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Scale dependence in the species-discharge relationship for fishes of the southeastern U.S.A.

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SUMMARY

1. Species-discharge relationships (SDR) are aquatic analogues of species-area relationships, and are increasingly used in both basic research and conservation planning. SDR studies are often limited, however, by two shortcomings. First, they do not determine whether reported SDRs, which normally use complete drainage basins as sampling units, are scale dependent. Second, they do not account for the effects of habitat diversity within or among samples.

2. We addressed both problems by using discrete fish zones as sampling units in a SDR analysis. To do so, we first tested for longitudinal zonation in three rivers in the southeastern U.S.A. In each river, we detected successive 'lower', 'middle', and 'upper' fish zones, which were characterized by distinct fish assemblages with predictable habitat requirements. Because our analyses combined fish data from multiple sources, we also used rarefaction and Monte Carlo simulation to ensure that our zonation results were robust to spurious sampling effects.

3. Next, we estimated the average discharge within each zone, and plotted these estimates against the respective species richness within each zone ($\log_{10}$ data). This revealed a significant, linear SDR ($r^2 = 0.83$; $P < 0.01$). Notably, this zonal SDR fit the empirical data better than a comparable SDR that did not discriminate among longitudinal zones. We therefore conclude that the southeastern fish SDR is scale dependent, and that accounting for within-basin habitat diversity is an important step in explaining the high diversity of southeastern fishes.

4. We then discuss how our zonal SDR can be used to improve conservation planning. Specifically, we show how the slope of the SDR can be used to forecast potential extinction rates, and how the zonal data can be used to identify species of greatest concern.

Keywords: Alabama fishes, fish conservation, habitat diversity, longitudinal zonation, species-area relationship

Introduction

Freshwater fishes throughout the world are contending with a host of anthropogenic threats, including climate change (Chu, Mandrak & Minns, 2005; Ficke, Myrick & Hansen, 2007), commercial and recreational harvest (Allan *et al*., 2005; Cooke & Cowx, 2006), nonnative species introductions (Cambray, 2003; Roll *et al*., 2007) and habitat degradation (Dudgeon *et al*., 2006; Ferreira *et al*., 2007). Ultimately, however, the most imminent threat to freshwater fishes may be an impending lack of water (Jackson *et al*., 2001b; Xenopoulos *et al*., 2005). For instance, in the southeastern United States (U.S.A.), urban and agricultural demands for fresh water have dramatically outpaced the available supply, leading

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to a number of court-moderated settlements and regulations that have little to do with the needs of aquatic biota (Stephenson, 2000; Dellapenna, 2005). This situation, which is an increasingly global problem (Jackson *et al.*, 2001b), is unfortunate because the southeastern U.S.A. is a hotspot of aquatic biodiversity (Lydeard & Mayden, 1995), with more than 550 described species of freshwater fishes (Warren *et al.*, 2000).

Recognizing that climate change and human development will continue to diminish aquatic resources in the foreseeable future (Naiman & Turner, 2000; Palmer *et al.*, 2008), Xenopoulos & Lodge (2006) sought to anticipate potential extinction rates of southeastern U.S.A. fishes. They began by regressing the number of fish species within each of 33 southeastern rivers against the mean annual discharge of each river (Xenopoulos & Lodge, 2006). The resulting species-discharge relationship (SDR) was, in essence, a species-area relationship (Rosenzweig, 1995), but was based upon a more explicit measure of aquatic habitat 'size' (i.e. discharge) than a two-dimensional surface area (Angermeier & Schlosser, 1989; Matthews, 1998). Xenopoulos & Lodge (2006) then used the slope and intercept of the SDR to predict how species richness might respond to a series of hypothetical flow reductions.

This forecasting method, which was modeled after the approach of Thomas *et al.* (2004), provides a quantitative tool for assessing extinction risk. But its utility is limited by two unresolved questions. First, Xenopoulos & Lodge (2006) did not determine whether their SDR is scale dependent; because their data were limited to major river systems [see Swift *et al.* (1986) for original data], it is unclear whether their results apply to smaller subunits, such as tributary streams (Palmer & White, 1994). Second, Xenopoulos & Lodge (2006) did not account for the variety of habitat types that occur within southeastern U.S.A. rivers (Ward, Harris & Ward, 2005). This is important because the ability to associate species with specific habitats is a key step in predicting which species are the most likely to become extinct (Botkin *et al.*, 2007).

Buckley's (1982) study of island plant diversity offers a potential solution to these two problems (i.e. scale dependence and habitat diversity). Buckley (1982) measured the areal extent of three major habitat types (white sand, red sand and limestone terrain) on each of several islands, surveyed the number of plant species within each habitat, then used these habitat-specific data as independent samples in a species-area analysis. By doing so, Buckley (1982) was able to track species-habitat associations, and to show that the resulting species-area relationship was scale dependent: a linear relationship did exist between total island area and total island richness (i.e. pooled habitat areas and species lists on each island), but the habitat-specific data (i.e. species richness within each habitat type) provided a better fit (see also Gotelli & Graves, 1996).

