A new composite abundance metric detects stream fish declines and community homogenization during six decades of invasions

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Abstract

Aim: We developed a new technique, utilizing species-specific counts of individuals from historical fish community samples, to examine landscape-level, spatio-temporal trends in relative abundance distributions. Abundance-based historical distribution analyses are often plagued by data comparability issues, but provide critical information about community composition trends inaccessible to those using analyses based only on species presence–absence. We established trends in native and non-native fish abundance and community homogenization, uniqueness and diversity to help local conservation managers prioritize targets and motivate similar studies globally to support fish conservation.

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

Methods: We compiled catch data from 61 years of fish community surveys (1958–2019) and tested for community homogenization by comparing data from repeatedly sampled sites (1900s versus 2000s samples) using dispersion analyses. We measured community uniqueness (site contributions to beta diversity) and species diversity (Shannon index) at sampled streams to identify potential conservation hotspots. We then used regression analyses and Wilcoxon signed-rank tests to examine species-specific basin-wide and local abundance trends and identify species of potential conservation concern.

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

Main conclusions: Our results indicate basin-wide community homogenization has occurred within the UMNR, but many unique and diverse communities persist. If conserved, these could help maintain regional fish diversity. We found basin-wide declines in four endemic species, as well as spread patterns of non-native and native species that were not detected by a presence–absence analysis applied within the
Due to myriad human-induced threats, freshwater fishes are in decline worldwide (Moyle & Leidy, 1992; Su et al., 2021). 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 1,000 times the background extinction rate (Burkhead, 2012; Ricciardi & Rasmussen, 1999). Imperilment of North American freshwater fishes is also on the rise; 700 fish taxa (species, subspecies and evolutionarily significant units) were listed as imperilled by the American Fisheries Society Endangered Species Committee in 2008, representing a 92% increase since 1989 (Jelks et al., 2008; Williams et al., 1989).

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 (Allan & Flecker, 1993; Dudgeon et al., 2006; Helfman, 2007; Miller et al., 1989). 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 can be difficult to assess the relative influence of each factor on decline of individual species. However, the cumulative effects of these threats may manifest in ecological signals across biotic communities at the landscape level (Milardi et al., 2018; Wilcove et al., 1998).

One landscape-level process associated with increases in anthropogenic threats is community homogenization. Also termed taxonomic homogenization, community homogenization refers to a process whereby two or more biotas increase in taxonomic similarity over a specified time period (Olden & Rooney, 2006). Oftentimes, community homogenization occurs because of changes in regional disturbance patterns that 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, specialist and sensitive taxa become less common, and communities become less distinct from one another, a pattern that has been documented globally across a wide range of ecosystems, taxa and disturbance types (Godet et al., 2014; Petrozzi et al., 2015; Rahel, 2000; Ribeiro-Neto et al., 2016; Smith et al., 2009). 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 spatio-temporal scales (Noss, 1990). Although taxonomic diversity has received the most focus in the past, recent studies have increasingly incorporated ecological and genetic diversity (also commonly termed functional and phylogenetic diversity respectively) alongside taxonomic diversity in landscape-level assessments of homogenization (Liang et al., 2019; Nowakowski et al., 2018).

Although assessing all components of biodiversity (taxonomic, genetic and ecological) across all hierarchical levels of organization (landscape, community, population, etc.) 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 spatio-temporal trends in fish community uniqueness and taxonomic species diversity 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 and diverse communities has both economic and ecological advantages over single-species approaches (Angermeier & Winston, 1999; Williams et al., 1989, 2011). Conservation based on community uniqueness and diversity, 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 (Angermeier & Schlosser, 1995; Franklin, 1993). In addition, unique and diverse 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 (Angermeier & Winston, 1999; Franklin, 1993).

To test the efficacy of a community uniqueness and diversity-based approach to targeting areas of conservation need, we chose to focus on the upper and middle New River (UMNR) basin in the Appalachian region of the eastern United States (Figure 1). 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, 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 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 time frame (NID (National Inventory of Dams), 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 (Buckwalter et al., 2018; Jenkins & Burkhead, 1994).

