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ARTICLE

Fragmentation alters ecological gradients and headwater fish assemblage composition relative to land use in a dendritic river system

Joshua P. Hubbell, Jake F. Schaefer, Peter Flood, Melvin L. Warren, Jr., and Ken A. Sterling

Abstract: The spatial and hydrological properties of headwaters allow dendritic systems to contribute to patterns of regional diversity. However, such ecological gradients may be disrupted as a result of habitat fragmentation. We tested the hypothesis that coarse-scale anthropogenic disturbances such as upstream land use and proximity to reservoirs can alter ecological gradients, thus influencing instream habitat, headwater fish assemblage composition, and species turnover in the Little Tallahatchie River system in north-central Mississippi. To test this hypothesis, we calculated species turnover coefficients, ordinated samples, and examined the correlations between assemblage composition and environmental and anthropogenic variables. Assemblage composition was strongly correlated with instream habitat and river system connectivity, and instream habitat was strongly associated with land use. Gradients in assemblage composition associated with ecological factors were altered due to land use. Our research highlights the importance of headwaters as distinctive habitat patches driving species turnover and the influence of land use in disrupting the ecological gradients that allow for the formation of these distinctive habitat types.

Résumé : Les propriétés spatiales et hydrologiques des cours d'eau d'amont permettent à des réseaux dendritiques de contribuer à définir les motifs de diversité régionale. La fragmentation des habitats peut toutefois entraîner la perturbation de tels gradients écologiques. Nous avons testé l'hypothèse selon laquelle des perturbations d'origine humaine d'échelle grossière telles que l'utilisation du sol en amont et la proximité de réservoirs peuvent modifier des gradients écologiques, influençant du coup les habitats dans les cours d'eau, la composition des assemblages de poissons des cours d'eau d'amont et le renouvellement des espèces dans le réseau de la rivière Little Tallahatchie, dans le centre-nord du Mississippi. Pour tester cette hypothèse, nous avons calculé des coefficients de renouvellement des espèces, ordonné des échantillons et examiné les corrélations entre la composition des assemblages et des variables environnementales et anthropiques. La composition des assemblages est fortement corrélée à la connectivité des habitats dans les cours d'eau et celle du réseau hydrographique, et il y a une forte association entre les habitats dans les cours d'eau et l'utilisation du sol. Des gradients de la composition des assemblages associés à des facteurs écologiques sont altérés en raison de l'utilisation du sol. Nos travaux font ressortir l'importance des cours d'eau d'amont en tant que parcelles d'habitats distinctifs modulant le renouvellement des espèces, ainsi que l'influence perturbatrice de l'utilisation du sol sur les gradients écologiques qui permettent la formation de ces types d'habitats distinctifs. [Traduit par la Rédaction]

Introduction

Typified by their branching pattern, headwaters, though small in size, are numerically abundant as the density of these habitats increases with increasing distance from the base of a dendritic system (Dodds and Rothman 2000). Headwaters account for 80% of the surface water within a river system (Colvin et al. 2019) and can be quite unstable (Ostrand and Wilde 2002; Grenouillet et al. 2004), while providing habitat to unique assemblages of fish (Paller 1994). These unique properties result in these systems contributing disproportionately to patterns of regional diversity. Historically, physical changes to habitat structure and associated variability in aquatic biota was viewed as a function of stream size (Vannote et al. 1980; Matthews and Robison 1998). However, the role of connectivity and flow directionality on dispersal patterns of headwater fishes is increasingly recognized (Olden et al. 2001; Le Pichon et al. 2009; Schmidt and Schaefer 2018).

Both stream fish assemblage structure and composition are influenced by the branching structure of a dendritic river system (Campbell Grant et al. 2007; Hitt and Angermeier 2008; Thornbrugh and Gido 2010). Attributes such as river system connectivity, centrality, and drainage density influence the roles of mass effects and species sorting on meta-assemblage dynamics (Altermatt et al. 2013) and species distributions (Göthe et al. 2013; Cañedo-Argüelles et al. 2015; Kärnä et al. 2015). Mass effects describe how dispersal along these lotic pathways facilitates the persistence of fishes even in degraded habitats. In opposition, species sorting explains how reduced dispersal encourages niche specialization as a consequence of the variation in the quality and arrangement of headwater patches. The links among fish assemblages, stream habitat, stream size, and river system connectivity are well established (Smith and Kraft 2005; Thornbrugh and Gido 2010). Confluences prompt abrupt changes in stream size and downstream habitat heterogeneity (Benda et al. 2004); therefore, these habitats likely function as important dispersal pathways of stream fishes within a dendritic river system (Campbell Grant et al. 2007). Consequently, rates of species turnover are strongly influenced by this dendritic structure (Taylor and Warren 2001; Labonne et al. 2008; Zbinden and Matthews 2017).

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Spatial turnover and nestedness are both components of beta diversity (Baselga 2010) and may account for the total variation in assemblage composition within a dendritic river system. Nestedness occurs when an assemblage consisting of few species is representative of a subset of a more speciose assemblage. Spatial turnover occurs when there is no overlap in composition between two assemblages, a likely outcome of habitat filtering (Qian et al. 2005; Baselga 2010). For example, a tributary would not contain fishes found within the main stem; instead, headwaters would be characterized by distinct assemblages. Headwater areas contribute to gamma diversity because of spatial turnover in species composition (Zbinden and Matthews 2017). Turnover can be a product of the spatial structuring of dendritic river systems, environmental filters, or both (Horwitz 1978; McNeely 1986).

Urban and agricultural land use fragment stream habitats (Leitão et al. 2018), and the deleterious effects associated with both land use types on aquatic ecosystems are widely recognized (Wang et al. 2001; Allan 2004; Leal et al. 2016). Increases in agricultural and urban land use degrades water quality and alters channel hydrology (Allan 2004), facilitating habitat fragmentation (Allan et al. 1997) and altering fish assemblage structure (Wang et al. 2001). Reservoirs can be deleterious anthropogenic structures on river systems, resulting in habitat fragmentation (Falke and Gido 2006). The homogenization of lotic habitats because of incised stream channels and the construction of impoundments has accelerated the dispersal rate of invasive species, furthering the instability of many North American stream fish assemblages (Gido and Brown 1999; Gido et al. 2009). Impoundments alter water chemistry and hydrological factors (e.g., discharge), which together modify resident fish assemblages (Herbert and Gelwick 2003; Guenther and Spacie 2006). Ecological research has examined the downstream effects of impoundments (Petts 1984; Collier et al. 2000), but there is a need to further understand upstream effects of reservoirs on headwater fish assemblages (Herbert and Gelwick 2003). The homogenization of headwater fish assemblages in littoral zones increases extirpation rates in isolated tributaries (Lienesch et al. 2000). Ultimately, habitat fragmentation, as a result of impoundment or urban and agricultural land use, degrades river system connectivity, limiting the dispersal ability of headwater fishes (Fagan 2002; Sterling et al. 2012; Fluker et al. 2014), thus altering assemblage structure and composition (Perkins and Gido 2012; Reuter et al. 2019). The long-term persistence of stream fishes may decline as a consequence of habitat fragmentation, though the influence of river system connectivity may mitigate the influence of reservoirs or fine-scale fragmentation (e.g., culverts, small impoundments; Falke and Gido 2006; Perkins and Gido 2012).

