- 1 Pinpointing biodiversity hotspots: local habitat and regional connectivity shape fish richness
- 2 within contrasting riverine metacommunities
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- 15 Running head: Biodiversity hotspots in riverine fish metacommunities

Keywords: Habitat Diversity, Metacommunity, Riverine Macrosystem, Species–Discharge
relationship, Tributary

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25 Abstract

26 1. Preserving biodiversity is a core goal of riverscape conservation planning. Although 27 connectivity and habitat diversity are generally deemed important for structuring riverine 28 communities, their specific contributions to riverine fish richness is often unclear. 29 2. We sampled fish communities along \geq 200 km of nonwadeable mainstems (fifth–seventh 30 order) and tributary branches of the Grand (low habitat diversity) and Meramec (high habitat 31 diversity) river systems (Missouri, USA). We asked whether local habitat diversity and regional 32 connectivity explained site-level fish richness of three stream-size preference guilds: core 33 species, large-river specialist (LRS) dispersers from the Mississippi and Missouri rivers, and 34 headwater specialists dispersing from network branch streams. We defined biodiversity hotspots as sites with high fish richness ($\geq 75^{\text{th}}$ percentile) and examined whether occurrences of LRS and 35 36 headwater species shifted hotspots longitudinally compared to hotspots defined solely by core 37 species. 38 3. Species richness peaked in mid- to lower-courses in both rivers (maximum richness = 3939 species in Grand River, 73 species in Meramec River) but not at either river mouth. Downriver

40 connectivity (distance from mouth of mainstem river) and habitat diversity predicted LRS- ($R^2 =$

41 0.44–0.91) and core-species ($R^2 = 0.37$ –0.57) richness, respectively. Densities of headwaters

42 within 25 fluvial km of sites positively related to headwater species richness in the Grand River

43 system ($R^2 = 0.85$) but not the Meramec River system, indicating rivers constrained headwater

44 species pools differently. Biodiversity hotspots based on core species extensively spanned sites

45 with high habitat diversity covering 63% (Grand) and 50% (Meramec) of mainstem sites. When

46 viewing all species collectively, contributions of LRS species (13–17 species) to hotspots

47 outweighed those of headwater species (≤6 species), causing hotspot spatial extents to narrow
48 and shift downriver.

49 4. Overall, riverine metacommunities contained downriver, core, and headwater constituents, and
50 thus, richness patterns were spatially explicit. Conserving hotspots likely requires strategies that
51 preserve both habitat diversity and connectivity to surrounding regional species pools.

52

53 Introduction

54 Accurate depictions of biodiversity are needed for conservation planning at regional 55 scales. For example, landscape-planning strategies often protect regional diversity by first 56 safeguarding areas supporting high local species richness (i.e., biodiversity hotspots; Smith et al., 57 2018). In stream networks, these hotspots function as reserves for species that recolonize 58 disturbed areas (Taylor & Warren, 2001) and often harbor rare and imperiled species (Miranda & 59 Kilgore, 2020). However, processes maintaining riverine biodiversity are often unclear, creating 60 uncertainty about which management actions to prioritize (Erős, 2017). Metacommunity 61 frameworks explicitly recognize that spatial (dispersal) and environmental (niche-based) 62 processes affect community composition throughout river networks, and thus, may help explain 63 biodiversity patterns (Erős, 2017). To date, however, few metacommunity-framed studies are set 64 in nonwadeable rivers, leaving uncertainty about community-assembly processes in rivers where 65 fish richness is typically highest (Erős, 2017; Vitorino Júnior et al., 2016). 66 Predictions about where fish richness peaks in rivers contrast among several river concepts. The River Continuum Concept (RCC, Vannote et al., 1980) continues to serve as a 67 68 useful touchstone for potential longitudinal changes in fish communities (Mee, Robins & Post,

69 2018; Vander Vorste et al., 2017). Although the RCC does not single-out fish, it predicts total

70 "biotic diversity" peaks in fourth-sixth-order rivers where spatiotemporal diversity in habitat, 71 thermal, and trophic resources are presumably maximized. Consequently, the RCC is inherently 72 niche-based, predicting community structuring by local environmental conditions (Roberts & 73 Hitt, 2010). Multiple studies spanning rivers report mid-course peak or "hump-shaped" fish 74 richness (Hughes & Gammon, 1987; Oberdorff, Guilbert & Lucchetta, 1993). However, 75 declining lower-course richness could also result from diminished ecosystem condition in industrialized areas along larger rivers and increasing sampling difficulty with traditional 76 77 surface- and bank-oriented sampling gears (i.e., electrofishing, seining) as rivers deepen and 78 widen downriver (Erős, 2017).

79 Alternatively, many studies report that fish species richness continuously increases 80 downstream (Matthews, 1998). This is partly attributed to the development of structurally 81 complex resource and refugium habitats (deep pools, off-channel areas) and increasingly 82 predictable flows downstream (Roberts & Hitt, 2010; Schlosser, 1987). Because of increasing 83 fish richness downriver, discharge is often used to identify species rich areas (Laub et al., 2018), 84 and simulate consequences of water abstraction (Xenopoulos et al., 2005) and climate change 85 (Xenopoulos & Lodge, 2006). Despite increasing use of discharge in conservation planning, few 86 studies investigate causal mechanisms underlying species-discharge relationships (SDRs; 87 McGarvey & Ward, 2008). Moreover, both the RCC and SDRs assume habitat diversity partly 88 explains fish richness at sites, meaning these concepts also have conflicting expectations for 89 where habitat diversity is greatest within rivers (RCC = mid-course, SDR = lower-course). Few 90 of the numerous studies examining longitudinal fish richness encompass nonwadeable rivers 91 (Jackson, Peres-Neto & Olden, 2001; Vander Vorste et al., 2017), and even fewer quantify

habitat diversity, meaning there are little data to validate predictions from either the RCC orSDRs in nonwadeable rivers.

94 Connectivity to regional species pools also affects species richness (Sarremejane et al., 95 2017). For example, fish dispersal from mainstems sometimes increases richness in downstream 96 reaches of adventitious tributaries, creating local hotspots near tributary mouths (Adventitious 97 Stream Concept, ASC; Osborne & Wiley, 1992). A generalized extension of the ASC is the 98 Network Position Hypothesis (NPH), which posits communities are increasingly structured by 99 spatial processes (i.e., dispersal) in more centrally (downstream) located streams in networks due 100 to greater accessibility to colonizers (Brown & Swan, 2010; Schmera et al., 2018). Support for 101 the NPH is context dependent, varying by taxa, species traits, watershed, and season (Henriques-102 Silva et al., 2019; Schmera et al., 2018). In mainstem rivers, however, Vitorino Júnior et al. 103 (2016) and López-Delgado et al. (2018) found elevated importance of spatial processes for 104 structuring fish communities than in more isolated tributaries, hence supporting the NPH. 105 Headwater species may also contribute to fish richness in mainstems. For example, 106 headwaters often support unique species (Meyer et al., 2007; Zbinden & Matthews, 2017), which 107 might drift downstream into mainstems during early life stages (Thornbrugh & Gido, 2010), and 108 mainstems could serve as temporary refugia for headwater fishes during flow intermittency 109 (Magoulick & Kobza, 2003; Meyer et al., 2007). Further, mainstems serve as corridors 110 connecting headwater fish populations throughout river networks. For example, landscape-111 genetic approaches indicate intervening mainstem dispersal distances, dams, and impoundments 112 spatially structure genetics of stream-fish populations (Fluker et al., 2014; Schmidt & Schaefer, 113 2018). Consequently, despite stream species often being disregarded as "waifs" when detected in

mainstems, these detections may indicate important spatial processes affecting basinwide genetic
exchange and metacommunity dynamics (McCluney et al., 2014; Van Looy et al., 2019).

116 We compared the mechanisms structuring longitudinal fish richness between two 117 nonwadeable river networks with contrasting habitat diversity and environmental gradients. We 118 had three objectives: 1) we examined SDRs to determine whether species richness increased 119 downriver (SDR), peaked mid-course (RCC), or showed other non-linear patterns indicative of 120 underlying heterogeneity or connectivity (Rosenfeld, 2017); 2) we disassembled fish 121 communities to ask whether habitat diversity and regional connectivity explained local richness 122 of core, downriver, and headwater species at sites; 3) finally, we asked whether longitudinal 123 positions and profiles of richness hotspots defined by core species were affected by occurrences 124 of species from downriver and headwater regional species pools.

125 Our study is among the largest coordinated tests of metacommunity theory in riverine 126 networks; because rivers were centrally located within river systems, we hypothesized "local" 127 fish communities were comingled species originating from disparate locations within river 128 systems and responsive to different assembly processes. Classic riverine theories often treat 129 mainstem rivers as uniform blocks without examining processes that give rise to among-river 130 differences in community-assembly mechanisms (e.g., NPH). In contrast, we solely focus on 131 rivers to highlight multiple processes that structure riverine communities and demonstrate how 132 structuring processes vary spatially within and between rivers.