**FIGURE 1** Major landmarks within and nearby the upper and middle New River basin (UMNR; black). Approximate longitude and latitude boundaries (decimal degrees) of the UMNR are −80.1° to −81.7° (east to west) and 37.5° to 36.1° (north to south). Fish collections from the lower New River basin (light grey) were not included in our analysis. Label Abbreviations: USA—Conterminous United States, NC—North Carolina, VA—Virginia, WV—West Virginia. Map projection: Albers equal area.
At least two types of spatio-temporal data are needed to prioritize unique assemblages for conservation in the face of biotic homogenization: species-specific 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 the work of Buckwalter et al. (2018) by analysing local (stream segment) and basin-wide (landscape) 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 landscape- 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 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; research suggests that different characteristics of invaders may be favourable 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 historical presence of unique species and communities within the UMNR, we expected to find evidence of basin-wide biotic homogenization. Thus, our study objectives were to 1. track spatio-temporal fish community uniqueness and diversity trends across the UMNR to determine whether biotic homogenization has occurred; 2. identify species-level abundance trends (spread or decline) that may have contributed to regional biotic homogenization over time and compare them to previously described trends in geographic spread/decline (based on the Buckwalter et al., 2018 occurrence-based analysis within the same study area); and 3. suggest priorities for new potential conservation targets, given population trends of native and non-native fishes and the distribution of unique and diverse communities across the basin.

## Methods

A glossary of acronyms and workflow diagram for the analyses described in this Methods section and referenced throughout the rest of the paper are included in Appendix B.

### 2.1 Study area

The New River flows north through the approximately 7620-km² UMNR study area (Figure 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-kilometres north of the border, effectively isolates fish communities within the UMNR from most West Virginia aquatic communities.

### 2.2 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, now VDWR), the Virginia Department of Environmental Quality (VDEQ), 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 and total fish collected) are detailed in Appendix A. Henceforth, the term "abundance" will be used to refer to species abundance (counts) within samples and indices derived from these counts. Sample abundances are not equivalent to overall population abundance, and no effort was made to estimate actual population abundance of species due to disparate sampling procedures and sparse information on catch per unit effort across input datasets.

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 (U.S. Environmental Protection Agency) (2019)], version 2 flowline), Strahler stream order (Figure 2a) and 10-digit Hydrologic Unit Codes (HUC10s), representing standardized drainage-defined subdivisions of the UMNR (USGS (U.S. Geological Survey), 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 site 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, 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 (C) within a site divided by the total number of individuals captured (T) at that site (Equation 3).

\[ TC_{ij} = \frac{C_{ij}}{C_{\text{Unsample}}} \]  

\[ RA_{ij} = \frac{(SR_j) - ((R_{ij}) - 1)}{SR_j} \]  

\[ PA_{ij} = \frac{C_{ij}}{T_j} \]  

One example of the efficacy of such an approach is the comparison of the abundance of a locally rare species X (rarity defined in this case as low abundance within individual samples) within 2 samples, one involving the sampling of a 100-metre section of a small, second-order stream (sample A) and one of a 200-metre section of a much larger fourth-order stream (sample B). Imagine that 10 individuals of locally rare species X were recorded within each survey, but that due to vastly different sample areas, 50 total fish were captured in sample A versus 250 in sample B. While the TC metric alone would identify the sites as having equal abundances of species X, the RA and PA metrics allow consideration of numeric dominance of species X in comparison with 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 X was more densely populated (Figure 3).

Composite species abundance scores 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 community-wide 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. Thus, local maxima in CSAs for numerically common species are unattainable by most uncommon species, even at the sites where they were most abundant. By scaling the CSAs, we eliminated these discrepancies in
To test for biotic homogenization across the UMNR, we first identified sites that had been sampled during two time periods: 1900s (1958–1999) and 2000s (2000–2019). We then ordinated these samples in SCSA space (Bray–Curtis distance) with non-metric multidimensional scaling (NMDS) (Kruskal, 1964), using the vegan package (Dixon, 2003; Oksanen et al., 2017) in program R (R Core Team, 2018). 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. To assess potential effects of non-native species and stream size on biotic homogenization trends, we compared dispersion across all sites sampled in both time periods. We conducted these comparisons with and without non-native species being included, and between groups of sites assigned to similar stream orders. For the purposes of this manuscript, we refer to "non-native" species as those whose historical distribution does not include the New River basin. This definition encompasses both "alien" species introduced from other continents and "native transplants" that are native to the United States, but have been unnaturally introduced intentionally or otherwise into the New River basin. Past researchers have attempted to resolve the native and non-native status of fishes occurring in the New River (Buckwalter et al., 2018; Jenkins & Burkhead, 1994), so we relied on native and non-native species lists from these publications to inform our native versus non-native classifications.