We examined the effects of ecological (stream size and river system connectivity) and anthropogenic (land use and impoundment) gradients on resident headwater fish assemblage composition and instream habitat within a dendritic river system. We contrast anthropogenic land use and the proximity of large-scale impoundment to distinguish whether either form of disturbance more strongly influences instream habitat variability and thus headwater fish assemblage composition. We tested the hypotheses that (*i*) headwater fish assemblage composition would be strongly related to instream habitat and river system connectivity gradients and, to a lesser extent, stream size, (*ii*) patterns of nestedness or spatial turnover in headwater fish assemblage composition would change along anthropogenic gradients, (*iii*) instream habitat will be strongly influenced by stream size and anthropogenic gradients, and (*iv*) variation in instream habitat as a consequence of anthropogenic gradients would aid in the degradation of natural stream size and river system connectivity gradients driving headwater fish assemblage composition. To test these hypotheses, we calculated species turnover coefficients (Baselga 2010), ordinated samples along environmental and anthropogenic gradients, and tested the correlations among headwater fish assemblage matrices (presence–absence), instream habitat variability, and coarse-scale environmental and anthropogenic variables.

Materials and methods

Study system

We conducted our study in the Little Tallahatchie River system (henceforth LTR), which is positioned within the upper Yazoo River basin in north-central Mississippi. The LTR system is characterized by the large (398 km^2) Sardis Reservoir. The Sardis Reservoir was constructed by the federal government following the Great Flood of 1927. Approximately 500.9 km^2 of the LTR system occurs within Holly Springs National Forest. Much of the surrounding land use is forested (64%); however, there is a strong agricultural and pastoral land use influence (28%). Therefore, the upper LTR serves as an excellent study system to explore discrepancies in the effects of land use and a large-scale impoundment on instream habitat and headwater fish assemblage composition. We sampled 58 streams (Fig. 1), all of which had a drainage area of ≤50 km^2 within the LTR, 23 of which were sampled between 1999 and 2003 (Sterling et al. 2013) and 35 that were sampled between 2015 and 2016 (Hubbell et al. 2020). Sampling procedures were identical between historical and contemporary surveys (Sterling et al. 2013), and prior analyses indicated that environmental variation between the two datasets attributed to temporal variation was minimal (Appendix A, Table A1). Previous work has also shown that stream fish communities in this system tend to be stable (Schaefer et al. 2012).

Data collection and organization

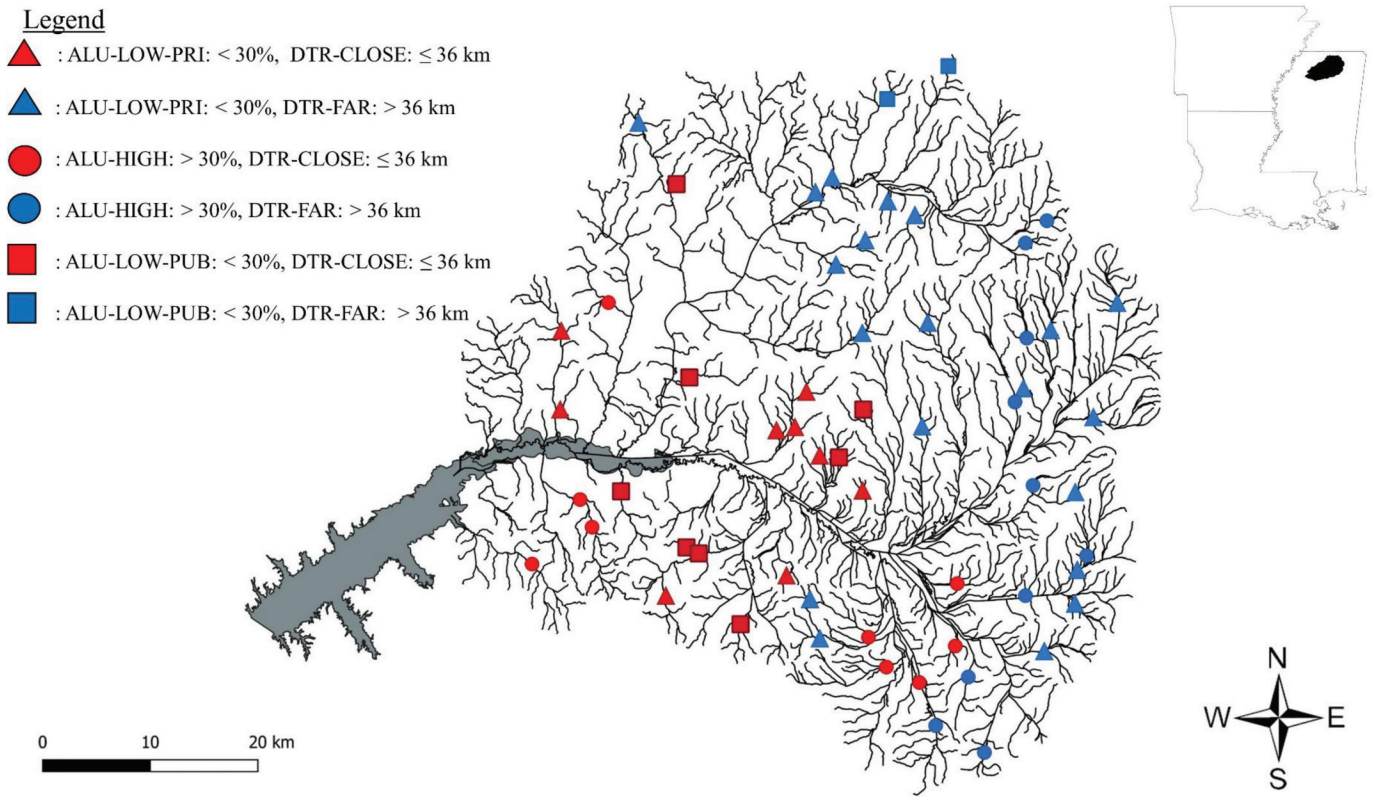
Field methods

We sampled fish assemblages in the late spring and summer (May–September). To ensure streams were sampled with similar effort with respect to the historical surveys (Sterling et al. 2013), stream sample lengths were 30 times the mean wetted width of the streambed, resulting in sample lengths between 120 and 300 m across all sites. Within each reach, backpack electrofishing and seining effort was standardized (eight seine hauls and 5 s·m^{-1} electrofishing), and an attempt was made to sample all available habitat types. Two seine hauls were performed within each subreach; spacing between seine hauls was dependent on habitat complexity.

Fishes were fixed in 10% formalin and later preserved to 70% ethanol, identified, enumerated, and deposited in the University of Southern Mississippi Ichthyological Collection. All sampling protocols were approved by an Animal Care and Use Committee (IACUC 09-007). We dropped rare species (≤3 total occurrences; Fig. A1), producing a 58 × 44 matrix (58 samples, 44 species). Rare species often represent shared zeros among communities and should therefore be removed prior to conducting any analyses (McCune et al. 2002).