133

134 Methods

135 Grand and Meramec river systems

136	We surveyed mainstems and nonwadeable principal tributary branches of two rivers with
137	contrasting environmental conditions (Missouri, USA; Figure 1; see Figure S1 for images). The
138	Grand River is a prairie river that drains 20,417 km ² of the Interior Plains region before joining
139	the lower Missouri River. Fine sediments predominate, creating high turbidity and unstable
140	channels reinforced by woody debris. Land-use is primarily row crops or pasture (drainage
141	agriculture = 76%; USGS, 2014). The system historically supported 79 fish species, consisting
142	mainly of habitat and physiological generalists (Missouri Department of Conservation, MDC,
143	unpublished databases, pre-2014).
144	The Meramec River system (drainage area = $10,270 \text{ km}^2$) is one of the most biologically
145	diverse systems in North America (129 fish species, MDC unpublished databases, pre-2014). It
146	is a tributary of the Mississippi River and drains the Ozark Plateau or "Ozarks" province, a
147	topographically diverse, upland region. High groundwater connectivity sustains baseflow,
148	moderates stream temperatures, and limits turbidity. Primary land uses are forest (68%) and
149	agriculture (22.5%), but much of the lower drainage is urban development (metro St. Louis
150	Missouri USA area; USGS, 2014). Channels are semi-confined by bluffs and consist of well-
151	defined alternating pools, shoals, and off-channel areas. Clear water and stable substrates
152	(gravel-boulder) afford abundant macrophytes along channel margins.
153	Both systems are rare examples of large free-flowing rivers and have similar network
154	architecture. For example, both mainstems span fifth-seventh orders and are joined mid-course
155	by two fifth-order tributary branches in close succession. Both rivers also join much larger rivers

- 156 with expansive floodplains (i.e., Mississippi or Missouri rivers), causing backwater-effected
- 157 hydrologic changes near river mouths. For clarity, we refer to the Mississippi and Missouri rivers
- as "great rivers," mainstems of the Grand and Meramec rivers as "mainstems," principal

tributaries of the Grand and Meramec rivers as "branches," and the Grand and Meramec river systems (mainstems plus branches) as "tributaries." We refer to the two most downriver sites in the Grand (river km [rkm] 1–24) and Meramec (rkm 1–30) rivers as "lower-course," the two most upriver sites as "upper-course" (Grand R. = rkm 151–200, Meramec R. rkm 215–244), and intermediate mainstem sites as "mid-course."

164 Similar river networks allowed us to replicate our study design by sampling sites 165 spanning the lower 200 (Grand R.) and 244 (Meramec R.) km of each system. Along the Grand 166 River mainstem, we placed eight sites approximately every 25 km and one site within lower 167 reaches of two branches (Shoal Creek and Thompson River; 10 sites). In the Meramec River 168 system, we placed nine sites every 30 km along the mainstem, and one site within lower reaches 169 of two principal branches (Bourbeuse and Big rivers). We also added a mainstem site (rkm 53) to 170 increase resolution into richness changes near confluences (12 sites). Access and navigability 171 were limited, so we adjusted sites to be within 5 km of the nearest access, but no sites overlapped 172 access points other than the lowermost 5-km-long sites. To minimize detections of random short-173 distance dispersers, we placed sites at least 1 km away from any major confluence (i.e., 174 mainstem-great river or branch-mainstem confluences).

175

176 *Fish and habitat sampling*

We surveyed fish at sites April through September 2016 using an intensive six-gear protocol developed for nonwadeable rivers that sampled all major habitats (exempting floodplain waterbodies without a surficial connection to the main channel). For comparison using 36 samples in Dunn and Paukert (2020), our methods detected 2.0 and 1.6 times more species per site as 500 m and 1,000 m of boat electrofishing-only effort commonly used in river assessments,

182 respectively. Sites ranged from 1.5–5.0 km depending on mean wetted-channel width (MWCW). 183 Because river size (i.e., discharge) varied by seven-fold across sites, we scaled effort with active-184 sampling gears to each site's MWCW, while keeping ratios of effort among active gears 185 approximately constant (Table S2). Total effort by each gear was divided into discrete sub-186 samples to distribute sampling spatially. Our active gears were boat electrofishing (550–1,600 m, 187 11–32 sub-samples per site), seining (7–25 hauls), and benthic trawling (150–500 m, 3–10 sub-188 samples). We complemented active gears with three passive gears fished overnight at each site: 189 one stationary trammel net (30.5 m long x 1.8 m deep) with 20.3-cm and 9.5-cm bar mesh outer 190 and inner panels, respectively; two hoop nets (1.2-m diameter) with 3.8-cm bar mesh; four mini-191 fyke nets (3.1-mm bar mesh). We set trammel and hoop nets in deep (>1.5 m) pools and mini-192 fyke nets in shallow off-channel slackwaters and/or structurally complex areas not effectively 193 sampled by active gears. Further details on fish sampling methods are in Dunn and Paukert, 194 (2020).

195 We recorded nine habitat variables contributing to local habitat diversity at sites 196 (September 26–October 27). Our habitat protocol was rapid (<1 d) and modified from USEPA 197 (2013). At each site, we placed 21 cross-sectional transects perpendicular to flow spanning the 198 main channel and off-channel habitats with surface-water connections. Along each transect, we 199 placed five equally spaced points (minimum of 105 points per site) and added a point at the 200 center of off-channel habitats intersected by transects. At each point, we measured five habitat 201 variables or documented if the point was in a shoal or pool (two variables). We mounted a sensor 202 (Hach FH950, Loveland, CO) to the base of a wading rod (wadeable) or sounding pole 203 (nonwadeable) to measure water-column velocity at approximately 60% depth or averaged 204 velocities at 20% and 80% for depths >1 m. Next, we used side-scan sonar imagery, corroborated

205	by a sounding pole or rope, to classify predominant substrate into six categories: silt/clay (1 =
206	≤ 0.06 mm), sand (2 = 0.07–2.0 mm), gravel/pebble (3 = 3–64 mm), cobble (4 = 65–256 mm),
207	boulder (5 = \geq 257 mm), and bedrock (6). We also used side-scan sonar to count woody debris (\geq
208	5 m long) and large boulders (\geq 1 m diameter) within a 5-x-5-m area centered at each point.
209	We recorded two habitat variables at the ends of each transect. We estimated the
210	percentage of shoreline covered by macrophytes within 10 m up- and down-river of each transect
211	$(0 = \le 5\%, 1 = 6 - 25\%, 2 = 26 - 50\%, 3 = 51 - 75\%, 4 = >75\%)$. We also visually estimated the
212	percentage of shoreline (lengthwise) with off-channel areas with surficial connections to the
213	main channel between consecutive transects, which was corroborated by satellite imagery. Off-
214	channel areas were often characterized by slackwater habitats; see Figure S2 for georeferenced
215	example of fish and habitat surveys). We averaged endpoint measurements on each transect to
216	summarize variables to transect.

218 Statistical analyses

219 *Objective 1. Examine where species richness peaks based on discharge*

220 We used stream-discharge gages within each river system to estimate the long-term mean 221 annual discharge at sites from drainage areas. First, we averaged annual discharge data at each 222 gage with near-continuous discharge data (years 1920–2016) in the Grand (5 gages) and 223 Meramec rivers (6 gages). Then we linearly regressed mean annual discharge against drainage 224 area from the National Hydrography Dataset (USEPA & USGS, 2012). High coefficients of variation ($R^2 > 0.99$) indicated discharge linearly reflected drainage area (Figure S3). Therefore, 225 226 we used system-specific models to predict mean annual discharge based on drainage areas at 227 lowermost boundaries of sites. Finally, we related fish richness at sites to predicted discharge

using Local Regression Smoothers (LOESS), which depicts nonlinear patterns via a movingwindow along an environmental gradient.

230

231 Objective 2: Determine whether habitat diversity and regional connectivity explained local
232 richness of core, downriver, and headwater species

233 We first classified each species potentially inhabiting river systems as a large-river 234 specialist (LRS), core, or headwater species by adapting classifications for "Big River" and 235 "Headwater" species lists in Pflieger (1989; Table S1). Historically, LRS species had populations 236 centered in the Missouri and Mississippi rivers in Missouri. (candidate LRS spp. = 35 in Grand 237 R., 42 in Meramec R.). Headwater species have distributions centered in small streams (first-238 third order; candidate headwater spp. = 14 in Grand R., 23 in Meramec R.). Non-LRS species 239 and non-headwater species were classified as "core" riverine species because they either 240 specialize in mainstem rivers or are common throughout mainstems and another stream-size 241 category.

242 We examined influences of habitat diversity and regional connectivity on local richness 243 of members of each stream-size guild. Habitat diversity reflects lateral, hydrogeomorphic, and 244 microhabitat variability, so we integrated the nine habitat variables into a single multivariate 245 index of habitat diversity (hereafter, habitat-diversity index). We organized habitat variables 246 summarized to transects (21 transects per site) into a correlation matrix and then performed a 247 Principal Component Analysis (PCA). We summarized the seven point-based variables to 248 transect by either averaging (continuous or ordinal) or summing occurrences (pool, riffle habitat) 249 along transects. Before performing PCA, we reduced skew by log(x + 0.1) or square-root 250 transforming six variables (Table 1). Finally, we calculated habitat diversity as the mean

Euclidean distance of transects to each site's multivariate centroid in PCA space (i.e., dispersion of transects around their centroid; Astorga et al., 2014). To assess whether the habitat-diversity index represented variability in habitat variables, we correlated the index to standard deviations of habitat variables (Pearson's product-moment, r). We also correlated standard deviations of habitat variables to mean annual discharge to assess whether discharge represented variability in habitat variables.

257 To validate that both habitat diversity (within-site habitat variability) and heterogeneity 258 (among-site habitat variability) were low and high in the Grand and Meramec river systems, 259 respectively, we performed two permutations analogous to one-sided two-sample *t*-tests with 260 unequal variances. The first permutation used values from the habitat-diversity index (response) 261 and river system (predictor) as inputs. The second permutation (habitat heterogeneity) tested 262 whether pairwise Euclidean distances among site PCA centroids were on average greater in the 263 Meramec River system than the Grand River system. Permutations were performed using the 264 'Deducer' package (Fellows, 2012) in program R.