McGarvey & Hughes (2008) recently used the habitat-specific approach of Buckley (1982) to assess the SDR for western U.S.A. fishes. Their analysis was premised on the fact that riverine fishes can often be partitioned into discrete assemblages, which are associated with specific types of habitats (Matthews, 1998). This partitioning is frequently described with a longitudinal zonation model, in which taxonomically and functionally distinct assemblages are successively oriented along river gradients (Hawkes, 1975; Rahel & Hubert, 1991). Because this zonation reflects species' affinities for major types of lotic habitats (e.g. 'headwater' versus 'mainstem' habitats; Hawkes, 1975; Schlosser, 1987), McGarvey & Hughes (2008) inferred that longitudinal zones are analogous to Buckley's (1982) habitat types, and could therefore be used as sampling units in a SDR analysis.

In this study, we applied the method of McGarvey & Hughes (2008) to fish assemblages in three rivers in the southeastern U.S.A. First, we used quantitative fish data to test for longitudinal zonation along each river. To ensure that these zonation test results were robust to spurious sampling effects (Gotelli & Colwell, 2001), we employed rarefaction and Monte-Carlo techniques. Next, we summed the total number of fish species within each zone, using a combination of point samples and regional atlases (Mettee, O'Neil & Pierson, 1996; Boschung & Mayden, 2004). Instantaneous discharge was estimated within each longitudinal zone, and plotted against its corresponding species richness value to obtain a SDR. Finally, we compared this zone-specific SDR with the complete basin SDR of Xenopoulos & Lodge (2006), noting differences in the slope, intercept, and fit and discussing ways such information can be used to enhance fish conservation efforts.

Methods

Study area and fish data assembly

We examined fish assemblage structure in three large southeastern U.S.A. rivers: the Cahaba, Black Warrior and Tallapoosa (Fig. 1). Each of these rivers occurs within the Mobile River Basin, which transects the states of Mississippi, Alabama, Georgia and Tennessee. For each river, we selected a discrete, continuous flow path (315–498 km total length) from the river's headwaters to its confluence with either the Tombigbee River (Black Warrior) or the Alabama River (Cahaba and Tallapoosa). Longitudinal profiles were then plotted for each river by querying select river segments from the U.S. Geological Survey's (USGS) National Hydrography Dataset (USGS, 2000) and superimposing them on 30-m resolution USGS National Elevation Dataset (Gesch *et al.*, 2002) grids (Fig. 2), using ArcInfo (Environmental Systems Research Institute, Redlands, CA, U.S.A.). Complete river descriptions are provided in Ward *et al.* (2005).

Because our aim was to detect longitudinal gradients in species' abundances, we required quantitative (i.e. numbers of individuals within samples, identified to species-level), spatially explicit fish assemblage data (Rahel, 1990). A suitable dataset was provided by the Geological Survey of Alabama, which included 394 samples [194 240 total individuals and 150 recognized species, based upon species designations in Boschung & Mayden (2004) and Nelson *et al.* (2004), and collected between 1979 and 1994], distributed along the three river profiles. Sampling methods were standardized according to habitat type: seines were used in shallow, wadeable habitats, while boat electrofishers were used in deep, non-wadeable habitats (see Mettee *et al.*, 1996 for complete sampling details). Backpack electrofishers were also used in combination with seines at some of the wadeable sites, but we did

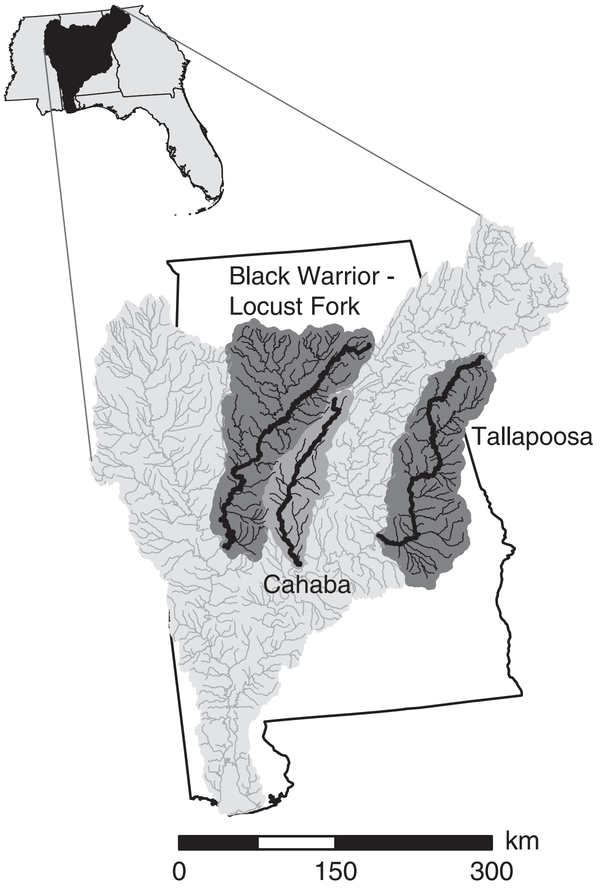

Fig. 1 Map of the Black Warrior (Little Cove Creek → Locust Fork → Black Warrior), Cahaba, and Tallapoosa (Wircher Creek → Tallapoosa) Rivers, shown as heavy black lines, with all rivers flowing south. Complete river networks and basin extents are also shown, within the greater Mobile River Basin (U.S.A.). States shown are (clockwise from upper left) Mississippi, Alabama, Georgia, and Florida.