We collected habitat variables along 12 evenly spaced transects perpendicular to flow at each site. At three points along each transect, depth (m), current velocity (m·s^{-1}), substrate size (modified Wentworth scale, eight categories; Cummins 1962), and the presence (binary variable) of both small wood (<10 cm diameter or <1.5 m in length) and large woody debris (>10 cm diameter or >1.5 m in length; Table A2) were measured. Substrate size represented the mean size of alluvial material at a site. The percent occurrence of small and large woody debris was condensed into one variable (woody structure) because the two variables were highly correlated ($r > 0.5$). We quantified mean values for all habitat variables within a site. Also at each site, we collected physiochemical variables, including dissolved oxygen (mg·L^{-1}), water temperature (°C), and conductivity (μS·cm^{-1}) (Table A2). Because these were the only water quality variables measured historically, we did not quantify any other water quality parameters during our current surveys. For model parsimony, dissolved oxygen and

Fig. 1. Site localities positioned within the Little Tallahatchie River System for all samples included in analyses. Colours represent our two distance to reservoir categories: red = ≤36 km and blue = >36 km. Shapes represent our three ALU (anthropogenic land use) categories: triangles = <30%, ALU-LOW-PRI; squares = <30%, ALU-LOW-PUB; and circles = ≥30% ALU-HIGH. See Materials and methods for description of terms. [Colour online.]



temperature were not included in any of our analyses due to their limited explanatory power. Thus, conductivity was the only water quality variable included in our analyses.

Data processing

Landscape variables were extracted from stream attributes (drainage area and confluence link) in the National Hydrography Data Plus (NHDPlus; USEPA and USGS 2006) using ArcGIS (version 10.0; ESRI 2011). We used the NHDPlus waterbody layer to delineate the mouth of Sardis Reservoir. River system connectivity (how sites differ in connectedness) was assessed as confluence link by counting the number of confluences downstream from each stream reach (Fairchild et al. 1998). Confluence link values decrease in a given watershed from extreme headwaters to the base of a stream network (mouth of Sardis Reservoir in this case). Distance to reservoir (DTR) was measured as the river distance between a site and the mouth of Sardis Reservoir. We extracted land use data for 2001 and 2011 from the National Land Cover Database (NLCD; https://www.mrlc.gov/data). These years were used because they were the closest when sites were sampled. NLCD data includes 20 classes of land use that we reclassified into five broad land use variables: forested, urban, wetland, open water, and agricultural. We then estimated the relative area of each land use type associated with each site's upstream drainage area. The percentage of anthropogenic land use (ALU) within a site's upstream drainage (from NHDPlus catchment layer) was then calculated as the sum of the percentages of urban and agricultural land use. We did not include open water because of the presence of small impoundments and Sardis Reservoir.

We classified sites with four categorical variables describing anthropogenic land use, distance to reservoir, drainage area, and confluence link. Because urbanization is limited (<4%) across the LTR system, agricultural land cover represents the dominant form of anthropogenic land use. Evidence suggests that drainages represented by >30% agricultural land use (Quinn 2000; Fitzpatrick et al. 2001) may be distinguished by markedly different instream habitat characteristics and thus distinct biotic communities, which we used as our breaking point. Therefore, we used two broad levels to delineate sites based on the percentage of anthropogenic land use in the upstream drainage (ALU-HIGH = ≥30% and ALU-LOW = <30%, 19 and 39 sites, respectively). Upstream drainages associated with reduced anthropogenic land use were further divided into two subcategories: drainages that were characterized by mostly forested, private (ALU-LOW-PRI) or public (ALU-LOW-PUB) land (29 and 10 sites, respectively), under the assumption that privately owned forested lands (e.g., pine plantations) would be subject to more intensive degradation. We used three levels of drainage area to define stream size categories (small ≤10 km^2, medium = 10 to 20 km^2, large ≥ 20 km^2, 22, 16, and 20 sites, respectively). We used two levels of confluence link to define river system connectivity gradients (C-link-LOW ≤ 10 confluences (i.e., large or streams draining directly into the main stem) between site and mouth of Sardis Reservoir; C-link-HIGH ≥ 10 confluences (i.e., headwaters) between site and mouth of Sardis Reservoir, 18 and 40 sites, respectively). Finally, we recognized two distance to reservoir categories (DTR-CLOSE = 0 to 36 km and DTR-FAR > 36 km, 27 and 31 sites, respectively).

Data analyses

Data transformations

We included river distance to reservoir (RIV-DTR), the percentage of anthropogenic land use (PCT-ALU), drainage area (DA), and confluence link (C-link) as continuous variables within our multi-

Table 1. Environmental and anthropogenic variables included in all partial Mantel tests.

Category	Variable	Unit	Transformation
Land use	PCT-ALU	Percentage in upstream drainage	Logit
Instream habitat	HAB1	PC1 axis scores	None
	HAB2	PC2 axis scores	None
Reservoir	RIV-DTR	km	$\log_{10}(x + 1)$
Connectivity	Confluence link	No. of confluences downstream	$\log_{10}(x + 1)$
Stream size	Drainage area	Drainage size in km^2	$\log_{10}(x + 1)$
Spatial	Geographic locality	Decimal-degrees	$\log_{10}(x + 1)$
	River distance between samples	km	$\log_{10}(x + 1)$

Note: Stream size and spatial distance matrices were included as confounding factors in all models.

variate analyses to distinguish gradients associated with land use, river system connectivity, and Sardis Reservoir. We log-transformed conductivity, current velocity, substrate size, depth, DA, RIV-DTR, and C-link to ensure these variables' distributions met the assumption of normality. We used the logit transformation on proportional variables (woody structure and PCT-ALU). We performed all analyses in R (version 3.2.4; R Development Core Team 2007).

Spatial turnover and nestedness

Beta diversity, or total species turnover, may be calculated for a whole region or for groups within a region (Baselga 2010). Species turnover (β_{sor}) is a summation of two additive components, (*i*) spatial turnover (β_{sim}) and (*ii*) nestedness (β_{nes}), whereby $\beta_{sor} = \beta_{sim} + \beta_{nes}$ (Baselga 2010). We used the package "betapart" (Baselga and Orme 2012) to quantify coefficients for β_{sim} and β_{nes} to distinguish whether the degree of spatial turnover or nestedness of headwater fish assemblage composition varied in relation to levels of our categorical, ecological (drainage area and confluence link), or anthropogenic factors (anthropogenic land use and distance to reservoir; Baselga 2010). We calculated coefficients for both components using beta.multi method. The beta.multi method calculates pairwise beta coefficients for all pairs of samples and then calculates the mean across all pairs of samples for both components. We chose a subsample size of $n = 10$ sites because this was the smallest sample size for any factor level. We set the number of iterations at 1000 bootstrap iterations.

Relations of instream habitat to ecological and anthropogenic gradients

We used principal components analysis (PCA) to reduce the dimensionality of our environmental data (substrate size, depth, current velocity, woody structure, conductivity) using a broken-stick method to determine how many axes to retain. We saved PC1 and PC2 (hereinafter HAB1 and HAB2) as descriptors of habitat. We performed Pearson's correlations between HAB1 and HAB2 variables and all coarse-scale continuous variables (i.e., RIV-DTR, PCT-ALU, DA, C-link).