265 Regional connectivity to downriver and headwater species pools was represented with a 266 suite of structural and functional connectivity indices (Tonkin et al., 2018). We used watercourse 267 distance of each site upriver from the Missouri (Grand) or Mississippi (Meramec) rivers as a 268 metric for downriver connectivity. Headwater connectivity is less straightforward because of 269 multiple potential sources of colonists within network branches, so we calculated structural 270 indices at three different spatial scales. We calculated densities of second-third-order stream 271 endpoints within 5 fluvial km (local scale) and 25 fluvial km (intermediate scale) of sites. 272 Endpoints either directly joined mainstems or were termini of second- and third-order streams 273 joining \geq fourth-order streams within network branches. We were uncertain about fish carrying

274 capacity and flow permanence of georeferenced first-order streams, so we excluded these as 275 sources of headwater fishes. At a basin-wide scale, we calculated average fluvial distances of 276 sites to all second-third-order stream endpoints. In case headwater fish were passively dispersed 277 into mainstems or had biased movements downriver (Peláez & Pavanelli, 2019), we also included an index that penalized basinwide upstream movements by an extra 0.2 km⁻¹, which 278 279 served as a conservative indicator of potential functional connectivity that could be further 280 explored if initially found important. Finally, we included discharge as a candidate predictor in 281 case headwater fishes were restricted to smaller nonwadeable sites rather than using structural or 282 functional corridors. All distances were calculated using a 1:100,000 stream network for 283 Missouri (Sowa, Annis, Morey, & Diamond, 2007).

We treated metrics of regional connectivity and habitat diversity as multiple competing hypotheses. All predictor variables were standardized by centering means on 0 and dividing by their standard deviations. We linearly regressed site-level richness of each stream-size guild to each predictor and evaluated relative support using Akaike's Information Criterion corrected for small sample size (AICc). We hypothesized local habitat diversity would predict core-species richness, whereas LRS- and headwater-species richness would respond to downriver and headwater connectivity, respectively.

291

292 *Objective 3: Do large-river and headwater species affect positions and profiles of hotspots?*

We used distributions of core-species along mainstems as references and examined how additions of LRS species and headwater species affected the longitudinal positions and profiles of hotspots. Our purpose was to simulate effects of lost up- and/or down-river connectivity on riverine richness. We constructed longitudinal-richness profiles of mainstem sites by relating

297 longitudinal positions of sites (distance to mouth) to richness via LOESS regression. We then 298 defined hotspots as mainstem sites with richness $\geq 75^{h}$ percentiles of predicted mainstem richness 299 within each system. We summarized longitudinal profiles and positions of hotspots by 300 calculating the number, mean distance upriver from the mouth, and longitudinal extent (km) of 301 mainstem sites qualifying as hotspots. 302

302

303 Results

304 Altogether 109 species and 46,696 individuals were collected across rivers. We detected 305 47 (60%) and 102 (79%) of 79 and 129 species historically occurring in the Grand and Meramec 306 river systems, respectively, demonstrating most species comprising regional species pools 307 occurred within nonwadeable rivers. Only six species were unique to the Grand River system, 308 and sites in the Meramec River system (57.9 species \pm 7.5 SD) supported nearly twice as many 309 species as those in the Grand River system (33.4 species \pm 4.4). 310 We observed large differences in habitat diversity (within-site habitat variability) and 311 among-site habitat heterogeneity between rivers (Figure 2). Sites in the Grand River system 312 lacked macrophytes, off-channel habitats, and boulders, and had lower variability in most other 313 habitat variables (see Table S3 for habitat data). Habitat diversity (mean \pm SD among sites) was 314 lower in the Grand River system (0.70 ± 0.14) than Meramec (1.04 ± 0.16) , permutation-based 315 one-sided t statistic = -5.3, p < 0.01). The habitat-diversity index positively correlated with 316 standard deviations of all habitat variables in both rivers, confirming it represented habitat 317 variability at sites (Table 1). Correlations between standard deviations of habitat variables and

318 discharge were generally positive for the Grand River system but mixed for the Meramec.

319 Habitat diversity was high throughout mid-sections of mainstems in both systems (Grand R. sites

320 rkm 24–145 \ge 0.77 habitat-diversity index; Meramec R. sites rkm 53–215 \ge 1.07 habitat-321 diversity index; Table S4).

322 Among-site habitat heterogeneity within the Meramec River system was nearly twice that 323 of the Grand (permutation-based one-sided t statistic, t = -4.9, p < 0.01, Figure 2), mainly 324 because Meramec River lower-course sites contrasted with mid- and upper-course sites. 325 Specifically, lower-course sites (rkm 1–30) in the Meramec River were deeper and had lower 326 water velocities, and limited macrophytes and shoal habitat (Table S3). Similar downriver 327 hydrogeomorphic changes (deepening, slowing) occurred in the Grand River, but to a lesser 328 degree, and changes were mainly apparent at the lowermost site near its confluence with the 329 Missouri River.

330

331 Species-discharge relationships

332 Changes in richness closely matched changes in discharge (Q) until richness peaked in 333 mid- to lower-course sites before dropping towards the mouths of both rivers, resulting in unimodal SDRs (pseudo- $R^2 = 0.60$ in Grand R., 0.71 in Meramec R.; Figure 3). Pseudo- R^2 was 334 335 calculated as the squared Pearson correlation coefficient between observed and predicted species 336 richness with LOESS regression curves. In the Grand River system, changes in species richness 337 were generally gradual. For example, richness ranged by only 13 species across sites, and two 338 sites contained 39 species (maximum richness) spaced 78 km apart. In contrast, richness steadily increased downriver in the Meramec River system from rkm 244 (51 spp., $Q = 19 \text{ m}^3 \text{s}^{-1}$) to rkm 339 53 (73 spp., $Q = 93 \text{ m}^3 \text{s}^{-1}$), until sharply declining by 23 species along the lower 53 km, despite 340 discharge increasing by 5 m³ s⁻¹ (50 spp. near the mouth with the Mississippi River). This decline 341 342 coincided with decreasing habitat diversity (habitat diversity at rkm 52 = 1.07 versus rkm 3 =

343 0.68). We observed a similar, albeit subtle, decline in species (8 spp.) in the Grand River system 344 between rkm 24 ($Q = 134 \text{ m}^3 \text{s}^{-1}$) and the mouth ($Q = 139 \text{ m}^3 \text{s}^{-1}$).

345

346 Distributions of core, headwater, and LRS species

347 We collected a mixture of core and non-core species at all sites across river systems. 348 Large-river specialist species were rare in the Meramec River system upriver of rkm 184 (≤ 2 349 LRS spp., $\leq 4\%$ of species), but increased sharply downriver (6–19 spp., 12–38% of species; 350 Figure 4). In contrast, percentages of LRS species comprising assemblages was $\geq 28\%$ (≥ 8 LRS 351 spp.) at all sites in the Grand River system. The highest percentages of LRS species were in 352 lower-courses of both rivers, comprising 48% (15 LRS spp.) and 38% (19 LRS spp.) of species 353 near the mouths of the Grand and Meramec rivers, respectively. 354 Patterns of headwater species diverged between systems in mid- to upper-course sites. In 355 the Meramec River system, headwater species richness was highest in sites upriver of rkm 184 356 and the Bourbeuse River (rkm 116, 7–10% of community, 4–5 spp.). In contrast, percentages of 357 headwater species richness in the Grand River system peaked in mid-course mainstem sites and 358 in branches (13–18%; 5–6 spp.).

359

360 Predictors of core, large-river, and headwater species

Processes structuring richness varied among stream-size guilds and slightly between systems (Figure 5). Habitat diversity was the best-supported variable explaining core-species richness in both systems (Grand R., Akaike weight of top model $w_I = 0.47$; Meramec R., $w_I =$ 0.85), but the relationship was stronger in the Meramec River system ($\hat{\beta} \pm$ standard error, $\hat{\beta} = 4.8$ $\pm 1.3, R^2 = 0.57$) than Grand River system ($\hat{\beta} = 1.3 \pm 0.6, R^2 = 0.37$; Table 2). In contrast, LRS richness was better explained by downriver connectivity than habitat diversity in both systems (support for downriver connectivity, $w_1 = 0.62$ in Grand R.; $w_1 > 0.99$ in Meramec R.) with diminishing LRS species richness as connectivity decreased upriver (effect size $\hat{\beta} = -1.8 \pm 0.7$ in Grand R.; $\hat{\beta} = -6.4 \pm 0.6$ in Meramec R.). Note distances to sources represent *isolation*, so signs of effects are reversed than if interpreted strictly as *connectivity*.

371 Variables explaining headwater richness varied between systems; in the Meramec River 372 system, no metrics for headwater connectivity were better supported than an intercept-only model. Instead, discharge was slightly better supported ($w_1 = 0.45$; $R^2 = 0.44$; slope = $\hat{\beta} = -1.0 \pm$ 373 374 0.5), indicating there were more headwater species at lower-discharge sites. In the Grand River 375 system, however, three of four headwater connectivity metrics were better supported than an 376 intercept-only model, indicating headwater richness increased with greater connectivity to headwater sources. Among headwater metrics, the number of second-third-order outlets within 377 378 25 fluvial km of sites garnered nearly all Akaike weight ($w_1 = 0.91$) and explained the most variation ($\hat{\beta} = 1.3 \pm 0.2$, $R^2 = 0.85$). The number of second–third-order outlets within 5 km (local 379 connectivity) had almost no support ($w_7 < 0.01$, $\hat{\beta} = 0.2 \pm 0.5$, $R^2 = 0.03$). 380

381 Occurrences of LRS species and headwater species affected the positions and spatial 382 extents of hotspots along each river (Table 3; Figure 6). If solely based on core species, both 383 systems would have broad hotspots (>55% of mainstem lengths sampled) encompassing mid-384 course reaches with high habitat diversity (mean hotspot position = rkm 83 in Grand R., rkm 102 385 in Meramec R.). However, headwater and LRS species shifted distributional centers of hotspots 386 upriver (14–19 km) and downriver (10–44 km), respectively. When hotspots were based on all 387 species, higher richness of LRS species than headwater species caused net downriver shifts (= 10 388 km in Grand R., 33 km in Meramec R.; Figure 6d, h). Moreover, because LRS species and

headwater species were not evenly distributed throughout either system, their occurrences
typically narrowed extents of hotspots relative to broad hotspots defined by core species. For
example, the Meramec River hotspot defined by all species spanned only three lower-midcourse
sites (rkm 53–91) with both high LRS and core species richness. Despite hotspots being
narrowed and shifted by non-core species, hotspots still contained 94% (44 spp.) and 80% (82
spp.) of species detected by our surveys in the Grand and Meramec river systems, respectively.