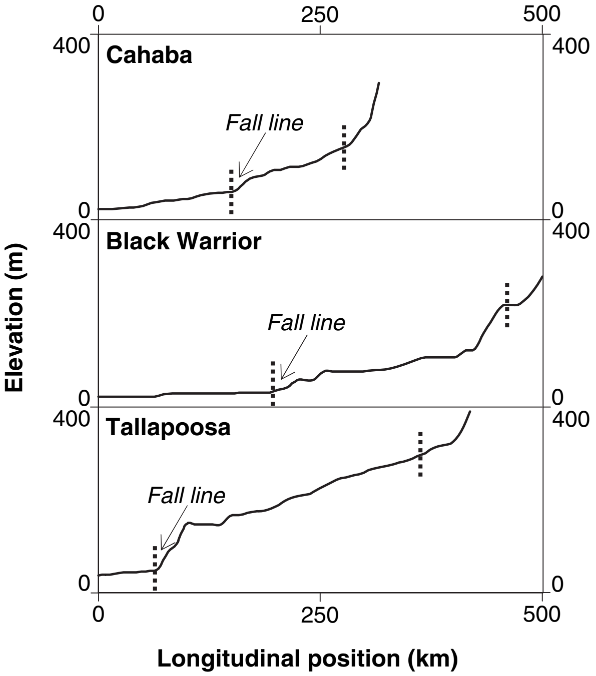

Fig. 2 Longitudinal profiles of the three study rivers. Dashed vertical lines indicate longitudinal zone boundaries, with 'lower', 'middle' and 'upper' zones oriented from left to right. On each river, the boundary between the lower and middle zones coincides with the 'Fall Line.'

not differentiate between the two methods, as they were often performed simultaneously, with the data pooled *in situ* (P. O'Neil, pers. comm.). To minimize seasonal effects, we constrained our analyses to April–September samples.

Longitudinal zonation tests

Prior to conducting longitudinal zonation tests, we standardized the abundance data for each river. This was necessary because sampling methods and sampling duration varied among sites (P. O'Neil, pers. comm.), and sites were not uniformly distributed along the three longitudinal profiles (Hofer, Bersier & Borcard, 1999; Gotelli & Colwell, 2001). To standardize the data, we first divided each profile into a series of contiguous, equal-length segments (Horwitz, 1978; Fagan & Stephens, 2006). Next, we pooled samples (i.e. consolidated all individuals) within each of the equal-length segments. We then used rarefaction to ensure that equal sample sizes were collected from each segment (Sanders, 1968), by randomly selecting a uniform number of individuals from the consolidated pool in each segment (Gotelli & Colwell, 2001). In this way, the rarefied abundance data within each of the equal-length segments became our basic sampling units. Rarefaction was performed with EcoSim 7.0 (Gotelli & Entsminger, 2006).

Having standardized the fish data, we next calculated Bray-Curtis similarity values for all adjacent river segments. Our objective was to detect abrupt changes, or 'faunal breaks' (*sensu* Matthews, 1986), in assemblage structure along each of the longitudinal profiles. Whenever Bray-Curtis similarity fell below an arbitrary threshold (e.g. ≤ 0.40; see 'Sampling effect tests' below), we inferred the location of a faunal break (Matthews, 1986). These breaks were assumed to indicate longitudinal zone boundaries, while river segments between breaks (i.e. Bray-Curtis similarity values equal to or greater than the threshold) were assumed to constitute continuous zones. We also used non-metric multidimensional scaling (NMDS; Bray-Curtis similarity, city-block distance) as a secondary indicator of longitudinal zonation: zonation was reflected by distinct clusters of adjacent segments within NMDS ordination plots (Cornelius & Reynolds, 1991; Walters *et al.*, 2003). NMDS was chosen because many species' relative abundance distributions along the longitudinal profiles were approximately unimodal, and NMDS is not constrained by the assumption of linearity (Fasham, 1977). Similarity calculations and NMDS were performed with SYSTAT 11.0 (SYSTAT Software, Inc., Richmond, CA, U.S.A.).

Mean similarity tests were then used to evaluate the significance of the longitudinal zonation results (Van Sickle & Hughes, 2000). We began by calculating the average Bray-Curtis similarity within each longitudinal zone (i.e. every equal-length segment within a given zone was compared to every other segment within that zone). We then computed the grand mean of all within-zone averages (W_{avg}) for each river. If only one segment was present within a zone, we drew a second, rarefied 'pseudosample' from the remaining pool of individuals within that segment, and used it to calculate a within-zone average similarity. Average between-zone similarities (B_{avg}) were also calculated for each river (i.e. mean of all among-zone similarities), and compared with the W_{avg} values. W_{avg} values that greatly exceeded B_{avg} values were considered evidence of longitudinal zonation (i.e. equal-length segments were highly similar within zones, but dissimilar among zones; Van Sickle & Hughes, 2000). Monte-Carlo simulations (1000 repetitions for each river) were then used to assess the significance of the zonation results. During each simulation, each of the equal-length segments was randomly shuffled among longitudinal zones (zone boundaries were preserved), and the resulting W_{avg} and B_{avg} values were recalculated. P-values were calculated as the proportion of simulations in which

$$(W_{\text{avg}} - B_{\text{avg}})_{\text{simulated}} \geq (W_{\text{avg}} - B_{\text{avg}})_{\text{empirical}}$$

thereby testing the null hypothesis of 'no zonal structure' (Van Sickle & Hughes, 2000). Mean similarity tests were performed with MEANSIM 6.0 (Van Sickle, 1998).