Relations between headwater fish assemblage composition and ecological and anthropogenic gradients

To control for the number of individuals collected, we calculated rarefied richness (Table A3) and then used two-way analysis of variance (ANOVA) to test for interactions and main effects of our categorical, anthropogenic, and ecological factors. We based rarefaction estimates on the random selection of a set number of individuals ($n = 151$ for this study) from a given sample and quantified the expected number of species only if that number of individuals was sampled at all sites. Our sample size was based on the number of individuals at which species accumulation appeared to asymptote.

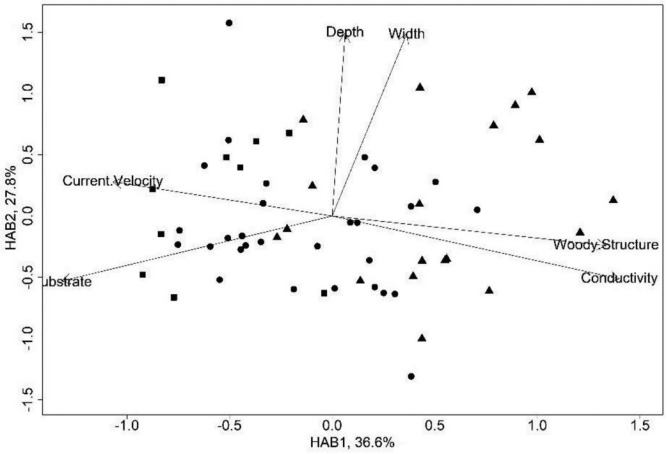
We performed nonmetric multidimensional scaling (NMDS) to explore how headwater fish assemblage composition (presence–absence matrix) varied in response to anthropogenic and environmental gradients. NMDS minimizes the difference between rank similarities in a distance matrix and rank Euclidean similarities in ordination space (McCune et al. 2002) using the "vegan" package (Oksanen et al. 2019). We calculated Jaccard dissimilarity distance of the assemblage composition data among all pairs of samples. We used the function metaMDS; this function implements several analyses to find the optimal solution with the lowest stress value ($k = 3$). We calculated the percent variation along each axis as the squared correlation between the ordination distance of the axis and the original distance matrix. We used the envfit function in the "vegan" package to fit environmental and anthropogenic gradients (HAB1, HAB2, DA, C-link, PCT-ALU, RIV-DTR) to NMDS scores. We used permutation tests ($n = 1000$) to distinguish the significance of vector fits with the ordination, and significant ($p < 0.05$) variables were included in the resulting biplots. Finally, using the results of our NMDS, we produced two plots to examine how headwater fish assemblage composition related to ecological (C-link and DA) and anthropogenic (i.e., anthropogenic land use and distance to reservoir) gradients.

We used partial Mantel tests (Mantel 1967) to test the predictions that headwater fish composition would be driven by instream habitat and river system connectivity gradients, and instream habitat variability would be intrinsically linked to coarse-scale environmental and anthropogenic gradients. We performed all partial Mantel tests using the package "ecodist" (Goslee and Urbana 2007). Mantel tests assess the probability that correlations between distance matrices are random using permutation procedures. We included the Jaccard's dissimilarity distance of headwater fish assemblage composition and the Euclidean distance of instream habitat variation (HAB1 and HAB2) as response matrices. We also produced two distance matrices to control for spatial effects. We used the earth.dist ("fossil" package; Vavrek 2011) function to quantify geographic distance between all pairs of sites and also included the Euclidean river distance (km) for all site pairings. River distance between pairs of sites was determined using GIS. We categorized predictor variables into broader groups (Table 1). Categories included instream habitat, land use, river distance to reservoir, spatial locality, stream size, and connectivity. We partialed out spatial variables during all tests; we only removed variance attributed to stream size when this variable was not included as a predictor.

Results

We collected a total of 8825 individuals representing 44 taxa. Sample size ranged from 4 to 640 individuals (mean ± SE, 136 ± 16.9). Across the 58 sites, the five most commonly sampled species were green sunfish (*Lepomis cyanellus*; 83% occurrence), bluegill (*Lepomis macrochirus*; 74%), creek chub (*Semotilus atromaculatus*; 66%), longear sunfish (*Lepomis megalotis*; 62%), and bluntnose minnow (*Pimephales notatus*; 59%). Interactions between distance to reservoir and anthropogenic land use ($F_{[2,51]} = 6.88$, $p < 0.01$) and between confluence link and anthropogenic land use ($F_{[2,52]} = 15.14$, $p < 0.01$) on headwater fish rarefied richness were significant. In that analysis, there were no significant interactions between drainage area and confluence link, anthropogenic land use, or distance to reservoir ($p > 0.05$). Finally, confluence link ($F_{[1,51]} = 13.03$, $p < 0.001$), anthropogenic land use ($F_{[2,51]} = 3.07$, $p < 0.05$),

Fig. 2. (A) Principal component analysis (PCA) of instream habitat and water quality variables for PC1 and PC2 axes. Arrows end at centroids for environmental variables. Shapes represent our three ALU (anthropogenic land use) categories: triangles = high (≥30% ALU), circles = low, private (<30% ALU) and squares = low, public (<30% ALU). (B) Plot of correlations between PCA scores (PC1 and PC2 axes) of instream habitat and our four coarse-scale vectors: stream size (drainage area, DA), C-link (confluence link), PCT-ALU (percent anthropogenic land use), and RIV-DTR (river distance to Sardis Reservoir). Arrows end at centroids for environmental vectors.



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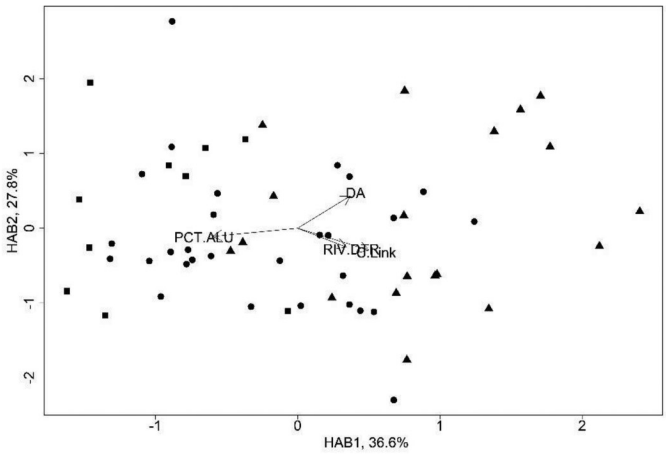


B

Table 2. Estimates of total fish turnover (β_{sor}), spatial turnover (β_{sim}), and nestedness (β_{nes}) for all sites and for each level of ALU (anthropogenic land use), DTR (distance to reservoir), DA (drainage area), and C-link (confluence link) factors.