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 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 $\beta_0 =$ intercept or scale and $\beta_1 =$ 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 in our dataset.

$$
\text{Intercept – only (null) model: } y = \beta_0 \tag{6}
$$

$$
\text{Linear model: } y = \beta_0 + \beta_1 x \tag{7}
$$

$$
\text{Exponential model: } y = \beta_0 e^{\beta_1 x} \tag{8}
$$

**FIGURE 3** Schematic diagram of two idealized fish samples, where fish bowls represent study stream segments with dissimilar sampled habitat volume (sample A and sample B, as referenced in text) and fish symbols indicate number of individuals (catch) of each species (1 fish symbol = 5 individuals). In this example, despite both samples containing the same total catch of Rare Species X, calculated CSA abundance of Rare Species X would be higher within sample A, owing to the higher proportional abundance of Rare Species X and its numerical dominance over Common Species Y within this sample. Fish graphics by Carrie Sleezer
In addition to basin-wide abundance trends, we examined species abundance trends within sites with repeat sample histories including at least one sampling event per time period (1900s and 2000s) by comparing mean SCSA values between time periods for species occurring within at least five repeatedly sampled sites. Nonparametric, paired Wilcoxon signed-rank tests were used to identify significant differences ($\alpha = 0.05$) in site-level mean abundance between time periods, as 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 spatial extents to quantify spread and level of potential threat to native species.

To pinpoint locations worthy of conservation consideration, we identified sampled sites that contributed most to biotic 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 & De Cáceres, 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 that often occurred at site-level SCSAs near basin-wide mean values. Therefore, in addition to analysing 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 highly unique (high LCBD) and locally diverse (high Shannon score) 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 which 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 SCSSAs were also mapped across the basin to identify areas relatively unimpacted by introductions, another potential criterion for prioritization of conservation targets.

$$U - D_j = \frac{\text{LCBD}_j}{\text{LCBD}_{\text{ave}}} + \frac{\text{Shannon}_j}{\text{Shannon}_{\text{ave}}} \quad (9)$$

3 | RESULTS

The final database included 638 unique fish community samples (1900s: $N = 180$ samples, 2000s: $N = 458$ samples) from 357 interconfluence stream segments (sites) across the UMNR (Figure 2b). Of these sites, 145 (23%) were sampled repeatedly (2 to 8 times) during the survey time frame and 56 (12%) were sampled during both the 1900s ($N = 67$) and the 2000s ($N = 109$). Average observed total species richness (natives and non-natives) within these 56 repeatedly sampled sites increased from 12.7 (1900s: range = 3–24) to 15.2 (2000s: range = 4–24) between time periods. Average non-native species richness also increased from 2.8 (1900s: range = 0–11) to 3.9 (2000s: range = 0–9). The number of repeatedly sampled sites with no non-natives detected decreased from 7 in the 1900s (12.5%) to 1 in the 2000s (1.8%). Repeatedly 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 SCSA space between the 1900s and the 2000s. Results of these dispersion tests (Table 1) and inspection of ordination plots (Figure 4) 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 the 2000s, regardless of stream size. The inclusion of non-native species in the data hindered detection of a significant homogenization trend only in small streams (1st and 2nd order).

Given our restrictive inclusion criteria, we were able to model basin-wide abundance trends for 64 species (36 natives and 28 non-natives) and site-level abundance trends for 42 of these (27 natives and 15 non-natives). Of the 42 taxa modelled at both spatial extents, 47.6% (20: 8 natives and 12 non-natives) tested as stable at both extents (Tables 2 and 3), with only two species displaying even marginal patterns ($0.1 > p > 0.05$) of spread or decline at the site level. 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 basin-wide trends, 17 best fit a linear model of spread or decline, while 13 best fit an exponential model. We observed basin-wide declines ($p < 0.05$) for 16 native species (44.4%), including four of five endemic species analysed (Candy Darter [Etheostoma osburni], New River Shiner [Notropis scabriceps], Appalachian Darter [Percina gymnocephala] and Kanawha Minnow [Phenacobius teretulus]). Basin-wide abundance spread was detected in six native species, most of which exhibited high prevalence 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 and 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 declining trend at the basin-wide extent.

To prioritize sites for potential conservation action across the UMNR, we separately mapped sites with communities ranked in the top 10 per cent (63 total) of LCBD (uniqueness) scores, Shannon (diversity) scores and sites within the top 10 per cent of both metrics (Figure 5a–c). We also mapped sites within the bottom 10 per cent of cumulative SCSAs for all non-native species (Figure 5d). 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.