Sampling effect tests

Our data standardization and zonation test methods entailed several arbitrary parameters (i.e. size of equal-length river segments, numbers of individuals within rarefied samples, gear type selection and faunal break thresholds), and were therefore vulnerable to spurious sampling effects (Gotelli & Colwell, 2001). For example, Angermeier & Smogor (1995), Paller (1995) and Hughes *et al.* (2002) showed that relative abundance distributions and richness estimates for fish assemblages are highly influenced by sampling effort, while Onorato, Angus & Marion (1998), Patton *et al.* (2000), and Cao, Hawkins & Storey (2005) documented the effects of differing gear types on sampling results. We therefore wished to ensure that our zonation results were robust. To do so, we performed a nested comparison of zonation test configurations, including four equal-length segment sizes (5-km, 10-km, 25-km and 50-km segments), two rarefied sample sizes (n = 150 individuals and n = the smallest total number of pooled individuals within a single segment), two gear type configurations (combined boat + seine data and seine-only data), and three faunal break thresholds (≤0.5, ≤0.4 and ≤0.3 Bray-Curtis similarity; see Appendix S1). These tests utilized the Cahaba River dataset because it was the most extensive, and it was not influenced by major discontinuities, such as dams (Ward *et al.*, 2005). The results of the sampling effect tests were then used to identify an optimal, standard set of procedures for longitudinal zonation tests on the Black Warrior and Tallapoosa Rivers.

Species-discharge relationship

To determine whether southeastern fishes would exhibit a strong SDR when longitudinal zones, rather than complete basins, were used as sampling units, we summed the total numbers of fish species occurring within each zone. We did not use the rarefied abundance data for this purpose, however, as rarefied data tend to omit many rare species, and can produce biased richness estimates (Cao *et al.*, 2007). Instead, we used the more comprehensive species' distribution maps in Mettee *et al.* (1996) and Boschung & Mayden (2004) to assess species richness within each zone. Longitudinal zone boundaries were superimposed on each species' distribution map, species' occurrences within each zone were noted, and the total number of species within each zone was summed.

We then estimated the mean annual discharge (Q) within each zone by creating elevation-specific discharge models for each of the three rivers. First, we identified every USGS gauging station that occurred along one of the river profiles and had ≥10 years (1996–2005) of continuous data on record. To ensure that the 1996–2005 data provided a reliable measure of the long-term Q at each station, we compared pre-1996 flow data with post-1996 data at stations with ≥50 years of continuous data on record; Mann–Whitney tests revealed no significant differences in Q between the two time periods (Cahaba, $P = 0.95$; Black Warrior, $P = 0.22$; Tallapoosa, $P = 0.52$). Q values (dependent variable) at each station were then regressed against their respective gauge elevations (independent variable). Logarithmic models of the Cahaba (nine stations; $\log_{10} Q = 2.3–1.0e^{-3}\ elevation$), Black Warrior (seven stations; $\log_{10} Q = 2.7–8.6e^{-3}\ elevation$), and Tallapoosa Rivers (five stations; $\log_{10} Q = 2.6–5.1e^{-3}\ elevation$) accounted for 98.1%, 87.8% and 88.0% of the variability in mean annual flow, respectively, and all models were highly significant ($P \leq 0.01$).

Next, we entered the midpoint elevation of each longitudinal zone (the mean of the maximum and minimum elevations, which were interpolated from Fig. 2) into the corresponding regression model equation to estimate Q within that zone. Mean channel slope within each zone was also interpolated from the longitudinal profiles (Fig. 2), and mean wetted channel width within each zone was estimated from aerial photographs (average of 10 transect widths), using the 'Ruler' tool in Google Earth (Google Inc., Mountain View, CA, U.S.A.). Lastly, we $\log_{10}$ transformed the species richness and Q values within each zone, and used linear regression to assess the resulting SDR (Rosenzweig, 1995; Drakare, Lennon & Hillebrand, 2006).

Results

Our longitudinal zonation analyses revealed a distinct zonation pattern on the Cahaba River, with contiguous 'lower', 'middle' and 'upper' zones (Figs. 2 & 3). Importantly, this zonation was robust to sampling effects. For example, when 25-km segments were used with faunal break thresholds of ≤0.5 and ≤0.4, the same three-zone pattern was observed for all sample size and gear type configurations (Appendix S2). Congruent zonation patterns (i.e. lower, middle and upper zone boundaries occurring at approximately equal locations along the river profile) were also observed for all sample size and gear type configurations when 10-km segments were used with a faunal break threshold of ≤0.4 (Fig. 3, Appendix S2). And 5-km segments produced congruent zonation at a faunal break threshold of ≤0.3, for combined gear