Group	β_{sor}	β_{sim}	β_{nes}
All sites	0.95	0.91	0.04
ALU-HIGH	0.87	0.75	0.12
ALU-LOW-PRI	0.91	0.84	0.07
ALU-LOW-PUB	0.79	0.70	0.09
DTR-CLOSE	0.89	0.81	0.08
DTR-FAR	0.92	0.85	0.07
DA-SMALL	0.89	0.81	0.08
DA-MEDIUM	0.85	0.78	0.07
DA-LARGE	0.87	0.75	0.12
C-link-LOW	0.85	0.78	0.07
C-link-HIGH	0.93	0.85	0.08

Note: See Materials and methods for full description of terms.

and distance to reservoir ($F_{[3,51]}$ = 3.49, p < 0.05) all exhibited significant main effects on headwater fish rarefied richness, while there was no effect of drainage area (p > 0.05).

Spatial turnover

Spatial turnover contributed more to changes in headwater fish assemblage composition than nestedness, for both ecological (drainage area, confluence link) and anthropogenic (anthropogenic land use or distance to reservoir) factors. Beta coefficients from pairwise comparisons for all samples were β_{sor} = 0.95, β_{sim} = 0.91, and β_{nes} = 0.04 (Table 2), suggesting that spatial turnover was responsible for 91% of the variance in species composition among samples, whereas only 4% was accounted for by nestedness. This pattern was also consistent across the levels of all factors, with beta coefficients yielding similar estimates (Table 2).

Relations of instream habitat to ecological and anthropogenic gradients

The PCA revealed two distinct gradients of physicochemical habitat characteristics (Fig. 2*a*). The strongest loading on PC axis 1 (36.6% variance explained) were conductivity (1.39), the percentage occurrence of woody structure (1.33), substrate size (–1.31), and current velocity (–1.06). The strongest loadings on PC axis 2 (27.8% variance explained) were depth (1.50) and width (1.49). Environmental vectors had a distinct effect on both the PC1 and PC2 components. Thus, PC1 represented a velocity–structural gradient, with negative scores along the PC1 axis characterized by lower conductivities, lower percentage of woody structure, larger substrate sizes, and faster current velocities. PC2 represented a width–depth gradient; samples with positive scores on the PC2 axis were deeper and wider relative to samples with negative scores.

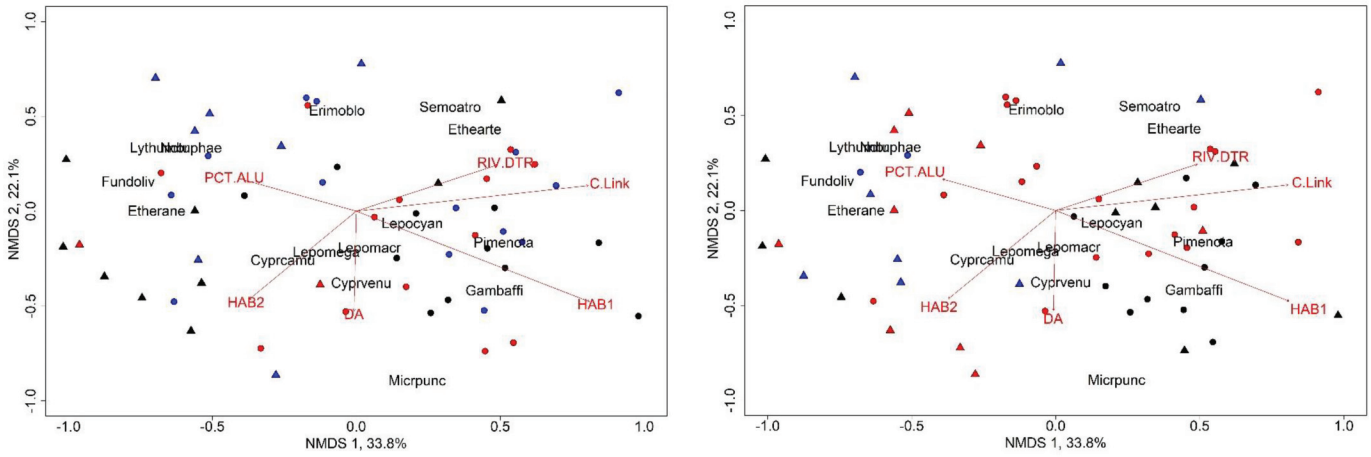
Pearson correlation coefficients suggest that instream habitat appears to be most tightly correlated to land use and stream size (Fig. 2*b*). PCT-ALU was negatively correlated with HAB1 scores (r = –0.60, p < 0.0001). HAB1 scores were also moderately correlated with C-link (r = 0.50, p < 0.0001), RIV-DTR (r = 0.34, p < 0.01), and DA (r = 0.37, p < 0.01). HAB2 scores were positively correlated with DA (r = 0.43, p < 0.001) and negatively correlated C-link (r = –0.29, p < 0.05), but no other variables.

Relations of headwater fish assemblage composition to ecological and anthropogenic gradients

The NMDS effectively summarized variability in headwater fish assemblage composition among samples (three-axis best solution, stress = 15.31%, 69 iterations; Figs. 3*a* and 3*b*). Axes 1 and 2 represented most of the variation (33.8% and 22.2%, respectively). Positive scores on axis 1 were associated with samples typified by an increase in the occurrence of western mosquitofish (*Gambusia affinis*), *P. notatus*, redspot darter (*Etheostoma artesiae*), and *S. atromaculatus*, whereas negative scores on axis 1 were linked to samples typified by an increase in the occurrence of Yazoo darter (*Etheostoma raneyi*), brown madtom (*Noturus phaeus*), redfin shiner (*Lythrurus umbratilis*), and blackspotted top minnow (*Fundulus olivaceus*). HAB1 and C-link gradients related moderately with the ordination of headwater fish composition (HAB1: r^2 = 0.59, p < 0.001; C-link: r^2 = 0.45, p < 0.001). In addition, PCT-ALU (r^2 = 0.24, p < 0.001), HAB2 (r^2 = 0.24, p < 0.001), RIV-DTR (r^2 = 0.20, p < 0.01), and DA (r^2 = 0.19, p < 0.01) were moderately correlated with headwater fish assemblage composition. Stream size groupings did not form distinct clusters in NMDS space (Fig. 3*a*).

Instream habitat was correlated with the percentage of anthropogenic land use, representing opposite ends of a habitat–land use gradient in NMDS space (Fig. 3*b*). Samples from catchments with more public land were mostly restricted to the upper left

Fig. 3. Nonmetric multidimensional scaling (NMDS) plots of headwater fish assemblage composition with fitted ecological and anthropogenic vectors envfit ($p < 0.05$). Weighted averages for the top 15 fishes based on rate of occurrence are plotted in NMDS space. Variables shown are HAB1, HAB2, C-link, DA, PCT-ALU, and RIV-DTR. See Materials and methods for further descriptions of terms. (A) Colours represent our three DA categories: black = LARGE (>20 km^2), red = MEDIUM (10 to 20 km^2), and blue = SMALL (<10 km^2). Shapes represent our two C-link categories: triangles = low (0 to 10 confluences between site and mouth of reservoir) and circles = high (>10 confluences between site and mouth of reservoir). (B) Colours represent our three ALU categories: black = high (≥30% ALU); red = low, private (<30% ALU); and blue = low, public (<30% ALU). Shapes represent our two DTR categories: DTR1: triangles = close (0–40 km) and DTR2: circles = far (>40 km). [Colour online.]



quadrant of the NMDS plot and had higher rates of occurrence of Yazoo darter, brown madtom, redfin shiner, and blackspotted top minnow. Samples from catchments with agricultural or urban land use had greater occurrence of green sunfish, western mosquitofish, and bluntnose minnow. Instream habitat does not appear to be strongly influenced by river distance to reservoir (Fig. 3*b*).