Community uniqueness and diversity varied among time periods and drainages. Although 1900s samples comprised only 180 of the 638 samples included in the analysis (28.2%), 1900s sites were disproportionately represented within the top 10 per cent of LCBD rankings (63.5%, Figure 5a) and in sites ranking within the top 10 per cent of both measures of uniqueness and diversity (52.9%, Figure 5c). The HUC10 drainages with the highest average uniqueness and diversity rankings tended to cluster in the north-eastern portion of the basin, specifically in the Little River and the upper and lower Big Reed Island Creek drainages. In addition, each of these drainages contained several sites where few non-native species occur, even in samples collected since 2000, and had low average non-native SCSA scores in comparison with most other HUC10s.

4 | DISCUSSION

4.1 | Biotic homogenization

Overall, results suggested widespread homogenization of UMNR fish communities over the study time frame (1958–2019). Coincident with this trend were basin-wide declines in abundance for 16 native fishes, including four endemics, and 10 instances of basin-wide abundance spread, mostly by non-native species and common, generalist natives. Locations that appear to have persisting unique and diverse communities, relatively unimpacted by non-native species, include the upper Little River and the upper and lower Big Reed Island Creek drainages. In addition, each of these drainages contained several sites where few non-native species occur, even in samples collected since 2000, and had low average non-native SCSA scores in comparison with most other HUC10s.

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**TABLE 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.

|                         | Number of sites | Mean distance to centroid | p-value |
|-------------------------|-----------------|---------------------------|---------|
|                         | 1900s           | 2000s                     |         |
| Natives only            |                 |                           |         |
| 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 |
| All species             |                 |                           |         |
| 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 |

Note: Tests were applied first across all sites sampled during both centuries and then within subsamples of these sites representing streams of different sizes, determined by Strahler stream order (Strahler, 1957). Significant p-values (p < .05) indicate significant differences between mean distances to point cloud centroids between 1900s and 2000s samples. Significant p-values in bold.
| Native Species                  | Basin-wide trends (Regression results) | Site-level trends (Paired Wilcoxon results) |
|--------------------------------|----------------------------------------|-------------------------------------------|
|                                | n     | Best model | Trend | n     | Trend |                      |
| Campostoma anomalum            | 576   | Lin        | ↑     | 52    | ↑     |                      |
| Catostomus commersonii         | 353   | Lin        | ↓     | 42    |       |                      |
| Chrosomus oreas                | 334   | Lin        | ↑     | 31    |       |                      |
| Clinostomus funduloides        | 388   | Null       |       | 39    |       |                      |
| Cottus sp.                     | 424   | Lin        | ↑     | 45    | ↑     |                      |
| Cyprinella spiloptera          | 63    | Lin        | ↓     | 6     | ↓     |                      |
| Etheostoma blemioides          | 191   | Lin        | ↓     |       |       |                      |
| Etheostoma caeruleum           | 15    | Exp        | ↑     |       |       |                      |
| Etheostoma flabellare          | 561   | Lin        | ↑     | 53    | ↑     |                      |
| Etheostoma kanawhae*           | 223   | Null       |       | 25    |       |                      |
| Etheostoma nigrum              | 24    | Null       |       |       |       |                      |
| Etheostoma osburni*            | 15    | Lin        | ↓     |       |       |                      |
| Etheostoma simoterus           | 19    | Null       |       |       |       |                      |
| Exoglossum laurae              | 102   | Null       |       | 21    |       |                      |
| Hypentelium nigricans          | 470   | Exp        | ↓     | 52    |       |                      |
| Ictalurus punctatus            | 12    | Null       |       | 16    |       |                      |
| Lepomis cyanellus              | 122   | Null       |       | 20    | ↑     |                      |
| Luxilus alboles/cornutus       | 234   | Null       |       | 27    | ↓     |                      |
| Luxilus chrysophealus          | 13    | Null       |       |       |       |                      |
| Lythrurus ardens               | 119   | Exp        | ↓     | 12    |       |                      |
| Nocomis sp.                    | 551   | Null       |       | 53    | ↑     |                      |
| Notropis rubellus/micropteryx  | 167   | Exp        | ↓     | 21    |       |                      |
| Notropis photogenis            | 112   | Null       |       | 16    |       |                      |
| Notropis scabriceps*           | 148   | Exp        | ↓     | 20    |       |                      |
| Notropis volucellus            | 68    | Exp        | ↓     | 8     |       |                      |
| Noturus insignis               | 190   | Null       |       | 21    |       |                      |
| Percina caprodes               | 14    | Lin        | ↑     |       |       |                      |
| Percina gymnocephala*          | 133   | Lin        | ↓     | 21    |       |                      |
| Percina oxyrhythenus           | 28    | Lin        | ↓     |       |       |                      |
| Phenacobiis teretulus*         | 167   | Lin        | ↓     | 22    |       |                      |
| Pimephales notatus             | 182   | Exp        | ↓     | 25    |       |                      |
| Pylodictis olivaris            | 30    | Exp        | ↓     |       |       |                      |
| Rhinichthys cataractae         | 300   | Null       |       | 42    |       |                      |
| Rhinichthys atratulus/obtusus  | 394   | Lin        | ↓     | 42    |       |                      |
| Salvelinus fontinalis          | 54    | Lin        | ↓     | 9     |       |                      |
| Semotilus atromaculatus        | 207   | Null       |       | 21    |       |                      |

