R. atratulus</i> = 366, <i>R. obtusus</i> = 28

*Reasons for Revision: 1 – Difficulty in identifying collected individuals to species in the field, 2 – Taxonomic revisions (Lachner and Jenkins 1971; Wood et al. 2002; Kinzinger et al. 2007) and uncertainty (Smith 2007) within the genus (affecting the New River basin) within the study timeframe, 3 – Genetic analyses indicate uncertainty between these species within the New River basin, 4 – Species records renamed due to likely misidentifications.

Appendix B: Physiography and land use.

Table B1. Physiographic and land-use covariates, derivations, and descriptions

Physiographic variables	Abbreviation	Units	Source*	Derivation	Purpose
Stream gradient	Stream_Slope	Meters per kilometer	NED	Subtraction of downstream from upstream endpoint elevation values of each given NHD* stream segment divided by the length of the segment	Partial proxy for erosion potential and average flow velocity
Relief	Relief	Meters	NED	Subtraction of the elevation at the most downstream endpoint of a stream segment from the highest elevation in its contributing watershed	Partial proxy for basin-wide erosion potential
Base elevation	Pour_Elev	Meters	NED	Raw elevation of the most downstream endpoint of a stream segment	Partial proxy for stream temperature
Soil matrix composition (particle size)	MEAN_Fine_Wshed MEAN_Fine_Rip	-	gSSURGO	Weighted average of soil texture descriptions in gSSURGO, given ordinal rank values in Table B2 (columns 1-2), derived across contributing watersheds (_Wshed) and 60-meter riparian buffers (_Rip) of sampled stream segments	Partial proxy for erosion and fine sediment deposition potential and type (size) of fine sediment inputs to sampled streams
Coarser particle inclusions in soil	MEAN_Coa_Wshed MEAN_Coa_Rip	-	gSSURGO	Weighted average of soil “textural modifier” descriptions in gSSURGO database, given ordinal rank values in Table B2 (columns 3-4), derived across contributing watersheds (_Wshed) and 60-meter riparian buffers (_Rip) of sampled stream segments	Partial proxy for larger substrate particles occurring on stream bottoms
Surficial geology	Sedi_pct	-	SGMC	Percentage of stream segment contributing watershed with sedimentary bedrock map units at the surface	Proxy for stream bed habitat, stream network characteristics given differing erosion patterns of different rock types, and water chemistry properties affected by bedrock
Stream order	Stream_Order	-	NHD+ V2	Strahler stream order (Strahler 1957; Pierson et al. 2008)	Partial quantification of stream size, as stream size affects characteristics ranging from habitat differentiation and volume, primary productivity, stream temperature, etc. that help determine size of fish populations

Watershed area	Wat_area_km2	km ²	NED	Area of contributing watershed, delineated in ArcGIS	Another partial stream size parameter which also helps describe potential for total runoff and thus sediment and other pollutant loads on sampled streams
Land use variables					
Land-use types across contributing watersheds	av_Urban av_Ag_Grass_Shrubland av_Water av_Wetland av_Barren	-	NLCD	Percent of urban, agriculture/grassland/shrubland, water, wetland, and barren land across upstream contributing watersheds of sampled segments	Proxies for instream habitat properties affected by erosion of stream banks and runoff from upstream sources
Land-use types adjacent to sampled segments	Rip_av_Urban Rip_av_Ag Rip_av_Water Rip_av_Wetland Rip_av_Barren	-	NLCD	Percent of urban, agriculture/grassland/shrubland, water, wetland, and barren land within 60-meter riparian buffers of sampled segments	Proxies for instream habitat properties affected by erosion of stream banks and runoff from surrounding land
Road-stream crossing covariates	X_Wshed_Dens X_Seg_Dens	Crossings per km ²	NHD+ V2 Tiger Lines	Number of road-stream crossings per square kilometer within contributing watersheds (_Wshed) and within 60-meter riparian buffers of sampled segments, tabulated using the intersection tool in ArcGIS	Proxies for chemical pollutants from roads and potential alteration of stream habitat and fish passage by bridges and culverts
Dam covariates	Dams_upstream MajDams_HUC10	-	NID WBD	Number of dams upstream of sampled segments (Dams_upstream) and number of dams on 4 th order streams and larger within HUC10 watersheds containing sampled stream segment	Proxies for estimating effects of nearby dams on fish passage and habitat properties both upstream and downstream of dams
NLCD lag	lag_LU	-	NLCD	Years of lag time between fish sample date and date of land-use data matched to each stream segment	Meant to balance out temporal disadvantage of land-use covariates against non-native species covariates, as non-native species were collected at the same time as natives for our analysis

*gSSURGO – gridded Soil Survey Geographic Database (USDA-NRCS 2016)

*NED – National Elevation Dataset (USGS 2016^B)

*NHD+ V2 – National Hydrography Dataset (USEPA 2019)

*NID – National Inventory of Dams (NID 2020)

*NLCD – National Landcover Dataset (Homer et al. 2019)

*SGMC – State Geologic Map Compilation (Horton et al. 2017)

*Tiger Lines – Tiger/Line roads dataset (US Census Bureau 2012)

*WBD – Watershed Boundary Dataset (HUC10 boundaries) (USGS 2013)

Table B2. Soil texture descriptor variables as proxies for in-stream substrate in fish abundance models (modified from Slezzer et al. 1998): Soil fine texture ranks - based on differences in soil matrix particle size (derived from textural classes in gSSURGO) and Coarse texture ranks - based on rock fragment modifiers of texture in gSSURGO.

Textural class	Fine texture rank	Modifier name	Coarse texture rank
Coarse sand	21		
Sand	20		
Fine sand	19	Bedrock	18
Very fine sand	18	Boulders	17
Loamy coarse sand	17	Extremely bouldery	16
Loamy sand	16	Very bouldery	15
Loamy fine sand	15	Bouldery	14
Loamy very fine sand	14	Stones	13
Coarse sandy loam	13	Extremely stony	12
Sandy loam	12	Very stony	11
Fine sandy loam	11	Stony	10
Very fine sandy loam	10	Cobbles	9
Loam	9	Extremely cobbly	8
Silt loam	8	Very cobbly	7
Silt	7	Cobbly	6
Sandy clay loam	6	Gravel	5
Clay loam	5	Extremely gravelly	4
Silty clay loam	4	Very gravelly	3
Sandy clay	3	Gravelly	2
Silty clay	2	N/A	1
Clay	1		