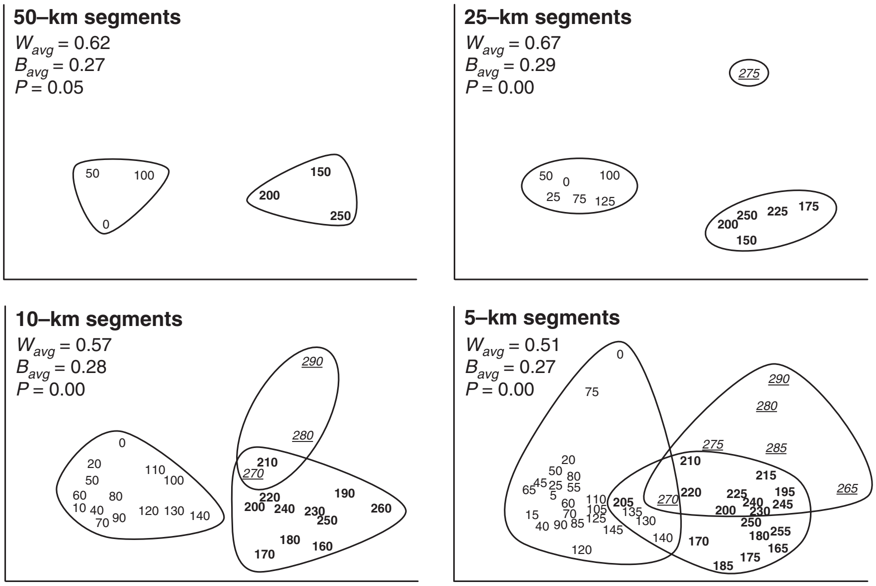

Fig. 3 NMDS ordinations and mean similarity test results for 50-km, 25-km, 10-km and 5-km segments on the Cahaba River. Segment lengths are shown in the upper left corner of each plot, with mean within-zone similarities (W_{avg}), mean between-zone similarities (B_{avg}), and mean similarity test P-values. Points within each plot are labeled according to their longitudinal positions (e.g. point '175' in the 25-km segment plot represents the 175–199 km segment). Longitudinal zonation results (i.e. clustering of segments within each zone) are indicated by ellipses and distinct label formats: plain, bold, and italicized/underscored labels represent lower, middle, and upper zone segments, respectively. Each ordination plot is based on 150 rarefied individuals per segment, drawn exclusively from seine samples, and a faunal break threshold of ≤0.4 (except the 5-km segment plot, which reflects a faunal break threshold of ≤0.3).

types and seine-only data (Fig. 3, Appendix S2). Only the 50-km segments failed to detect a congruent zonation pattern; faunal break thresholds of ≤0.5 and ≤0.4 reflected lower and middle zones, which were separated by the 'Fall Line' (see Discussion), but failed to detect a third, upper zone (Fig. 3, Appendix S2).

Mean similarity tests confirmed the statistical significance of the longitudinal zonation results. W_{avg} values (i.e. average within-zone similarities) were approximately twice as high as their corresponding B_{avg} values (i.e. average among-zone similarities) for all segment lengths, and all P-values were ≤0.05 (Fig. 3). Given this statistical significance, and the congruence between 25-km, 10-km and 5-km segment results (Fig. 3, Appendix S2), we selected 25-km segments for use in our zonation tests. We also determined that rarefied samples of 150 individuals, drawn exclusively from seine samples, were sufficient to detect longitudinal zonation when a faunal break threshold of ≤0.4 was used (Appendix S2). This was a non-intuitive result because many of the large, mainstem fishes that occur only below the Fall Line (e.g. southeastern blue sucker, *Cycleptus meridionalis* Burr & Mayden, and paddlefish, *Polyodon spathula* [Walbaum]) are most effectively sampled with boat electrofishers (Mettee *et al.*, 1996). Inspection of the complete taxa lists within each zone (Appendix S3) revealed, however, that many of the species that are endemic to the lower zones are small-bodied fishes that occur primarily in shallow, wadeable habitats (Mettee *et al.*, 1996; Boschung & Mayden, 2004). Hence, much of the unique diversity within the lower zones can be described with basic seining methods.

When the zonation testing procedures described above (i.e. 25-km segments, 150 individuals randomly drawn from seine samples only, and a faunal break threshold of ≤0.4) were applied to the Black Warrior and Tallapoosa River datasets, we obtained similar results. Lower, middle, and upper zones were detected along each river (Fig. 2), and mean similarity test results showed this zonation to be highly significant (Fig. 4). To minimize the effects of several large impoundments, we removed all inundated segments from the Black Warrior (the 0-km to 75-km and 200-km to 300-km segments) and Tallapoosa (the 75-km to 100-km and 225-km to 250-km segments) datasets. We also lacked abundance data for several

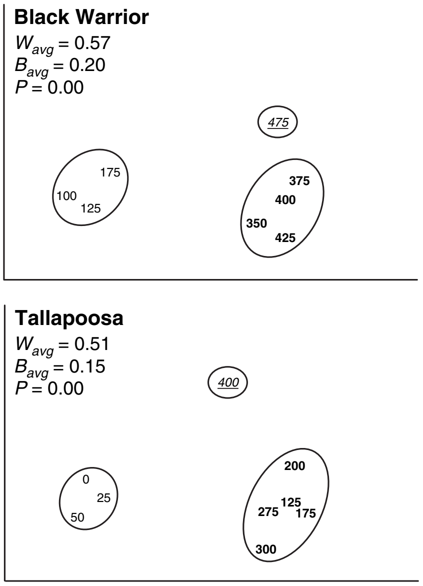

Fig. 4 NMDS ordinations and mean similarity test results for the Black Warrior and Tallapoosa Rivers, using rarefied abundance data (150 individuals per segment, drawn from seine samples only) within 25-km segments and a faunal break threshold of ≤0.4. Mean similarity test results and equal-length segment labels are as shown in Fig. 3.

non-impounded segments in the upper reaches of the Tallapoosa River. These data gaps limited our ability to identify the precise locations of some zone boundaries, but did not prevent us from detecting longitudinal zonation; whenever a faunal break coincided with a data gap, we inferred a zone boundary at the longitudinal midpoint of the gap.