Note: 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 with 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 (α = 0.05), comparing SCSAs between 1900s and 2000s samples. Black arrows indicate significant spread (↑) and decline (↓). Site-level trends suggested by marginal p-values (0.05 < p < 0.10) are also shown (grey arrows).

↑—spread, ↓—decline, ↑—marginal spread, ↓—marginal decline, *—endemic species
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TABLE 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 repeatedly sampled sites where each species occurred)

| Non-native Species | Basin-wide trends (regression results) | Site-level trends (paired Wilcoxon results) |
|--------------------|----------------------------------------|--------------------------------------------|
|                    | n          | Best model | Trend | n | Trend |
| Ambloplites rupestris | 421        | Null       |       | 47 |
| Ameiurus natalis     | 12         | Null       |       |    |
| Ameiurus nebulosus   | 15         | Null       |       |    |
| Cyprinella galactura | 89         | Null       |       | 13 |
| Cyprinus carpio      | 10         | Exp        | ↑     |    |
| Etheostoma olmstedi  | 26         | Null       |       |    |
| Etheostoma rufilinatum | 10       | Exp        | ↑     |    |
| Exoglossum maxillina | 32         | Null       |       |    |
| Hybopsis hyspinotus  | 10         | Exp        | ↑     |    |
| Lepomis auritus      | 233        | Lin        | ↓     | 26 | ↑    |
| Lepomis gibbosus     | 40         | Null       |       | 6  | ↓    |
| Lepomis macrochirus  | 131        | Null       |       | 18 |
| Luxilus cerasinus    | 140        | Null       |       | 16 |
| Luxilus coccogenis   | 74         | Null       |       | 13 | ↑    |
| Micropterus dolomieu | 260        | Lin        | ↓     | 33 |
| Micropterus punctulatus | 28        | Lin        | ↓     |    |
| Micropterus salmoides | 54         | Null       |       | 10 |
| Moxostoma cervinum  | 26         | Null       |       |    |
| Notropis chiliticus  | 50         | Null       |       | 8  |
| Notropis hudsonius   | 41         | Null       |       | 7  |
| Notropis leuciodus   | 15         | Null       |       |    |
| Notropis proce     | 13         | Null       |       |    |
| Notropis rubricorpus | 152        | Null       |       | 17 |
| Notropis telescopus | 116        | Null       |       | 18 | ↑    |
| Oncorhynchus mykiss | 147        | Null       |       | 26 |
| Percina roanoka     | 32         | Exp        | ↓     |    |
| Pimephales promelas | 19         | Exp        | ↑     |    |
| Salmo trutta        | 199        | Null       |       | 20 |

Note: 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 with 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 (α = 0.05), comparing SCSAs between 1900s and 2000s samples. Black arrows indicate significant spread (↑) and decline (↓). Site-level trends suggested by marginal p-values (0.05 < p < .10) are also shown (grey arrows).

↑—spread, ↓—decline, ↑—marginal spread, ↓—marginal decline.

have generally taken one of two paths to describe these trends:
1. compiling historical species lists at multiple hierarchical spatial extents (sub-watersheds, watersheds, countries, continents and global) and relying on changes in species occurrences across these areas to isolate potential homogenization trends (Castaño-Sánchez et al., 2018; Kirk et al., 2020; Peoples et al., 2020; Rahel, 2000; Sommerwerk et al., 2017; Vargas et al., 2015; Villéger et al., 2011); and
2. collecting contemporary data to compare communities subject to different environmental conditions in terms of species richness, proportional abundance or occurrence of resident species (Clavero & Hermoso, 2011; Dai et al., 2020; Scott & Helfman, 2001). The first method ignores signals of homogenization that are undetectable by analysis of occurrence data alone, including shifts in numerical abundance of component species that may signify ongoing invasions and impacts from non-native taxa, population expansions by native taxa that are resistant to pervasive human-induced habitat changes, and declines in populations of sensitive native species in response to relevant stressors that have not yet caused extirpation. The second suffers from lack of historical information key for quantifying the extent and rate of regional
species spread and decline that contribute to homogenization. Our methods took advantage of both historical and contemporary fish collections to analyse homogenization at spatial scales relevant to regional managers, thereby enabling assessments of changes in fish population abundance and proportional abundances of taxa across communities.
Such abundance-based analyses of homogenization between past and present communities and/or abundance spread or decline in component species have been conducted in reservoirs, where historical commercial catch data are available (Brito et al., 2020), and in other vertebrate taxa where established abundance survey protocols are in place (i.e., the Breeding Bird Survey; La Sorte & McKinney, 2007). However, such analyses have been avoided in lotic, freshwater environments, presumably due to a lack of such standardized protocols available across sources of historical data. As such, our study provides unique insight and unique methods for quantifying the severity of biodiversity losses over time within lotic freshwater ecosystems.