Discussion

397 Our study revealed nonwadeable, free-flowing rivers supported most species comprising 398 regional species pools. Instead of conforming to a single river concept, the distribution of fish 399 richness in both systems supported elements of spatial- (e.g., Adventitious Stream, Network 400 Position) and niche-based (River Continuum) river concepts. Moreover, support for specific 401 concepts varied spatially within and between river systems. Overall, our results indicate riverine 402 fish communities are likely metacommunities blended from core, downriver, and headwater 403 species pools. Superimposition of regional connectivity and local habitat diversity contributed to 404 uneven distributions of species, thereby affecting the positions of hotspots and shaping the 405 longitudinal profiles of richness within each system.

406

407 Scale dependency of species-discharge relationships

Species richness did not continuously increase downriver in either system, and in the
Meramec River system, the fewest species were detected at the highest-discharge site (i.e.,
Meramec River mouth). We hypothesize discrepancies between our findings and existing linear
or linearized SDRs partly result from the finer resolution of our observational units (1.5–5.0 km).

412 In comparison, most investigations of SDRs aggregate fish-collection data from multiple 413 collections across coarse spatial grains (e.g., \geq 50 km, sub-basins), which may not reflect finer-414 grain habitat changes within units (McKerrow et al., 2018). For example, if sites in the Meramec 415 River system were aggregated into three groups based on discharge (4 sites per group), richness 416 would appear to increase downriver, thereby masking lower-course declining richness (lowest 417 discharge group = 77 spp., medium discharge = 79 spp., highest discharge = 90 spp.). Similarly, 418 McGarvey and Hughes (2008), and McGarvey and Ward (2008) reported scale-dependent SDRs 419 arising from within-river heterogeneity in geology, elevation, and temperature. Although coarse-420 grained SDRs have useful basinwide applications, their predictions may not provide realistic 421 baselines of richness that match observations at finer spatiotemporal scales typical of most site-422 level ecological assessments. Instead, finer-grained SDRs or other reach-based classification 423 schemes (e.g., Troia & Mcmanamay, 2020) might be more applicable for monitoring and 424 management at local scales.

425

426 *Core species-habitat diversity relationships*

427 Core-species richness increased with habitat diversity in both river systems, indicating 428 many riverine fishes exploit locally available resources. These positive species-habitat diversity 429 relationships implicate habitat homogenization as a potential contributor to widespread declines 430 in riverine biodiversity (Koel, 2004; Peipoch et al., 2015), while indicating habitat diversity is 431 likely an important ingredient for successful river restoration (Palmer, Menninger & Bernhardt, 432 2010). In contrast to the SDR expectation of continuously increasing habitat diversity downriver, 433 habitat diversity decreased towards both river mouths due to lost variability in water velocities 434 (both rivers), substrates (Meramec R.), and aquatic vegetation (Meramec R.). Consequently,

435 lower habitat diversity and fewer core species likely underpinned declining total richness near436 both river mouths.

437 Our finding that species richness declined near river mouths contrasts with biodiversity 438 hotspots often reported near the mouths of wadeable streams (Boddy, Booker & McIntosh, 2019; 439 Hitt & Angermeier, 2008). This discrepancy might result from broader hydrogeomorphic 440 changes upstream of river confluences compared to those in smaller wadeable streams. For 441 example, river flows in lower-course reaches of our focal rivers are influenced by backwater 442 effects mediated by surface elevations of the Missouri and Mississippi rivers (Brown & Coon, 443 1994). Backwater effects slow water velocities, meaning the availability of habitat for fluvial-444 dependent species varies spatially and temporally in backwater-affected sites. These backwaters 445 are symptomatic of ecotones or "confluence zones" that develop in tributaries upstream of 446 confluences (Rice, 2017; Thornbrugh & Gido, 2010). Extensive homogenous ecotones in low-447 gradient rivers could result in low fish richness above large-tributary mouths, whereas rapid 448 dispersal (i.e., mass effects) across smaller ecotones in streams may manifest as hotspots above 449 small tributary mouths (e.g., Hitt & Angermeier, 2008; Miyazono & Taylor, 2013). Our findings 450 also contrast with Fernandes, Podos, and Lundberg (2004), who found backwater-affected areas 451 increased fish richness in nonwadeable Amazon River tributaries by concentrating food 452 resources, albeit only for electric fishes (Gymnotiformes). Thus, "tributary effects" may be 453 taxon- or system-specific.

Lower fish richness in lower-course sites also likely resulted from accumulating effects of local and regional stressors downriver. Locally, lower-courses of both rivers are partially channelized and the Grand River is extensively leveed, which can reduce habitat diversity and mainstem-floodplain connectivity (Koel, 2004). Despite channel and floodplain modifications, a

458 few isolated floodplain waterbodies remain along lowercourses of both rivers. We did not sample 459 these waterbodies, but these waterbodies undoubtably would have contributed to riverine habitat 460 diversity. Similarly, we detected many floodplain-inhabiting species by sampling off-channel 461 lentic habitats with surficial water connections and backwater-effected main channels, but there 462 were a few species that we might have detected had we sampled isolated waterbodies (e.g., 463 Mississippi silvery minnow *Hybognathus nuchalis*, bullhead catfishes *Ameiurus* spp.). These 464 species would not have off-set observed declines in core species downriver, but it is important to 465 recognize some lateral habitat diversity of our focal systems was not sampled, and natural lateral 466 habitat diversity in our systems has been compromised by channel and floodplain modifications. 467 Effects of channel modifications may have been compounded by near- and up-stream 468 intensive land uses. For example, we observed a 12-species decline between rkm 53 and rkm 30, 469 coinciding with intensifying urban land use (St. Louis metropolitan area near rkm 30). Most 470 species underpinning declines (e.g., bleeding shiner Luxilus zonatus, rainbow darter Etheostoma 471 *caeruleum*, black redhorse *Moxostoma duquesnei*) are core species reliant on upland habitats 472 (silt-free shoals, aquatic vegetation), which are often lost in rivers with urban watersheds (Allan, 473 2004). Our findings parallel those of other studies in nonwadeable rivers, which attribute 474 declining downstream richness to byproducts of intensifying land use, including diminished 475 water and habitat quality (Hughes & Gammon, 1987; Oberdorff et al., 1993; Troia & 476 Mcmanamay, 2020). Specifically, Argentina, Freeman, and Freeman (2010) found fewer benthic 477 fishes in downriver reaches of a southeastern river (USA) corresponding to intensifying land use, elevated turbidity, and fewer macrophytes. Together our work and these studies suggest that 478 479 biodiversity hotspots in nonwadeable rivers might be artificially truncated by accumulating 480 stressors downriver, especially in intensively used landscapes.

482 Large-river and headwater species

483 Downriver declines in core richness in lower-courses were partially masked by increasing 484 richness of LRS species downriver, demonstrating that spatial processes linked to the ASC and 485 NPH theories partially explain fish richness patterns in nonwadeable rivers. Upriver dispersal 486 appears more extensive in our systems than existing examples of the ASC and NPH. For 487 example, LRS richness was ≥ 13 species throughout the lower 90 km of mainstems in both 488 systems, and we detected at least one LRS species at our uppermost sites (≥ 200 km from great 489 rivers). Ferreira et al. (2019) indicated riverine species were restricted from accessing upstream 490 reaches by environmental conditions in smaller streams, and our results suggest these resistance 491 mechanisms may subside as river size increases. Similarly, Hitt and Angermeier (2008) and 492 Grenouillet, Pont, and Hérissé (2004) indicated upstream dispersal by mainstem fish increased in 493 larger branches of river networks. Although richness did not peak at either river's mouth within 494 our study, the extensive reach of LRS species contributed to total richness in mid- to lower-495 course sites causing the extent of mid-course hotspots to skew downriver.

496 Despite maximized headwater connectivity in mid-courses of both rivers, headwater fish 497 richness only peaked mid-course in the Grand River system, indicating headwater fishes interact 498 with mainstems differently across river systems. Further, Grand River headwater species 499 richness was predicted by headwater sources at intermediate (≤ 25 km) rather than local (≤ 5 km; 500 Stoll et al., 2013) scales, indicating headwater fish disperse broadly in this prairie system. Prairie 501 headwaters typically have highly variable flows prone to drying (Dodds et al., 2004). Thus, long-502 term persistence of headwater fishes in prairie river systems likely depends on periodic usage of 503 nonwadeable rivers for refuge and as corridors to recolonize re-wetted sites. This aligns with

504 Hudman and Gido (2013), who found a headwater fish, Creek Chub Semotilus atromaculatus, 505 likely disperses throughout mainstems of prairie rivers in the absence of dams and impounding. 506 Although we are unaware of community-focused studies examining use of nonwadeable prairie 507 rivers by headwater fishes, both Falke et al. (2012) and Whitney et al. (2015) found fish 508 community dynamics in ephemeral prairie streams depended on re-colonization from 509 downstream sources. Hence, convergent findings from riverine and headwater perspectives 510 indicate dispersal and connectivity are particularly important for structuring prairie fish 511 metacommunities.