A similar progression of physical habitats was observed along each of the longitudinal profiles (Table 1). Marginal increases in mean channel slope occurred between the lower and middle zones, while more rapid increases occurred between the middle and upper zones. Mean annual discharge also increased at an accelerating rate, but in the downstream direction. And mean wetted channel width increased in the downstream direction, at a nearly constant rate. Generally speaking, the lower zones of each river were large, deep, slow flowing habitats with predominately sandy substrates, abundant woody debris and extensive riparian and floodplain connections (Ward *et al.*, 2005). The upper zones were small, shallow, high gradient habitats that were largely confined to bedrock channels with coarse substrates. The middle zones were transitional habitats, featuring broad, shallow channels with limited floodplain connections (Ward *et al.*, 2005).

In all rivers, species richness increased in the downstream direction (Table 1). Each of these linear richness gradients reflected a nested series of species additions (Rahel & Hubert, 1991; Matthews, 1998): most of the fishes occurring in the upper and middle zones were also found in the lower zones (e.g. blacktail shiner, *Cyprinella venusta* Girard, and bluegill, *Lepomis macrochirus* Rafinesque). Many species were, however, endemic to either the deep, 'mainstem' habitats (e.g. bowfin, *Amia calva* Linnaeus, and highfin carpsucker, *Carpiodes velifer* Rafinesque) or the sandy-bottom and backwater habitats (e.g. southern sand darter, *Ammocrypta meridiana* Williams, and silverside shiner, *Notropis candidus* Suttkus) of the lower zones (Appendix S3). Finally, when the species richness of each zone was plotted against its corresponding *Q* estimate (Table 1), we observed a distinct SDR with a linear model equation of $\log_{10}$ *richness* = 1.48 + 0.26 · $\log_{10} Q$ (Fig. 5).

Discussion

Longitudinal zonation

While many authors have documented local fish-habitat associations in southeastern U.S.A. rivers (e.g. Wood & Bain, 1995; Bowen, Freeman & Bovee, 1998; Tabit & Johnson, 2002), this study is, to the best of our

Table 1 Physical habitat characteristics and species richness within each of the lower (L), middle (M) and upper (U) zones of the Cahaba, Black Warrior and Tallapoosa rivers

	Cahaba			Black Warrior			Tallapoosa		
Variable	L	M	U	L	M	U	L	M	U
Mean channel slope (% grade)	0.04	0.06	0.41	0.01	0.07	0.15	0.05	0.08	0.24
Mean annual discharge ($m^3 s^{-1}$)	64.6	14.2	1.0	173.8	25.1	3.1	209.9	29.2	7.6
Mean wetted channel width (m)	56	27	<10	135	40	<10	105	46	<10
Species richness	123	64	37	108	73	28	99	67	46

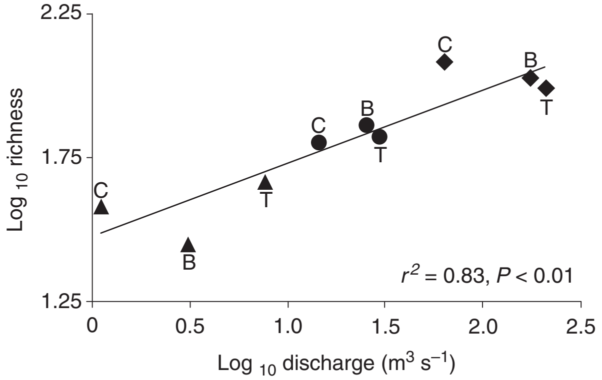

Fig. 5 The combined species-discharge relationship for Cahaba (C), Black Warrior (B), and Tallapoosa (T) River fishes, including linear regression results. Lower, middle, and upper zone samples are shown as diamonds, circles, and triangles, respectively. The linear model equation is log_{10} *richness* = 1.48 + 0.26 · (log_{10} *discharge*).

knowledge, the first to quantify zonal structure along complete (>300 km) river profiles within the region. Using a spatially extensive, abundance dataset, we detected lower, middle and upper zones in three large, southeastern rivers. Along each river, the transition between the lower and middle zones coincided with the Fall Line (Fig. 2). The Fall Line is the physiographic boundary between the Coastal Plain and the Appalachian Highlands, and its biogeographic influence is apparent throughout the region (Mettee *et al.*, 1996; Boschung & Mayden, 2004; Ward *et al.*, 2005). For instance, 41 species (27% of the complete dataset) occur exclusively below the Fall Line (i.e. within the lower zone). The boundaries between the middle and upper zones were less readily apparent, as few species were entirely restricted to either zone. Our quantitative data did, however, detect peaks in the abundances of many species within the upper zones (e.g. scarlet shiner, *Lythrurus fasciolaris* [Gilbert], and burrhead shiner, *Notropis asperifrons* Suttkus & Raney). Thus, by using abundance data we were able to detect gradients in species' distributions that presence–absence data would likely have missed (Rahel, 1990). Also, the physical habitats that typify the upper zones (e.g. steep gradients with coarse substrates) favour the ecologies of many small-bodied, benthic species (Wood & Bain, 1995; Mettee *et al.*, 1996; Walters *et al.*, 2003; Boschung & Mayden, 2004). We are therefore confident that the upper and middle zones are distinct fish assemblages (Hawkes, 1975; Schlosser, 1987).