### 4.2 Species-level abundance trends

Past studies within the UMNR have emphasized declines of a few resident species (Buckwalter et al., 2018; Dunn & Angermeier, 2019), but none has indicated the mass community- and species-level losses in fish diversity suggested by our analysis. 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 species as spreaders or decliners (as a function of occurrence-based range size within HUC12 drainages), but many of their detected species-specific trends differ from ours. Comparing basin-wide models of fish distribution between our study and Buckwalter et al. (2018), 50% of spreaders but only 10% of decliners in our study were assigned the same trend (spread or decline) as in the Buckwalter et al. (2018) analysis. Only two native species were classified as decliners in both studies (Rosyface Shiner [Notropis rubellus] and Sharpnose Darter [Percina oxyrhynchus]), hereafter referred to as concordant decliners.

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 ongoing non-native invasions. Because our basin-wide abundance trends are based on mean 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 abundance decline we observed for non-native Redbreast Sunfish. The Buckwalter et al. (2018) occurrence-based study identified Redbreast Sunfish as a strong geographic spreader, and this provides important perspective when interpreting our results. Rather than signifying a range-wide decline, it appears that the establishment of new, small populations of Redbreast Sunfish along the periphery of the species’ expanding range within the UMNR is the primary driver of perceived decrease in average abundance across occupied sites. In other words, the rate of local population increase seems to be lagging behind the establishment of new populations in the ongoing invasion of Redbreast Sunfish. Lag phases in spatial expansion and local population increase are common in non-native species invasions (Crooks, 2005), but this particular finding could be elucidated only by pairing range-wide spatio-temporal presence–absence and abundance analyses within the same study area. Another plausible but untested explanation for this pattern is that Redbreast Sunfish is spreading into suboptimal habitats where local populations will remain small. In any case, understanding the pattern and process of ongoing non-native invasions is crucial to anticipating and mitigating potential impacts to native fauna.

Integration of abundance and occurrence methods is needed to adequately summarize distribution trends of all types of conservation-relevant species. These species include declining natives, spreading non-natives and spreading native generalist populations that may signal degradation in habitat or water quality. Examples of each of these three groups of species (Figure 6) 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 sometimes 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 abundance of this species across the UMNR (Figure 6b). Non-native Warpaint Shiner (Figure 6c) and Redbreast Sunfish both exhibited site-level abundance increases in our analysis, and occurrence analyses indicate modest to large 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 6a), whose distribution may be limited by riparian canopy that limits sunlight penetration and thus the periphyton 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.
Studies analogous to Buckwalter et al. (2018), identifying range expansions and contractions of native and non-native species, are relatively common in the literature (Allen & Mandrak, 2019; Habit et al., 2010; Olden & Poff, 2005), but these studies are very rarely paired with abundance-based analyses similar to our SCSA approach. A positive relationship between abundance and occupancy has been documented for a wide variety of species groups, suggesting that occupancy is a useful proxy for abundance in descriptions of species distributions (Gaston et al., 2000). However, past research suggests that analyses of abundance may have advantages over presence–absence analyses in the context of detecting species’ responses to landscape-scale stressors, thereby offering insight into mechanisms of range expansions and contractions. For example, in Maryland (USA) streams, Utz et al. (2010) found that sensitivity of several fish species to land use (urban and agricultural) was only detectable when using an abundance-weighted response metric. Similarly, Steel et al. (2012) found that spawner abundance of Coho Salmon (Oncorhynchus kisutch) in the Oregon Coastal Province (USA) correlated more strongly with known landscape-scale stressors than did presence–absence of spawners. Although linking patterns of species abundance to specific environmental stressors was outside the scope of our study, incorporating some measure of abundance in studies of current and past spatial distributions of fish species could help illuminate trends in species responses to relevant landscape stressors that may not be apparent when focusing on occurrence patterns alone.