512 In the Meramec River system, headwater species richness was highest in smaller upper-513 course sites, indicating larger mainstems might function as dispersal barriers for headwater 514 fishes. The discrepancy in headwater fish richness patterns between the Grand and Meramec 515 river systems likely resulted from stronger environmental gradients in the Meramec River 516 system, causing marked contrasts between headwaters and larger mainstems. Accordingly, 517 mainstem environmental conditions (e.g., presumably warmer temperatures, lower dissolved 518 oxygen, higher turbidity) might have filtered headwater fishes, especially upland species in the 519 Meramec River system that prefer cool water, high dissolved oxygen, and/or low turbidity 520 (Smale & Rabeni, 1995). Similarly, Kanno et al. (2012) detected few headwater species in 521 mainstems of an upland southeastern river system (USA), and Schmidt and Schaefer (2018) 522 found large rivers restricted genetic connectivity (and presumably among-population dispersal) 523 of two headwater fishes in southern Mississippi River drainages. If temperature and dissolved 524 oxygen contributed to mainstem-filtering, then warming temperatures may intensify mainstem-525 filtering strength, thereby further inhibiting headwater fishes from tracking climatic changes 526 within upland river systems (Troia, Kaz, Niemeyer, & Giam, 2019).

528

Riverine fish communities as metacommunities

529 Both spatial and niche-based mechanisms likely contributed to longitudinal riverine 530 richness, demonstrating the utility of metacommunity theory for explaining patterns in riverine 531 communities (López-Delgado et al., 2018; Vitorino Júnior et al., 2016). Spatial processes (e.g., 532 mass effects, long-distance dispersal) are thought to predominate community dynamics in larger 533 streams and rivers (Brown & Swan, 2010; Erős, 2017; Vitorino Júnior et al., 2016). However, we 534 found core species richness responded to local habitat diversity, demonstrating niche-based 535 processes (e.g., species-sorting) structure substantial percentages of riverine fish richness. 536 We also showed that the relative importance of spatial and niche-based processes varies 537 longitudinally along mainstems and among river systems. For example, spatial processes are 538 likely more important in lower-course reaches with lower channel habitat diversity but have 539 higher accessibility to downriver regional species pools (Ferreira et al., 2019; Peláez & 540 Pavanelli, 2019). Similarly, finds from the Grand River system indicated regional connectivity 541 and dispersal are likely more important in structuring communities within systems spanning 542 weak environmental gradients (i.e., Grand River). Unfortunately, few metacommunity-framed 543 studies are set in nonwadeable rivers to corroborate our findings; greater representation in 544 literature may further clarify mechanisms and contingencies of community assembly within free-545 flowing rivers.

546

547 Implications for conserving riverine hotspots

548 Riverine biodiversity is increasingly managed at riverscape scales, and conserving
549 hotspots is at the core of many landscape conservation strategies (Smith et al., 2018). However,

550 specific contributions of underlying processes to riverine richness, and thus biodiversity

hotspots, are often unclear (Erős, 2017). Our findings suggest some common actions may benefit conserving hotspots in both systems. For example, LRS species comprised large percentages of species (23–44%) inhabiting hotspots in both rivers, demonstrating the importance of downriver connectivity to riverine fish richness (King et al., 2017). Lower-course river reaches often harbor unique species (Kanno et al., 2012; Miranda & Kilgore, 2020), and our results indicate many of these species are likely linked to downriver species pools.

557 Other aspects of conservation planning strategies may need unique tailoring for different 558 systems with varying levels of habitat diversity and connectivity. Habitat diversity contributed to 559 hotspots in both systems, but species-habitat diversity relationships were far stronger in the 560 Meramec River system. Consequently, protecting the narrowly distributed Meramec River 561 hotspot likely depends on prioritizing actions that maintain mid-course habitat diversity, such as 562 minimizing channel modifications and mitigating impacts from intensive upstream land uses 563 (Abell et al., 2017). In contrast, preserving hotspots in temporally variable systems with low 564 habitat diversity, such as prairie rivers, may require prioritizing watershed connectivity via 565 targeted barrier removals and modifying dispersal-inhibiting infrastructure (e.g., Perkin & Gido, 566 2012).

567

568 *Conclusions*

569 Our study indicated riverine richness is spatially explicit and highly integrated with both 570 downriver and headwater surrounding species pools. We support contentions of Vitorino Júnior 571 et al. (2016), and López-Delgado et al. (2018) that riverine fishes within nonwadeable rivers are 572 likely organized as metacommunities. Regional connectivity contributed LRS species and

headwater species along longitudinal gradients in habitat diversity to generate river-specificprofiles of fish richness.

575 Rivers are among the most globally impacted ecosystems, and relatively few free-flowing 576 rivers remain in industrialized nations (Grill et al., 2019). Given local riverine richness is also 577 regionally sourced, diminished connectivity and habitat diversity may shift and misshapen 578 longitudinal patterns of riverine richness. For example, diminished downriver connectivity could 579 re-center hotspots upriver, and broad habitat homogenization might flatten longitudinal profiles 580 of local richness. Thus, longitudinal profiles not only reflect richness but also key mechanisms 581 governing resiliency of riverine macrosystems to stressors operating across scales (Van Looy et 582 al., 2019).

583

584 Acknowledgements

585 The Missouri Department of Conservation (MDC) funded this research through Project 358. We 586 thank MDC personnel for input on the study design. Bob Hrabik provided advice on stream-size 587 preference guilds. Dr. Steve Miranda provided constructive comments on an early draft. We 588 thank all crewmembers that collected data for this project. This study was performed under the 589 auspices of University of Missouri protocol 8532. Any use of trade, firm, or product names is for 590 descriptive purposes only and does not imply endorsement by the U.S. Government. The 591 Missouri Cooperative Fish and Wildlife Research Unit is jointly sponsored by the Missouri 592 Department of Conservation, the University of Missouri, the U.S. Geological Survey, the U.S. 593 Fish and Wildlife Service, and the Wildlife Management Institute.

594

595	Data availability statement: Underlying data are in Tables S3–S4. Distributional data were
596	added to databases maintained by the Missouri Department of Conservation.
597	

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824 Tables

TABLE 1 Habitat variables recorded at 21 transects at each of the 22 sites in the Grand (N = 10) and Meramec (N = 12) rivers in 2016 (Missouri, USA). Habitat diversity at sites were from transformed or raw ("-") variables based on average dispersion of transects around site centroids in principal component space. Pearson product-moment correlations (r) were between standard deviations of habitat variables at sites and habitat diversity or discharge. Positive correlations indicate multivariate habitat diversity and discharge represent variability in specific habitat variables. No sampled macrophytes prevented us from calculating correlations in the Grand

832 River system

			Habitat diversity (r)		Disc	narge (<i>r</i>)	
Variable	Unit	Transformation	Grand	Meramec	Grand	Meramec	
Boulder	Count	Log(x + 0.1)	0.56	0.66	0.66	0.04	
Depth	m	Log(x + 0.1)	0.63	0.71	0.15	-0.39	
Large wood	Count	Log(x + 0.1)	0.69	0.66	0.66	-0.48	
Macrophyte	Ordinal	Log(x + 0.1)	-	0.70	-	-0.44	
Off-channel	%	Log(x + 0.1)	0.37	0.49	0.02	0.05	
Pool	Count	-	0.75	0.61	0.11	-0.36	
Shoal	Count	-	0.61	0.80	0.10	-0.49	
Substrate size	Ordinal	-	0.40	0.17	0.21	0.27	
Velocity	$m s^{-1}$	\sqrt{x}	0.72	0.83	-0.04	-0.44	

833	TABLE 2 Parameters (K) and evaluation criteria of competing linear regression models
834	(hypotheses) explaining fish species richness of three stream-size fish guilds in the Grand ($N =$
835	10 mainstem and branch sites) and Meramec ($N = 12$ mainstem and branch sites) rivers in 2016
836	(Missouri, USA). Lower delta Akaike Information Criterion values (Δ AICc) and higher weights
837	(w_i) are better-supported models. Exempting intercept-only (null) models, each model included
838	both an intercept $(\hat{\beta}_0)$ and slope $(\hat{\beta}_1) \pm$ standard error (SE). Habitat diversity was the average
839	dispersion of habitat transects around multivariate centroids. Downriver isolation is the
840	watercourse distance (km) to the mouth of each river. Headwater (HW) isolation (basinwide) is
841	the mean distance (km) to second-third-order outlets within each river system. Headwater
842	isolation (directional) is basinwide headwater isolation penalized for upstream movements.
843	Headwater sources are numbers of second-third-order outlets within specified watercourse
844	distances of sites (km)

845

		Log-			2	<u>^</u>	•		
Hypothesis	Κ	likelihood	ΔAICc	W_i	R^2	$\hat{\boldsymbol{\beta}}_0 \pm \mathrm{SE}$	$\hat{\beta}_1 \pm SE$		
Grand: Core species									
Habitat diversity	2	-18.5	0.0	0.47	0.37	17.0 ± 0.5	1.3 ± 0.6		
Intercept-only (null)	1	-20.9	0.4	0.38	< 0.01	17.0 ± 0.6	-		
Downriver isolation	2	-20.3	3.6	0.08	0.11	17.0 ± 0.7	$\textbf{-}0.7\pm0.7$		
HW isolation (basinwide)	2	-20.4	3.7	0.08	0.10	17.0 ± 0.7	$\textbf{-}0.6\pm0.7$		
		Merame	c: Core	species	5				
Habitat diversity	2	-33.8	0.0	0.85	0.58	46.0 ± 1.3	5.0 ± 1.3		
HW isolation (basinwide)	2	-35.9	4.1	0.11	0.41	46.0 ± 1.5	-4.2 ± 1.6		
Intercept-only (null)	1	-39.0	6.7	0.03	0.00	46.0 ± 1.9	-		
Downriver isolation	2	-38.5	9.2	0.01	0.09	46.0 ± 1.9	2.0 ± 2.0		
Grand: Large-river species									
Downriver isolation	2	-20.8	0.0	0.62	0.44	12.5 ± 0.7	-1.8 ± 0.7		
Intercept-only (null)	1	-23.7	1.5	0.29	< 0.01	12.5 ± 0.9	-		
Habitat diversity	2	-22.7	3.9	0.09	0.18	12.5 ± 0.8	1.1 ± 0.9		
Meramec: Large-river species									
Downriver isolation	2	-24.7	0.0	1.00	0.91	9.8 ± 0.6	-6.4 ± 0.6		
Intercept-only (null)	1	-39.4	25.7	0.00	0.00	9.8 ± 1.9	-		
Habitat diversity		-37.7	26.1	0.00	0.24	9.8 ± 1.8	-3.3 ± 1.9		
5									