Numerous studies have reported longitudinal zonation, comparable to the pattern presented here. For example, Humphries *et al.* (2008) characterized fish assemblage structure within three river zones in Australia (Murray-Darling Basin), while Edds (1993) described four longitudinal fish zones within the Gandaki River (Nepal). Balon & Stewart (1983) and Ibanez *et al.* (2007) each detected four fish zones in large African rivers (Congo Basin and Gabon regions, respectively). Ibarra & Stewart (1989) detected four fish zones within each of two rivers in the Amazon Basin, while Petry & Schulz (2006) reported two fish zones in the Sinos River (southern Brazil). Esselman, Freeman & Pringle (2006) detected three fish zones in the Monkey River (Belize). Fish zonation is also common throughout Europe, where distinct zones have been described in England and Wales (Noble, Cowx & Starkie, 2007), France (Ibarra *et al.*, 2005), Poland (Przybylski, 1993), Lithuania (Virbickas & Kesminas, 2007) and the Iberian Peninsula (Ferreira *et al.*, 2007). Moreover, many authors have reported similar zonation patterns for aquatic macroinvertebrates, in streams and rivers throughout the world (e.g. Statzner & Higler, 1986; Kilgour & Barton, 1999; Santoul *et al.*, 2004; Reese & Batzer, 2007). We therefore suggest that our methods have broad relevance for studies of riverine diversity.

To ensure that our zonation results were robust, we performed a rigorous series of sampling effect tests (Gotelli & Colwell, 2001). These tests increased our confidence in the zonation results, as did the overall congruence between our results and the work of others (see previous paragraph). Nevertheless, our methods did preclude the detection of more local-scale patterns (Shea & Peterson, 2007). For instance, in their reach-scale (20–40 channel widths) study of Etowah River fishes (a tributary of the Coosa River, which lies between the Cahaba and Tallapoosa Rivers), Walters *et al.* (2003) rejected a longitudinal model in favour of a 'Process Domains Concept' model, in which assemblage structure was determined primarily by locally realized geomorphic constraints, rather than longitudinal habitat gradients. Following Jackson, Peres-Neto & Olden (2001a), who challenged fish ecologists to integrate results across scales, we readily acknowledge the limitations of our coarse-scale, regional analysis. But we also point out that the sampling sites of Walters *et al.* (2003) would fit entirely within our upper zone category. Thus, Walters *et al.* (2003) provide insight to the dynamics within a particular zone, and are therefore complimentary, rather than contradictory, to our zonation results (see also Wood & Bain, 1995).

Given the generality of the zonal pattern, we propose that longitudinal zones may be ideal conservation and management units. For example, the primary mechanisms for protecting imperiled fishes in the southeastern U.S.A. are currently species-specific recovery plans (Bibb, 2003), which are implemented under the U.S. Endangered Species Act (National Research Council, 1995). But with 187 species considered endangered, threatened, or vulnerable throughout the region, it is difficult to provide comprehensive protection (Warren *et al.*, 2000). A more efficient approach was outlined by Angermeier & Winston (1999), who proposed expanding conservation priorities from individual species to complete 'community types', noting that assemblage and community-level function are essential components of biodiversity. Because longitudinal zones are defined by complete fish assemblages, which have predictable trophic, life-history, and habitat requirements (Hawkes, 1975; Schlosser, 1987; Aarts & Nienhuis, 2003), they have much in common with the community types of Angermeier & Winston (1999). Longitudinal zones are already a central component of the European Union (EU) Water Framework Directive, which provides a standard protocol for assessing the ecological integrity of all freshwaters within the EU (Noble & Cowx, 2002; Schmutz *et al.*, 2007). We therefore encourage fisheries managers and conservation planners in other regions to consider the benefits of a zonal perspective.

Species-discharge relationship

Using longitudinal zones as sampling units, we detected a distinct, highly significant SDR for southeastern U.S.A. fishes. Xenopoulos & Lodge (2006) also reported a linear SDR ($\log_{10}$ data) for southeastern fishes, but used complete river basins as sampling units. This difference is important because it demonstrates that the SDR is scale dependant. Our zonal SDR had a steeper slope (0.26 vs. 0.16), a lower intercept (1.48 vs. 1.59), and a higher coefficient of determination (0.83 vs. 0.61) than the SDR of Xenopoulos & Lodge (2006). Moreover, we verified that these differences were not artifacts of the unequal samples sizes (9 vs. 33) in the two analyses: when Monte Carlo simulations (1000 iterations) were used to equalize the sample sizes (i.e. nine samples repeatedly selected from the total set of 33, then used in independent regression analyses), the slope, intercept, and r^2 of our SDR were still significantly different than the averages of Xenopoulos & Lodge's (2006) complete-basin model (mean slope = 0.16, SD = 0.04; mean intercept = 1.59, SD = 0.08; mean r^2 = 0.60, SD = 0.16).