4.3 Unique and diverse communities: Prioritizing future conservation targets in the UMNR

In addition to species-level trends, our study also revealed several unique and diverse communities that may warrant conservation. While we revealed more uniqueness and diversity among UMNR fish communities during the 1900s, several highly unique and diverse communities still persist, especially 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 reported declines in UMNR range size (Rosyface Shiner and Sharpnose Darter; Buckwalter et al., 2018). The previously named hotspot drainages each contain recent occurrences of at least four of these species.

Recognizing hotspot drainages may be a first step towards 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 use of live bait and stocking non-native species within the waters it occupies (USFWS (U.S. Fish and Wildlife Service), 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 (North Carolina Wildlife Resources Commission), 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 already been suggested (Angermeier & Schlosser, 1995; Angermeier & Winston, 1999; Collares-Pereira & Cowx, 2004; Rohlf, 1991), yet application of community conservation approaches is hindered by a number of factors. Perhaps the most influential factor discussed by the authors cited above is the fact that community conservation has no direct legal mandate in the United States. As such, species-specific approaches, as mandated under the federal Endangered Species Act and state analogs, currently rule most fish conservation in the United States. Consequently, actions 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 (Burlakova et al., 2011; Collares-Pereira & Cowx, 2004).

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 adverse effects of intensive land use on adjacent stream communities, ranging from increased sediment loading to altered stream temperatures, and live-bait restrictions to limit the spread of non-native species (NCWRC (North Carolina Wildlife Resources Commission), 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 (Sukki & 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.
4.4 | Transferability and efficacy of methods

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

Globally, recent efforts suggest a trend towards making existing fish community data more readily available to researchers conducting landscape-level analyses on fish biodiversity. So far, efforts to compile global fish community databases have focused on establishing species lists (occurrence records) for the world's major drainage basins (Tedesco et al., 2017) and isolating data from long-term monitoring sites (stream reaches) subject to routine sampling visits and standardized sampling and data management practices (Comte et al., 2020). Consistent, repeatable long-term monitoring, such as what has occurred in those sites included in the Comte et al. (2020) RivFishTIME database, remains the gold standard for time series ecological analyses. However, in most areas across the globe, sites subject to such intensive sampling remain scarce at spatial scales relevant to local fish conservation efforts. Similarly, the lack of abundance information and coarse spatial resolution of species lists in the Tedesco et al. (2017) database limit its utility to inform studies at finer spatial grains. Future efforts to compile all publicly available historical freshwater fish community data worldwide, regardless of sampling method or sample history, would be required to adequately assess where studies like ours are possible.

Limited availability of long-term monitoring sites necessitates use of data collected using disparate sampling methods and fragmented survey efforts to track spatio-temporal fish community trends in study areas similar to the UMNR. 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, Teixeira-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 electrofishing methods (which make up the bulk of the data included), as suggested by these studies, we use of novel SCSAs should have reduced the inherent differences in sampling efficiency and intensity by modifying catch-based abundance estimates using community proportion and abundance ranks. Furthermore, Pritt and Frimpong (2014) suggested that sampling intensity tends to correlate positively with observed numerical abundance of rare species but negatively with proportional abundance estimates of these same rare species. Thus, the adoption of composite metrics, such as SCSAs, may help provide abundance estimates for particularly conservation-relevant, rare species more reflective of actual abundance and less influenced by variations in sampling intensity.

5 | CONCLUSIONS

Available historical fish community data in the UMNR suggest basin-wide community homogenization, widespread instances of decline in specific native species, and spread of non-native and common native species over the past six decades. These phenomena suggest an overall trend of degrading aquatic communities, but highly unique and diverse communities still remain, providing opportunity for conservation of remaining biodiversity in these potential conservation hotspots across the UMNR. Unique and diverse communities tend to contain species that may be considered at risk of extinction in the near future, so focusing conservation efforts on these areas may prevent future losses of unique aquatic fauna. SCSAs, as a new method for utilizing historical species abundance information to assess community- and species-level trends, allow comparison of historical samples collected using different techniques and sampling intensities, help identify unique and diverse communities and may be more sensitive to early signatures of species declines than other commonly used response metrics. Many other drainages in the United States and around the globe face similar fish conservation and data management challenges as the UMNR and, as such, could make use of similar study protocols to assist with prioritization of their own freshwater conservation targets.

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CONFLICT OF INTEREST
The authors report no conflict of interest.

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**BIOSKETCH**

Logan J. Sleezer is an MS graduate from Virginia Tech. He developed an MS thesis focusing on spatio-temporal abundance trends of native freshwater fish species and the effects of non-native species and land use on these species at multiple spatial scales.