Correlations occurred between both headwater fish assemblage composition and local drainage attributes; instream habitat was related to land use and stream size (Table 3). Headwater fish assemblage composition was moderately correlated with river system connectivity (Mantel $r = 0.21$, $p < 0.001$) and instream habitat (Mantel $r = 0.19$, $p < 0.001$) and weakly correlated with stream size (Mantel $r = 0.10$, $p < 0.05$) (consistent with NMDS results above), but no other predictor variables (Table 3). Instream habitat was moderately correlated with land use (Mantel $r = 0.26$, $p < 0.001$) and stream size (Mantel $r = 0.19$, $p < 0.001$).

Discussion

In this study, we assessed the influence of ecological and anthropogenic gradients on instream habitat and headwater fish assemblage composition within a Gulf Coastal Plain dendritic river system. Our analyses yielded five primary findings on headwater fish assemblage composition within a dendritic river system: (*i*) instream habitat and river system connectivity gradients regulate headwater fish assemblage composition; (*ii*) spatial turnover is driving differences in headwater fish assemblage composition; (*iii*) instream habitat is strongly associated with stream size and land use gradients; (*iv*) assemblages do not appear to represent nested subsets regardless of which coarse-scale factor is used to group samples; and (*v*) instream habitat, as a consequence of anthropogenic land use, has altered natural ecological gradients driving headwater fish assemblage composition. No association was detected between instream habitat or headwater fish assemblage composition and proximity to Sardis Reservoir. Our results highlight the influence of river system connectivity, instream habitat, stream size, and land use gradients on instream habitat and headwater fish composition. However, these results should be interpreted cautiously for two reasons. First, we recognize that the assemblage dataset used for this study was small (58 sites), thus reducing the number of replicates within groups (3–15). Secondly, we did not use direct gradient multivariate techniques (e.g., canonical correspondence analysis or redundancy analysis) because we wanted to assess the strength of relatedness between all ecological and anthropogenic gradients and headwater fish composition and instream habitat. The analyses used in this study (NMDS, envfit, partial Mantel tests) allowed us to identify the strength and significance of correlations (r^2 and Mantel r) between response matrices (headwater fish assemblage and instream habitat) and ecological and anthropogenic gradients. Quite often, r^2 is referred to as the percent variance "explained", but may be more aptly interpreted as the percent variance "represented" (McCune et al. 2002). Therefore, while we found strong evidence supporting our findings, these results should not be interpreted as conclusive evidence for the inferences made below.

Headwater fish assemblage composition, instream habitat, and river system connectivity

Our results support recent findings that headwater fish assemblage composition in dendritic river systems is mostly influenced by connectivity and instream habitat gradients (Campbell Grant et al. 2007; Hitt and Angermeier 2008; Brown and Swan 2010). Our finding that adventitious streams, designated as streams that are at a minimum three stream orders smaller than the streams they drain into (Gorman 1986), harbored more diverse species pools than true headwater streams (Fig. 3*a*) is well supported (Schaefer and Kerfoot 2004; Hitt and Angermeier 2008). Within a dendritic river system, Brown and Swan (2010) indicate that dispersal within headwater streams is commonly reduced for organisms confined to within-branch movements. Thus, meta-assemblages in headwaters are arranged by species-sorting processes whereby fishes inhabit patches of high habitat suitability, as opposed to larger streams and adventitious streams that are more likely to be regulated by mass effects (Leibold et al. 2004; Hitt and Angermeier 2008; Thornbrugh and Gido 2010). Headwater streams were driven by an increase in the relative occurrence of small stream specialists (e.g., redspot darter and creek chub), whereas adventitious streams were associated with an increase in the relative occur-

Table 3. Mantel correlations between headwater fish assemblage composition (Sørenson–Dice of presence–absence data) and environmental and anthropogenic predictors.

Data matrix	Dissimilarity index	Predictor	Mantel *r*	*p*
Presence–absence	Jaccard's	Connectivity	0.21	0.001**
		Instream habitat	0.19	0.0009***
		Stream size	0.10	0.01*
		Land use	0.07	0.16
		Reservoir	0.11	0.12
Instream habitat	Euclidean distance	Connectivity	0.06	0.36
		Stream size	0.19	0.0003***
		Land use	0.26	0.0001***
		Reservoir	–0.08	0.94

Note: *, significant at the 0.05 level; **, significant at the 0.01 level; and ***, significant at the 0.001 level.

rence of rheophilic fishes such as bluntnose shiner, Yazoo darter, brown madtom, and redfin shiner (Sterling et al. 2013) (Fig. 3*a*).

The spatial positioning of headwaters within a dendritic river system may regulate the strength of species-sorting effects as a result of immigration and extinction risk (Fagan 2002; Campbell Grant et al. 2007). Local abundance (Taylor and Warren 2001) and drought tolerance (Adams and Warren 2005) have been identified as key traits that may determine a fish's colonization probability in headwaters. Redspot darters, creek chubs, and bluntnose minnows were characteristic of samples collected from headwater (i.e., C-link > 10) streams. In this study, creek chub and bluntnose minnow were the most abundant fishes collected from small streams (i.e., drainage area < 10 km^2, 344 and 266 individuals, respectively) and the second (bluntnose minnow) and third (creek chub) (775 and 745 individuals, respectively) most abundant fishes sampled from headwater streams. Consistent with our results, the redspot darter is known to recolonize headwaters following extreme drought conditions (Adams and Warren 2005).

C-link was likely an important hydrological factor in describing headwater fish assemblage composition because it helps distinguish between headwaters and adventitious streams (Gorman 1986; Smith and Kraft 2005). Confluences affect the rate at which changes to channel morphology occur and habitat boundaries are defined (Benda et al. 2004). Confluences of geomorphic significance (Benda et al. 2004) are known to alter rates of longitudinal biodiversity among stream fish assemblages (Kiffney et al. 2006).

Spatial turnover

Spatial turnover, and not nestedness, may be more correlated to headwater fish assemblage composition within a dendritic river system. Local assemblages along spatial gradients tended to be compositionally distinct from one another, regardless of the influence of stream size. These results signify that the compositional distinction of local headwater fish assemblages may play a large role in contributing to gamma (regional) diversity within a dendritic river system. Neither of our ecological or anthropogenic gradients appear to influence the magnitude to which spatial turnover nor nestedness contribute to headwater fish assemblage composition in our study basin. Therefore, our results suggest resident headwater fish assemblages represent isolated assemblages as a result of dendritic structuring within this river system (Schmidt and Schaefer 2017).