Grand: Headwater species							
HW sources (25 km)	2	-7.8	0.0	0.91	0.85	3.9 ± 0.2	1.3 ± 0.2
HW isolation (basinwide)	2	-10.6	5.6	0.06	0.74	3.9 ± 0.2	-1.2 ± 0.3
HW isolation							
(directional)	2	-11.0	6.5	0.04	0.72	3.9 ± 0.3	-1.2 ± 0.3
Intercept-only (null)	1	-17.4	14.9	0.00	0.00	3.9 ± 0.5	-
Discharge	2	-15.5	15.5	0.00	0.31	3.9 ± 0.4	-0.8 ± 0.4
Habitat diversity	2	-17.2	18.7	0.00	0.04	3.9 ± 0.5	0.3 ± 0.5
HW sources (5 km)	2	-17.2	18.9	0.00	0.03	3.9 ± 0.5	0.2 ± 0.5
	N	Ieramec:]	Headwa	ter spec	cies		
Discharge	2	-21.3	0.0	0.45	0.32	2.0 ± 0.5	-1.0 ± 0.5
Intercept-only (null)	1	-23.6	0.9	0.29	0.00	2.0 ± 0.5	-
HW isolation							
(directional)	2	-23.2	3.7	0.07	0.07	2.0 ± 0.5	0.5 ± 0.6
HW sources (5 km)	2	-23.4	4.1	0.06	0.04	2.0 ± 0.5	0.3 ± 0.6
HW sources (25 km)	2	-23.6	4.5	0.05	0.01	2.0 ± 0.5	-0.1 ± 0.6
Habitat diversity	2	-23.6	4.5	0.05	0.01	2.0 ± 0.5	0.1 ± 0.6
HW isolation (basinwide)	2	-23.6	4.5	0.05	0.00	2.0 ± 0.5	0.0 ± 0.6

846	TABLE 3 Longitudinal positions and spatial extents of mainstem sites with fish species richness
847	\geq 75th percentile ("hotspot minimum") in the Grand ($N = 8$ mainstem-only sites) and Meramec
848	(N = 10 mainstem-only sites) rivers in 2016 (Missouri, USA). Hotspot sites were first defined
849	solely by core species and then with additional stream-size guilds (Table S1). Inclusion of
850	headwater and/or large-river specialist species caused hotspot zones to shift and narrow relative
851	to hotspots defined by core species (except core + headwater species). Positions are watercourse
852	distances to respective mouths of each river. Downriver and Upriver are downriver and upriver
853	limits of hotspot zones, respectively

Stream-size guilds	Hotspot minimum (species)	Hotspot sites	Mean position (km)	Hotspot extent (km)	Down- river (km)	Up- river (km)	Hotspot shift	Hotspot extent
Grand River								
Core (reference)	18	5	83	120	24	145	-	-
Core + headwater	23	2	96	15	89	104	Upriver	Narrowed
Core + large-river	32	3	72	80	24	104	Downrive	r Narrowed
All species	35	3	72	80	24	104	Downrive	r Narrowed
			Merar	nec Rive	r			
Core (reference)	49	5	102	132	53	184	-	-
Core + headwater	51	6	121	162	53	215	Upriver	Widened
Core + large-river	61	4	59	61	30	91	Downrive	r Narrowed
All species	62	3	69	39	53	91	Downrive	r Narrowed

855 Figures

856



FIGURE 1 Nonwadeable mainstem and tributary branch sites in the Grand (*N* = 10, Prairie

region) and Meramec (N = 12, Ozark region) river systems surveyed for fish in 2016 (Missouri,

- USA). Insets: sites extended at least 1 km away from major confluences, and watercourse
- 860 distances (river km) are upriver of each river system's mouth. Gray areas are municipal
- 861 boundaries indicative of urban development



FIGURE 2 Left: within-site habitat diversity at sites in the Grand (N = 10) and Meramec (N =
12) rivers in 2016 (Missouri, USA). We calculated habitat diversity by first performing a
principal component (PC) analysis of nine habitat variables summarized to 21 transects. Habitat
diversity at each site was the average Euclidean distance of transects to a site's centroid in PC
space (units = PC axes). Right: among-site habitat heterogeneity (dissimilarity) was the pairwise
Euclidean distances among site habitat centroids in PC space (y-axis units = Euclidean distances
in PC space). Horizontal bars are averages



FIGURE 3 Species-discharge relationships between mean annual discharge and fish species richness in the Grand (N = 10 sites; bottom) and Meramec (N = 12 sites; top) rivers from 2016 (Missouri, USA). Pseudo- R^2 values (squared Pearson correlation [r] between observed and predicted) are from Local Regression Smoothers (dashed lines \pm 90% confidence intervals) and were 0.60 and 0.71 for models in Grand (lighter shaded symbols) and Meramec rivers (darker symbols), respectively. Labels are watercourse distances (km) from the Missouri (Grand R.) and Mississippi (Meramec R.) rivers





879 **FIGURE 4** Relationships between mean annual discharge and fish species richness within three

stream-size preference guilds (Table S1) for the Grand (left, N = 10 sites) and Meramec (right, N

881 = 12 sites) rivers in 2016 (Missouri, USA). The top horizontal axis is watercourse distance

- 882 upriver from a respective river mouth to the Missouri River (Grand River) or Mississippi River
- 883 (Meramec River) (km). "B" = sites in tributary branches



884

FIGURE 5 Habitat and spatial predictors of fish species richness (\pm 90% confidence interval) for three river-size guilds in the Grand (N = 10 sites) and Meramec (N = 12 sites) rivers in 2016 (Missouri, USA). Habitat diversity and distance-from-mouth were best-supported hypotheses explaining richness for core and large-river specialist species in both river systems, respectively. Headwater sources within 25 km of sites was the best-supported hypothesis explaining headwater species for the Grand River system, but not the Meramec River system. Classifications for headwater and large-river species are in Table S1



FIGURE 6 Longitudinal profiles of fish species richness for three stream-size guilds in the Grand (N = 8 mainstem sites) and Meramec (N = 10 mainstem sites) rivers in 2016 (Missouri, USA). Profiles are Local Regression Smoothers (LOESS) between distance from river mouth and richness. Hotspots (white) and coolspots (black) are sites where observed richness was \geq 75th and <75th percentile of predicted richness (dashed line), respectively. Shading reflects longitudinal richness profiles constructed from different stream-size guilds. Profiles were

- 899 developed for four combinations of stream-size guilds: (a, e) core species, (b, f) core + headwater
- 900 species, (c, g) core + large-river specialist species, and (d, h) all species. Horizontal error bars
- 901 show means and ranges of sites within hotspot zones

902 Appendices

TABLE S1 Memberships of fish species to three stream-size preference guilds for the Grand and 903 904 Meramec river systems (Missouri, USA). Headwater fishes were likely sourced in $\leq 3^{rd}$ -order 905 streams. Their membership designations were slightly modified from the "headwater zone" 906 (Pflieger 1989) to include updated records from Missouri Department of Conservation 907 unpublished databases (Fish community database pre-2010; Resource Assessment and 908 Monitoring program 1994–2014). Large-river specialist species in tributaries likely dispersed 909 from, and/or have high population connectivity to, the Missouri (Grand R.) or Mississippi 910 (Meramec R.) rivers. Large-river specialist designations were slightly modified from the "Big 911 River" fishes group (Pflieger 1989) to include diadromous species, introduced large-river 912 species, and lowland species associated with the Mississippi River floodplain. Species were 913 either detected (1) or undetected (0) by our sampling in 2016, or unknown to occur (-) within a 914 river system

	Stream-size	Grand	Meramec
Species	guild	River	River
Banded Darter Etheostoma zonale	Core	-	1
Bigeye Chub Hybopsis amblops	Core	-	1
Bigeye Shiner Notropis boops	Core	-	1
Black Crappie Pomoxis nigromaculatus	Core	1	1
Black Redhorse Moxostoma duquesnei	Core	0	1
Blackspotted Topminnow Fundulus olivaceus	Core	-	1
Bleeding Shiner Luxilus zonatus	Core	-	1
Bluegill Lepomis macrochirus	Core	1	1
Bluntnose Minnow Pimephales notatus	Core	1	1
Brook Silverside Labidesthes sicculus	Core	0	1
Carmine Shiner Notropis percobromus	Core	-	1
Channel Catfish Ictalurus punctatus	Core	1	1
Chestnut Lamprey Ichthyomyzon castaneus	Core	-	1
Common Carp Cyprinus carpio	Core	1	1
Crystal Darter Crystallaria asprella	Core	-	1
Flathead Catfish Pylodictis olivaris	Core	1	1
Freckled Madtom Noturus nocturnus	Core	-	1
Freshwater Drum Aplodinotus grunniens	Core	1	1
Gilt Darter Percina evides	Core	-	1
Gizzard Shad Dorosoma cepedianum	Core	1	1
Golden Redhorse Moxostoma erythrurum	Core	0	1
Golden Shiner Notemigonus crysoleucas	Core	1	1
Gravel Chub Erimystax x-punctatus	Core	-	1
Green Sunfish Lepomis cyanellus	Core	1	1
Greenside Darter Etheostoma blennioides	Core	-	1
Highfin Carpsucker Carpiodes velifer	Core	0	1
Largemouth Bass Micropterus salmoides	Core	1	1