Author contributions: L.J.S. compiled data, conducted all supplementary fieldwork and led writing efforts. All authors contributed to editing the manuscript, with P.L.A. acting as chief editor. L.J.S., E.A.F. and P.L.A. all contributed heavily to project design. E.A.F. and B.L.B. assisted L.J.S. with statistical analyses and interpretation of results.

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

- Data inclusion standards
  a. Collections must include over 100 individual fish.
  b. Collections must include more than one species.
  c. Collections must contain non-game species (species within the Catostomidae, Cottidae, or Cyprinidae families and/or species within the Etheostoma, Percina, or Noturus genera).
  d. Collections where metadata suggest sampling only occurred in 1 habitat type (riffle, run, or pool) were excluded.
  e. 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.
  f. 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 per cent of records changed)
  a. Mismatches between "GNIS_name" field for nearest stream segment in the NHDPlus V2 flowlines shapefile (USEPA, 2019) and the stream name in the metadata for the collection record.
  b. 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.

b. 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: there were 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 oregas, 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, Notemigenus crysoleucus, Notropis photogenis, Notropis scabriceps, Notropis telescopis, Percina caprodes, Phenacobius teretulus, Pomoxis annularis, Pomoxis nigromaculatus, Pylodictis olivaris, Sander vitreus

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**TABLE A1** Taxonomic resolution edits and correction decisions for final New River species abundance database

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

*Reasons for Revision: 1—Difficulty in identifying collected individuals to species in the field; 2—taxonomic revisions (Lachner & 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 time frame; 3—genetic analyses indicate uncertainty between these species within the New River basin; and 4—species records renamed due to likely misidentifications.*
APPENDIX B

GLOSSARY OF ACRONYMS AND WORKFLOW DIAGRAM

Location terms
UMNR—Upper and middle New River basin

Fish collection data sources: Data-contributing organizations
VDGIF (now VDWR)—Virginia Department of Game and Inland Fisheries
VDEQ—Virginia Department of Environmental Quality
NCDEQ—North Carolina Department of Environment Quality
NCWRC—North Carolina Wildlife Resources Commission

Sample abundance response metric, component metrics and equation terms
Equation Terms: C—total sample catch (count), SR—species richness, R—sample abundance rank, T—total number of individual fish, i—species, j—sites
TC—scaled total catch metric (one of three components of SCSAs; 
\[ TC_{ij} = \frac{C_{ij}}{C_{i_{\text{max}}}} \]
RA—rank abundance metric (one of three components of SCSAs; 
\[ RA_{ij} = \left( \frac{\text{SR}_j - (R_{ij} - 1)}{\text{SR}_j} \right) \]
PA—proportional abundance metric (one of three components of SCSAs; 
\[ PA_{ij} = \frac{C_{ij}}{T_j} \]
CSAs—composite species abundance scores (unscaled site abundance scores for species; \[ CSA_{ij} = [TC_{ij} + RA_{ij} + PA_{ij}]_j \]
SCSAs—scaled composite species abundance scores (final response metric, consisting of scaled site abundance scores for species; \[ SCSA_{ij} = \frac{CSA_{ij}}{CSA_{i_{\text{max}}}} \]

Other input data, data display and analysis methods
NHDplus—National Hydrography Dataset flowlines (stream segments; used as sampling units)
HUC10—Hydrologic Unit Code 10 watersheds (watershed units used to compile and display spatio-temporal variability in fish diversity and uniqueness across the UMNR; Figure 5)
NMDS—non-metric multidimensional scaling
AICc—Akaike’s information criterion (second-order)
LCBDs—local contributions to regional beta diversity
U-D index—uniqueness and diversity index (combination of site-level LCBD ranks (uniqueness) and Shannon diversity ranks (diversity); Figure 5c)

FIGURE B1  Workflow diagram displaying linkages between data inputs, response metric calculation and project endpoints (statistical analyses and hotspot maps) for the current manuscript. Grey shapes represent integral components of the study (rectangles—datasets, circles and ovals—calculated metrics, diamonds—models, triangles—statistical analysis methods, pentagons—plots and maps), and block arrows represent linkages between these components. The black ribbon contains novel equations for derived response metrics and models, along with references for the more commonly used models and metrics (white text). Equation terms: C—total sample catch (count), SR—species richness, R—sample abundance rank, T—total number of individual fish, i—species, j—sites.
Conservation terms
- SCGN—species of greatest conservation need
- FPAs—freshwater protected areas

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