Instream habitat, land use, and stream size

Land use may be more associated with the alteration of instream habitat as opposed to stream size within the headwaters of a dendritic river system. Headwater fish assemblage composition changed along land use and instream habitat gradients (Fig. 3*b*). We note that land use disproportionately influenced instream habitat in relation to stream size, which may be expected due to the restricted size of streams sampled (<50 km^2). Historical studies have documented similar findings on the effects of channel incision and habitat fragmentation in headwater streams as a result of extensive land use within the LTR system (Shields et al. 1994). Drainages characterized by an increase in agricultural or urban land use were associated with a greater relative occurrence of tolerant fishes (Fig. 3*b*), whereas drainages characterized by mostly public land were often distinguished by a higher relative occurrence of lotic and benthic specialists, such as Yazoo darters, brown madtoms, and redfin shiners (Fig. 3*b*). Environmental parameters measured for streams impacted by anthropogenic land use, in relation to streams flowing through or adjacent to public land, exhibited higher conductivities, smaller mean substrate sizes, and reduced current velocities (Table A2 and Fig. 3*b*), physiochemical habitat characteristics that are typical of degraded streams as a result of urban and agricultural land use (Wang et al. 2001; Paul and Meyer 2001; Meyer et al. 2005).

Land use disrupts ecological gradients in headwaters

The cumulative effect of anthropogenic land use on instream habitat appears to disrupt the natural stream size and river system connectivity gradients promoted in less perturbed watersheds, and thus these coarse-scale, ecological variables may no longer serve as strong predictors of assemblage composition and structure within highly degraded streams (Fig. 3*b*). Headwater fish assemblages of anthropogenically impacted drainages appear to be quite similar and were characterized by a greater relative occurrence of tolerant and generalist fishes. In opposition, headwater fish assemblages of streams characterized by forested, private land appear to be extremely varied, whereas headwater fish assemblages of streams flowing through public land appear to be fairly stable and typified by an increase in the relative occurrence of specialists. We suggest that fine-scale habitat fragmentation such as local channel incision, lipped culverts, and box culverts as a result of increasing urban and agricultural land use is reducing river system connectivity. There is much evidence that these sources of fine-scale fragmentation cause these assemblages to become isolated, and thus local composition is determined chiefly by local factors (Angermeier and Winston 1998; Matthews and Robison 1998). Such isolated assemblages may diverge from assemblages that maintained connectedness with the surrounding dendritic river system because of biotic (e.g., predation or competition) or nonbiotic (e.g., channel incision) factors. Population growth in Lafayette County (county seat, Oxford) increased by 22.76% from 2010 to 2017 (US Census Bureau 2017), thus promoting an increase in the number of culverts and extensive channel incision throughout the river system as road densities multiplied.

Influence of distance to Sardis Reservoir

Our finding suggests that headwater fish assemblage composition within a large, dendritic river system may not be influenced by coarse-scale impoundments. Other studies have observed that headwater fish assemblages may be influenced by reservoirs as a consequence of declining recolonization (Falke and Fausch 2010) and increasing extirpation rates of stream fishes (Minckley and Cross 1959) or decreasing species richness (Winston et al. 1991). Samples collected either in close proximity or far away from Sardis Reservoir were not compositionally distinct (Fig. 3*b*). If a res-

ervoir effect were present, we would expect to see an increase in the relative occurrence of macrohabitat generalists in streams that were in closer proximity to Sardis Reservoir. Instead, streams located in close proximity to the reservoir were not typified by an increase in the relative occurrence of generalist fishes such as green sunfish or western mosquitofish (Fig. 3*b*).

Basin size, stream size, and the connectedness among drainages alter the strength of a reservoir effect (Taylor et al. 2001; Gido et al. 2002; Falke and Gido 2006) in river basins. As the size of a river basin decreases, the effect of impoundment on assemblages increases (Pigg et al. 1998; Taylor et al. 2001; Gido et al. 2002). Herbert and Gelwick (2003) and Reuter et al. (2019) described a reduced effect of distance to reservoir on headwater fish assemblages and concluded that this result was a likely effect of the dominance of generalist fishes throughout these drainages. In opposition, Guenther and Spacie (2006) documented an increase in the relative abundance of fluvial generalists and decline in the relative abundance of fluvial specialists within medium to large drainages fragmented by impoundment (mean drainage area = 79 km^2). Taylor et al. (2001) described an alteration in assemblage structure from cyprinid-dominated assemblages to centrarchid-dominated assemblages; however, the drainage area of the study system was fairly small (135 km^2). Gido et al. (2002) noted that the large basin area of their study system (24 900 km^2) likely played a key role in maintaining fish assemblage stability despite it having been impounded for almost half a century. The LTR system is fairly large (3231 km^2 at the mouth of Sardis Reservoir), and our results most closely align with those of Gido et al. (2002), suggesting that basin size likely reduced any reservoir effect on headwater fish assemblage composition. The dispersal ability of many reservoir specialists is likely promoted in larger streams and rivers because of the pervasiveness of lentic-like habitats and the proximity of these lotic systems to the impoundment. Finally, connectedness among drainages influences the strength of a reservoir effect; streams that are indirectly connected to a large-scale impoundment display a reduced reservoir effect (Falke and Gido 2006). Although the number of confluences was reduced for samples collected in close proximity to Sardis Reservoir in relation to samples collected further away from the impoundment (Table A2), our results do not suggest that upstream headwater fish assemblages with the upper LTR system are currently being altered as a result of this structure. We suggest that a strong reservoir effect may only be observed within upstream drainages that are directly connected to a reservoir or within larger streams and rivers that may provide more suitable dispersal routes for reservoir specialists (Gido et al. 2009; Falke and Gido 2006).

Management implications

Headwater fish assemblage composition in dendritic river systems appears to be driven by network connectivity and local stream habitat. The influence of these ecological gradients on headwater fish assemblage composition may be altered as a result of changing land use. The proximity of Sardis Reservoir does not appear to currently influence headwater fish assemblage composition, or at least it had no influence on the samples collected during the study period. Drainages adjacent to or constrained to public land appear to be maintaining their compositional distinctness; however, drainages typified by anthropogenic land use (predominately agricultural) are characterized by low species richness. As a consequence of their isolation, headwater fish assemblages often contribute to regional diversity; thus, the conservation of these ecosystems is vital to ensure the biological integrity of dendritic river systems. Therefore, it is important for conservation managers to (*i*) recognize the importance of headwater streams as distinctive habitat patches and (*ii*) identify the extent to which upstream land use alters the ecological gradients driving species diversity in these systems and make necessary improvements to impaired streams to buffer against these effects.

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Appendix A

Appendix Fig. A1 and Tables A1–A3 appear on the following pages.

Fig. A1. Frequency histograms of the number of species occurrences: (A) histogram of species occurrences with no species dropped; (B) histogram of species occurrences after dropping species with ≤3 occurrences.