Table S1 continued: species stream-size pr	reference memb	erships	
	Stream-size	Grand	Meramec
Species	guild	River	River
Largescale Stoneroller <i>Campostoma oligolepis</i>	Core	-	1
Logperch Percina caprodes	Core	0	1
Longear Sunfish Lepomis megalotis	Core	0	1
Longnose Gar Lepisosteus osseus	Core	1	1
Meramec Saddled Darter Etheostoma erythrozonum	Core	-	1
Mimic Shiner Notropis volucellus	Core	-	1
Mooneye Hiodon tergisus	Core	0	1
Northern Hogsucker Hypentelium nigricans	Core	-	1
Northern Studfish Fundulus catenatus	Core	-	1
Orangespotted Sunfish Lepomis humilis	Core	1	1
Quillback Carpiodes cyprinus	Core	0	1
Rainbow Darter Etheostoma caeruleum	Core	-	1
Red Shiner Cyprinella lutrensis	Core	1	1
Redear Sunfish Lepomis microlophus	Core	-	1
River Redhorse Moxostoma carinatum	Core	-	1
Rock Bass Ambloplites rupestris	Core	-	1
Sand Shiner Notropis stramineus	Core	1	1
Shorthead Redhorse Moxostoma macrolepidotum	Core	1	1
Silver Redhorse Moxostoma anisurum	Core	-	1
Slenderhead Darter Percina phoxocephala	Core	-	1
Smallmouth Bass Micropterus dolomieu	Core	-	1
Smallmouth Buffalo Ictiobus bubalus	Core	1	1
Spotfin Shiner Cyprinella spiloptera	Core	-	1
Spotted Bass Micropterus punctulatus	Core	0	1
Spotted Sucker Minytrema melanops	Core	-	1
Steelcolor Shiner Cyprinella whipplei	Core	-	1
Stonecat Noturus flavus	Core	1	1
Striped Shiner Luxilus chrysocephalus	Core	-	1
Suckermouth Minnow <i>Phenacobius mirabilis</i>	Core	1	0
Walleye Sander vitreus	Core	1	1
Warmouth Lepomis gulosus	Core	-	1
Wedgespot Shiner Notropis greenei	Core	-	1
Western Mosquitofish Gambusia affinis	Core	1	1
White Crappie Pomoxis annularis	Core	1	1
Yellow Bullhead Ameiurus natalis	Core	1	0
Banded Sculpin Cottus carolinae	Headwater	-	1
Bigmouth Shiner <i>Notropis dorsalis</i>	Headwater	1	0
Black Bullhead Ameiurus melas	Headwater	0	0
Blackside Darter <i>Percina maculata</i>	Headwater	-	Õ
Blackstripe Topminnow Fundulus notatus	Headwater	-	Õ

Table S1 continued: species stream-size p	reference memb	erships	
	Stream-size	Grand	Meramec
Species	guild	River	River
Brassy Minnow Hybognathus hankinsoni	Headwater	0	-
Central Stoneroller Campostoma anomalum	Headwater	1	1
Common Shiner Luxilus cornutus	Headwater	0	-
Creek Chub Semotilus atromaculatus	Headwater	1	1
Creek Chubsucker Erimyzon claviformis	Headwater	-	0
Fantail Darter Etheostoma flabellare	Headwater	-	1
Fathead Minnow Pimephales promelas	Headwater	1	0
Grass Pickerel Esox americanus	Headwater	-	1
Hornyhead Chub Nocomis biguttatus	Headwater	-	1
*Johnny Darter Etheostoma nigrum	Headwater	1	-
Least Brook Lamprey Lampetra aepyptera	Headwater	-	0
Mottled Sculpin Cottus bairdii	Headwater	-	1
Northern Brook Lamprey Ichthyomyzon fossor	Headwater	-	0
Orangethroat Darter Etheostoma spectabile	Headwater	0	1
Ozark Minnow Notropis nubilus	Headwater	-	1
Redfin Shiner Lythrurus umbratilis	Headwater	0	0
Silverjaw Minnow Notropis buccatus	Headwater	-	1
Slender Madtom Noturus exilis	Headwater	-	1
Southern Redbelly Dace Chrosomus erythrogaster	Headwater	-	1
Stippled Darter Etheostoma punctulatum	Headwater	-	0
Topeka Shiner Notropis topeka	Headwater	0	-
Trout-Perch Percopsis omiscomaycus	Headwater	0	-
White Sucker Catostomus commersonii	Headwater	1	0
Alabama Shad Alosa alabamae	Large-river	0	1
Alligator Gar Atractosteus spatula	Large-river	-	0
American Eel Anguilla rostrata	Large-river	0	1
Bighead Carp Hypophthalmichthys nobilis	Large-river	1	1
Bigmouth Buffalo Ictiobus cyprinellus	Large-river	1	1
Black Buffalo Ictiobus niger	Large-river	1	1
Blue Catfish Ictalurus furcatus	Large-river	1	1
Blue Sucker Cycleptus elongatus	Large-river	1	1
Bowfin Amia calva	Large-river	-	1
Bullhead Minnow Pimephales vigilax	Large-river	1	1
Channel Shiner Notropis wickliffi	Large-river	0	1
Emerald Shiner Notropis atherinoides	Large-river	1	1
Flathead Chub Platygobio gracilis	Large-river	0	0
Ghost Shiner Notropis buchanani	Large-river	0	1
Goldeye Hiodon alosoides	Large-river	1	1
Grass Carp Ctenopharyngodon idella	Large-river	1	1
Lake Sturgeon Acipenser fulvescens	Large-river	0	0

Table S1 continued: species stream-size preference memberships						
	Stream-size	Grand	Meramec			
Species	guild	River	River			
Mississippi Silvery Minnow Hybognathus nuchalis	Large-river	-	0			
Mud Darter Etheostoma asprigene	Large-river	-	1			
Paddlefish Polyodon spathula	Large-river	0	0			
Pallid Sturgeon Scaphirhynchus albus	Large-river	0	0			
Plains Minnow Hybognathus placitus	Large-river	1	0			
River Carpsucker Carpiodes carpio	Large-river	1	1			
River Darter Percina shumardi	Large-river	-	1			
River Shiner Notropis blennius	Large-river	0	1			
Sauger Sander canadensis	Large-river	0	1			
Shoal Chub Macrhybopsis hyostoma	Large-river	1	1			
Shortnose Gar Lepisosteus platostomus	Large-river	1	1			
Shovelnose Sturgeon Scaphirhynchus platorynchus	Large-river	1	1			
Sicklefin Chub Macrhybopsis meeki	Large-river	0	0			
Silver Carp Hypophthalmichthys molitrix	Large-river	1	1			
Silver Chub Macrhybopsis storeriana	Large-river	1	1			
Silver Lamprey Ichthyomyzon unicuspis	Large-river	0	1			
Silverband Shiner Notropis shumardi	Large-river	0	0			
Skipjack Herring Alosa chrysochloris	Large-river	1	1			
Spottail Shiner Notropis hudsonius	Large-river	-	0			
Sturgeon Chub Macrhybopsis gelida	Large-river	0	0			
Threadfin Shad Dorosoma petenense	Large-river	0	0			
Western Sand Darter Ammocrypta clara	Large-river	-	1			
Western Silvery Minnow Hybognathus argyritis	Large-river	0	0			
White Bass Morone chrysops	Large-river	1	1			
Yellow Bass Morone mississippiensis	Large-river	0	1			

* Johnny Darters are specialized for headwaters in the Grand River system, but not the Meramec River system where the species is common in larger streams and rivers (Pflieger 1997).

- **FIGURE S1.** Images of Grand River (left column) and Meramec River (right column). Row one:
- 919 confluence zones of Grand (rkm 3) and Meramec (rkm 3) rivers. Row two: sites upriver, beyond
- 920 confluence zones of Grand (rkm 24) and Meramec (rkm 52) rivers. Row three: mid- to upper-
- 921 course sites in Grand (rkm 145) and Meramec (rkm 244) rivers



- 928 FIGURE S2 Imagery and georeferenced fish and habitat (inset) survey of a site (rkm = 120) of
- 929 the Meramec River in 2016 (E = 686710, N = 4246362, UTM zone 15 N Missouri, USA).
- Habitat data were collected at five equidistant points along 21 transects plus additional points if
- transects intersected off-channel areas (≥ 105 points per site). Image was obtained from 2012
- 932 National Agriculture Imagery Program
- 933





TABLE S2 Fish sampling effort per site for the Grand (N = 10 sites) and Meramec (N = 12 sites)
river systems in 2016 (Missouri, USA). Sampling effort was proportional to mean wetted
channel width (MWCW). Distances are to the mouths of the Missouri (Grand R.) and Mississippi
(Meramec R.) rivers. Total distances electrofished (Electro) and trawled per site were
accumulated from individual 50-m runs. M-fyke = mini-fyke net