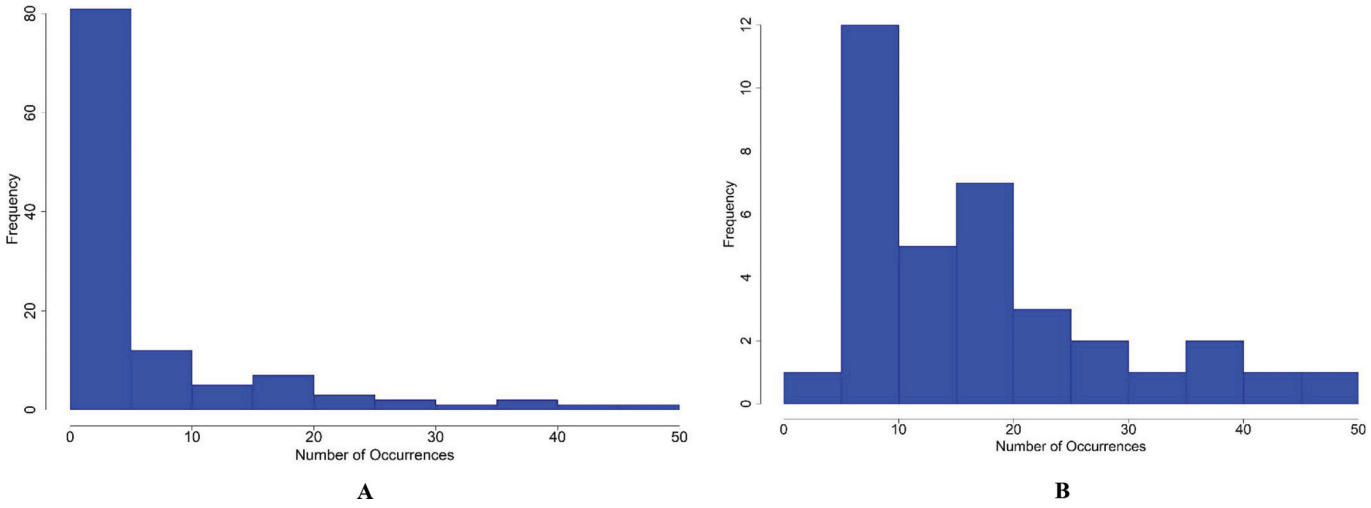

Table A1. Summary of NP-MANOVA (n = 1000) results on principal components analysis scores (PC1 and PC2, 59% variation explained) of all site-scale habitat variables.

Variable	df	Pseudo-F	R^2	p
Land use	2	2.81	0.05	0.019
Time	1	1.53	0.01	0.218
Land use × Time	2	1.76	0.03	0.158

Note: Time (historical or contemporary) and land use (small: 1–10 km²; medium: 10–20 km²; or large: >20 km²) were included as fixed factors, and stream size (small: 1–10 km²; medium: 10–20 km²; or large: >20 km²) was included as a blocking factor to test the hypotheses that there was not a significant effect of time on environmental variation between the two datasets.

Table A2. Summary of environmental variables for each level of each factor (DA, ALU, DTR, C-link) level used in the analyses of the influence of stream size, connectivity, land use, and proximity to reservoir on headwater fish assemblage composition.

Group	Temperature (°C)	Conductivity (μS·cm⁻¹)	Dissolved oxygen (mg·L⁻¹)	% Woody structure	Substrate size	Current velocity (m·s⁻¹)	Depth (cm)	Wetted width (m)	Drainage area (km²)	Confluence link
ALU-HIGH	23.36±3.98	231.59±218.53	7.38±2.06	3.10±3.40	2.23±0.69	0.13±0.17	18.64±15.51	4.29±2.64	23.07±14.40	34.00±17.61
ALU-LOW-PRI	23.33±3.00	92.11±88.17	7.13±1.05	3.60±0.13	2.95±0.66	0.15±0.16	15.49±11.99	3.09±1.48	13.90±10.20	30.06±16.03
ALU-LOW-PUB	24.40±3.25	40.38±12.35	6.27±1.01	3.57±0.11	3.17±0.34	0.44±0.35	17.34±10.80	3.66±1.79	14.60±13.55	18.80±10.65
DTR-CLOSE	23.91±3.28	81.81±101.99	6.98±1.27	13.33±11.76	2.95±0.79	0.31±0.31	18.61±14.94	4.03±1.99	20.49±14.15	15.04±11.12
DTR-FAR	23.26±3.44	162.12±179.79	7.13±1.63	12.96±13.56	2.60±0.65	0.11±0.10	15.60±11.38	3.26±2.01	14.58±11.33	39.55±11.07
DA-SMALL	22.93±3.22	92.91±96.91	6.72±1.45	2.66±0.30	3.03±0.75	0.19±0.20	14.48±11.00	2.51±0.82	5.90±2.80	27.59±13.84
DA-MEDIUM	23.57±3.62	122.10±109.06	6.92±1.60	2.63±0.29	2.48±0.69	0.13±0.15	20.88±15.95	3.33±1.07	14.72±3.44	36.31±15.80
DA-LARGE	24.16±3.35	173.88±224.11	7.56±1.34	2.51±0.19	2.66±0.65	0.25±0.30	16.21±11.99	4.98±2.67	31.10±10.98	25.90±18.48
C-link-HIGH	23.55±3.66	168.29±174.95	7.24±1.61	2.58±0.29	2.54±0.68	0.11±0.10	16.12±12.67	3.41±2.01	15.93±12.05	38.37±10.78
C-link-LOW	23.49±2.66	41.31±12.70	6.67±1.07	2.65±0.19	3.22±0.61	0.38±0.32	18.45±13.71	3.96±2.04	19.46±14.37	9.50±5.21

Table A3. Means and standard deviations for rarefied richness for all possible sample groupings based on our anthropogenic (DTR and ALU) and ecological (DA and C-link) factors.

Factor 1	Factor 2	Mean	SD
ALU	**DTR**		
HIGH	CLOSE	5.46	2.36
LOW-PRI	CLOSE	8.08	0.88
LOW-PUB	CLOSE	6.35	0.87
HIGH	FAR	5.41	1.73
LOW-PRI	FAR	5.41	1.77
LOW-PUB*	FAR	8.17	1.16
DA	**C-link**		
SMALL	LOW	7.17	1.30
MEDIUM	LOW	6.46	0.17
LARGE*	LOW	7.72	1.17
SMALL	HIGH	5.16	1.81
MEDIUM	HIGH	5.70	2.34
LARGE	HIGH	5.47	1.75
DA	**ALU**		
SMALL	HIGH	4.57	1.17
MEDIUM	HIGH	5.21	2.23
LARGE	HIGH	5.96	2.14
SMALL	LOW-PRI	5.87	2.18
MEDIUM	LOW-PRI	5.69	2.10
LARGE	LOW-PRI	6.74	1.77
SMALL	LOW-PUB	6.58	1.10
MEDIUM*	LOW-PUB	7.95	2.27
LARGE	LOW-PUB	7.32	1.16
DA	**DTR**		
SMALL	CLOSE	6.84	1.53
MEDIUM	CLOSE	5.87	2.15
LARGE*	CLOSE	6.79	2.23
SMALL	FAR	5.37	1.83
MEDIUM	FAR	5.76	2.32
LARGE	FAR	6.17	1.48

*Sample grouping associated with greatest mean rarefied richness for each combination of factors.