Location	Category	Length	Electro	M-fyke	Ноор	Seine	Trammel	Trawl		
(rkm)	(MWCW)	(km)	(m)	(nets)	(nets)	(hauls)	(net)	(m)		
Grand River										
3	>95	5.0	1800	4	2	25	1	500		
24	75-84	4.0	1450	4	2	20	1	400		
52	75-84	4.0	1450	4	2	20	1	400		
89	65-74	3.5	1250	4	2	17	1	350		
^b 103.6	45-54	2.5	900	4	2	12	1	250		
103.9	45-54	2.5	900	4	2	12	1	250		
145	45-54	2.5	900	4	2	12	1	250		
151	45-54	2.5	900	4	2	12	1	250		
199	35–44	2.0	700	4	2	10	1	200		
^b 92	25-34	1.5	550	4	2	7	1	150		
			Merai	mec Rive	er					
3	>95	5.0	1800	4	2	25	1	500		
30	85–94	4.5	1600	4	2	22	1	450		
53	75-84	4.0	1450	4	2	20	1	400		
63	65-74	3.5	1250	4	2	17	1	350		
91	65-74	3.5	1250	4	2	17	1	350		
120	55-64	3.0	1100	4	2	15	1	300		
145	45-54	2.5	900	4	2	12	1	250		
184	45-54	2.5	900	4	2	12	1	250		
215	45-54	2.5	900	4	2	12	1	250		
^b 62	35–44	2.0	700	4	2	10	1	200		
244	35–44	2.0	700	4	2	10	1	200		
^b 116	25-34	1.5	550	4	2	7	1	150		

941 ^b branch site: rkm = 92 Shoal Creek, 103.6 Thompson River, 62 Big River, 116 Bourbeuse River

FIGURE S3 Linear relationships ± 90% confidence intervals (shaded in gray) between
watershed area (WSA) and mean annual discharge (years 1920–2016) measured at six and five
USGS stream gages in the Grand and Meramec river drainages, respectively. Estimated
relationships were used to predict mean annual discharge at sites without discharge gages. Grand
River gage IDs: 06899500, 06902000, 06897500, 06901500, 06899700, 06900000; Meramec
River gage IDs: 07013000, 07014500, 07019000, 07018500, 07016500



950 **TABLE S3** Means (standard deviation) for habitat variables summarized to transects (*N* = 21

951 transects per site) at sites in the Grand and Meramec river systems in 2016 (Missouri, USA).

952 Distances are to the mouths of each system

953

			Large	Macro-	Off			Sub-		
Location	Boulder	Depth	wood	phytes	channel	Pool	Shoal	strate	Velocity	
(rkm)	(count)	(m)	(count)	(ordinal)	(%)	(count)	(count)	(ordinal)	$(m s^{-1})$	
Grand River										
3	0.6 (1.9)	2.7 (0.5)	0.5 (0.7)	0.0 (0.0)	0 (0)	4.8 (1.1)	0.0 (0.0)	1.1 (0.3)	0.1 (0.0)	
24	1.7 (4.4)	1.4 (0.8)	0.7 (1.1)	0.0 (0.0)	0 (0)	1.0 (2.0)	0.7 (1.8)	1.8 (0.3)	0.2 (0.1)	
52	1.4 (3.9)	1.3 (0.4)	0.9 (1.3)	0.0 (0.0)	1 (3)	1.4 (2.3)	1.7 (2.4)	1.8 (0.3)	0.4 (0.1)	
89	0.4 (1.7)	0.8 (0.2)	1.0 (1.5)	0.0 (0.0)	1 (2)	0.5 (1.5)	1.2 (2.2)	1.9 (0.3)	0.3 (0.1)	
^b 92	0.4 (1.7)	1.0 (0.3)	0.9 (0.8)	0.0 (0.0)	0 (0)	3.3 (2.4)	0.0 (0.0)	1.4 (0.4)	0.1 (0.1)	
^b 103.6	0.0 (0.2)	0.4 (0.1)	0.3 (0.6)	0.0 (0.0)	0(1)	0.0 (0.0)	0.6 (2.0)	1.9 (0.1)	0.3 (0.1)	
103.9	0.0 (0.0)	0.7 (0.2)	0.5 (0.7)	0.0 (0.0)	0 (0)	1.4 (2.3)	1.0 (2.0)	1.8 (0.1)	0.3 (0.1)	
145	0.4 (0.9)	0.8 (0.3)	0.3 (0.5)	0.0 (0.0)	1 (3)	1.0 (2.0)	0.8 (1.7)	1.9 (0.4)	0.2 (0.1)	
151	0.0 (0.0)	0.6 (0.2)	0.2 (0.7)	0.0 (0.0)	0 (0)	0.2 (1.1)	0.3 (1.3)	2.1 (0.3)	0.3 (0.1)	
199	0.1 (0.7)	0.4 (0.2)	0.2 (0.4)	0.0 (0.0)	0(1)	0.2 (1.1)	0.7 (1.8)	2.0 (0.2)	0.3 (0.1)	
Mean	0.5 (1.5)	1.0 (0.3)	0.5 (0.8)	0.0 (0.0)	0 (0)	1.4 (1.6)	0.7 (1.5)	1.8 (0.3)	0.3 (0.1)	
				Meran	ec Rive	r				
3	1.3 (3.3)	3.5 (0.4)	0.4 (0.7)	0.0 (0.0)	4 (5)	5.0 (0.0)	0.0 (0.0)	2.2 (0.4)	0.0 (0.0)	
30	2.6 (3.4)	1.8 (0.6)	0.7 (0.8)	0.0 (0.0)	2(4)	3.3 (2.4)	1.0 (1.8)	3.0 (0.6)	0.2 (0.1)	
53	4.0 (4.6)	1.2 (0.4)	0.5 (0.8)	0.9 (1.1)	7 (13)	1.2 (2.2)	1.4 (2.3)	3.0 (0.4)	0.3 (0.1)	
^b 62	1.8 (2.4)	1.0 (0.4)	0.8 (0.9)	0.9 (1.1)	0 (0)	1.7 (2.4)	1.5 (2.2)	3.0 (0.5)	0.3 (0.2)	
63	3.7 (4.3)	0.9 (0.3)	0.5 (1.0)	1.2 (1.1)	2 (6)	0.5 (1.5)	1.3 (2.0)	3.1 (0.4)	0.4 (0.1)	
91	4.3 (7.0)	1.1 (0.4)	0.9 (1.3)	1.0 (0.9)	3 (5)	1.4 (2.2)	1.4 (2.2)	2.8 (0.4)	0.3 (0.2)	
^b 116	0.3 (1.1)	0.7 (0.3)	0.6 (1.2)	0.5(0.7)	3 (8)	1.7 (2.4)	1.2 (1.9)	3.0 (0.3)	0.2 (0.1)	
120	7.1 (8.4)	1.2 (0.7)	0.8 (0.8)	1.1 (1.1)	11 (18)	2.8 (2.5)	1.5 (2.1)	3.0 (0.5)	0.3 (0.2)	
145	3.9 (6.6)	0.9 (0.3)	1.0 (1.3)	1.2 (1.0)	9 (18)	0.9 (1.8)	1.7 (2.3)	2.8 (0.4)	0.3 (0.1)	
184	8.0 (10.5)	0.7 (0.4)	1.0 (1.6)	1.1 (0.9)	12 (20)	0.7 (1.8)	2.4 (2.6)	2.9 (0.5)	0.4 (0.2)	
215	4.0 (8.3)	0.9 (0.4)	0.6 (0.8)	0.7 (0.9)	5 (13)	1.4 (2.3)	1.5 (2.4)	2.8 (0.5)	0.3 (0.1)	
244	3.8 (4.2)	0.7 (0.2)	0.6 (0.7)	1.2(0.5)	5 (13)	0.5 (1.5)	1.0 (1.9)	2.8(0.3)	0.2 (0.1)	
Mean	3.7 (5.4)	1.2 (0.4)	0.7 (1.0)	0.8 (0.8)	5 (10)	1.8 (1.9)	1.3 (2.0)	2.9 (0.4)	0.3 (0.1)	

954 ^b branch sites. km = 92 Shoal Creek, 103.6 Thompson River, 62 Big River, 116 Bourbeuse River

(Missouri, USA). Headwater (HW) sources were counts of 2^{nd} - and 3^{rd} -order stream	stems in 2016
and	ream outlets
within specified distances. System connectivity is mean distance of sites to $2^{nd}-3^{nd}$	-3 rd -order stream
outlets across each river system. Upriver distances were penalized 20% (distance x	ce x 1.2 km) for
directional (system) connectivity. LRS = large-river specialist species (spp.). Abbr	bbreviations: Spp.
960 = species, conn = connectivity	

					Dis-		HW	HW	System	Directional	
Location	Total	Core	LRS	HW	charge	Habitat	sources	sources	conn.	conn.	
(rkm)	spp.	spp.	spp.	spp.	$(m^{3}s^{-1})$	diversity	(5 km)	(25 km)	(km)	(km)	
Grand River											
3	31	15	15	1	139	0.54	3	10	182	182	
24	39	19	17	3	134	0.85	4	18	160	160	
52	34	18	13	3	122	0.91	3	22	138	138	
89	38	19	13	6	98	0.78	6	27	116	118	
^b 92	28	15	8	5	11	0.68	4	27	115	117	
^b 103.6	34	17	12	5	39	0.55	1	25	113	115	
103.9	39	19	15	5	47	0.77	1	26	112	115	
145	34	18	12	4	40	0.77	1	20	122	130	
151	30	17	10	3	39	0.49	1	18	124	132	
199	27	13	10	4	22	0.61	2	18	144	159	
					Meran	iec River					
3	50	30	19	1	97	0.68	6	10	207	208	
30	61	44	17	0	95	0.96	4	18	181	181	
53	73	55	17	1	93	1.07	4	17	161	162	
^b 62	55	42	13	0	24	0.98	1	19	155	156	
63	68	50	16	2	69	1.01	2	17	153	154	
91	65	51	13	1	67	1.10	3	17	144	147	
^b 116	51	41	6	4	20	0.96	4	14	145	150	
120	60	51	7	2	43	1.22	1	17	141	146	
145	51	45	6	0	39	1.21	2	26	144	152	
184	55	50	1	4	37	1.29	7	23	154	167	
215	54	48	2	4	33	1.07	3	26	166	182	
244	51	45	1	5	19	0.91	2	12	182	203	

^b branch sites: rkm = 92 Shoal Creek, 103.6 Thompson River, 62 Big River, 116 Bourbeuse River