This scale dependence has at least two implications for the conservation of regional fish diversity. First, the steeper slope of our SDR suggests that species' extinctions might occur more rapidly than Xenopoulos & Lodge (2006) predict (see also Drakare *et al.*, 2006). For instance, the SDR of Xenopoulos & Lodge (2006) forecasts *c.* 6 extinctions if mean annual discharge is reduced from 300 to 200 $m^3 s^{-1}$. But our zonal SDR attributes *c.* 13 extinctions to the same flow reduction. Similarly, the SDR of Xenopoulos & Lodge (2006) predicts that mean annual discharges of 200, 100 and 50 $m^3 s^{-1}$ will support 93, 83 and 74 species, respectively, while our zonal SDR predicts that the same discharges will support 119, 99, and 83 species. Caution is necessary when applying this forecasting tool, as it involves statistical extrapolation (Prairie, 1996) and untested assumptions regarding future habitat distributions and species-habitat associations (Botkin *et al.*, 2007; see also below). For the moment, however, it is one of the more promising tools available for anticipating species extinctions at large spatial scales (Rosenzweig, 1999; Thomas *et al.*, 2004).

The scale dependence of the SDR also highlights the need to account for specific habitat types in conservation planning (Botkin *et al.*, 2007). For example, Xenopoulos & Lodge (2006) suggest that the SDR reflects special vulnerability on the part of large-bodied, mainstem species, reasoning that flow reductions will discriminately reduce or eliminate the primary habitats of these large fishes. They do not, however, consider the effects of flow reductions on other habitats and their resident species. This is a critical area for future research because many of the rarest fishes in southeastern U.S.A. rivers are small-bodied, headwater species that occur in only one or several locations, despite having physical access to adjacent systems (e.g. Warrior bridled darter, *Percina* sp. cf. *P. macrocephala* 'A' [Boschung & Mayden, 2004], within the Sipsey Fork of the Black Warrior River, Winston County, Alabama). It is therefore unfounded to assume that these endemic species could, in the event of significant habitat losses (i.e. flow reductions), survive by migrating to alternative habitats (Angermeier, 1995; Turner & Trexler, 1998; Knouft & Page, 2003; Lake, 2003). Our longitudinal zonation results, which delineate geographically and ecologically distinct assemblages within each of three river basins, provide an objective means of establishing habitat-specific conservation priorities, and of linking the SDR with particular habitat types.

Another benefit of the zone-specific approach is that it suggests a causal mechanism. Figure 5 shows that the SDR is driven by differential richness among the three longitudinal zones: the three samples within each type of zone cluster together, so that the regression line is primarily a result of among-zone differences, rather than within-zone variation. We therefore submit that among-zone habitat shifts are an important determinant of the observed SDR. This mechanistic interpretation is analogous to Buckley's (1982) study of plant diversity in that it infers a causal relationship between the heterogeneity of key habitat types (i.e. longitudinal zones) and the richness of the associated communities (see also Báldi, 2008). It is an incomplete explanation, however, as we lack sufficient data to perform a comprehensive test of habitat structure. Our data do demonstrate among-zone differences in several key habitat parameters (Table 1), but they omit many other habitat variables that have been shown to affect fish assemblage structure (e.g. Wood & Bain, 1995; Bowen *et al.*, 1998). Nevertheless, we conclude that the longitudinal zone perspective taken here is an essential step in accounting for the high diversity of southeastern U.S.A. fishes, and that it may prove useful in other regions.

Assessing the generality of our SDR is currently difficult because most studies have been performed at the basin level, and do not consider intra-basin habitat units (e.g. Oberdorff, Guégan & Hugueny, 1995; Xenopoulos & Lodge, 2006). Two exceptions, however, are the analyses of McGarvey & Hughes (2008) and Angermeier & Schlosser (1989). McGarvey & Hughes (2008) analysed zonal fish assemblages in three Pacific Northwest (U.S.A.) rivers and detected a strong SDR (slope = 0.19; r^2 = 0.94; $\log_{10}$ data). Notably, when their discharge estimates are recalculated with the method used here (McGarvey & Hughes (2008) point out that their discharge estimates, which incorporate the length of each zone, are actually volumetric measurements), the slope of the Pacific Northwest SDR becomes 0.27 – nearly identical to the 0.26 slope of the southeast SDR. Angermeier & Schlosser (1989) studied fish richness within discrete pools and riffles in midwestern U.S.A. streams, finding significant relationships between the size and richness of each habitat type. Because their analyses were based upon water volume, rather than discharge, the results of Angermeier & Schlosser (1989) are not directly comparable to our own. It is, however, instructive to note that the slope of the among-habitat (i.e. all pool and riffle data from midwestern streams combined) species-volume relationship is 0.38 (Angermeier & Schlosser, 1989; all data $\log_{10}$ transformed). This steeper slope shows that species richness can accumulate among discrete microhabitats at a comparatively rapid rate.

Finally, we note that the slope of our SDR is similar to the average slope (0.24; 95% CI = 0.02) reported by Drakare *et al.* (2006). Drakare *et al.* (2006) obtained this average through a meta-analysis of 794 species-area studies, which included a variety of aquatic and terrestrial assemblages, and a range of habitat sizes that encompassed *c.* 20 orders of magnitude. Given its comprehensiveness, we believe the meta-analysis of Drakare *et al.* (2006) is an appropriate baseline for assessing individual studies. We therefore find the similarity between their average slope and our SDR slope compelling, and suggest that future studies, when conducted with a zonal perspective, may reveal common, fundamental constraints on global fish diversity.

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Supporting Information

Additional Supporting Information may be found in the online version of this article.

Appendix S1. Outline of the sampling effect tests.

Appendix S2. Sampling effect test results for the Cahaba River.

Appendix S3. Species distributions within the lower (Low), middle (Mid), and upper (Up) zones of the Cahaba, Black Warrior, and Tallapoosa Rivers.

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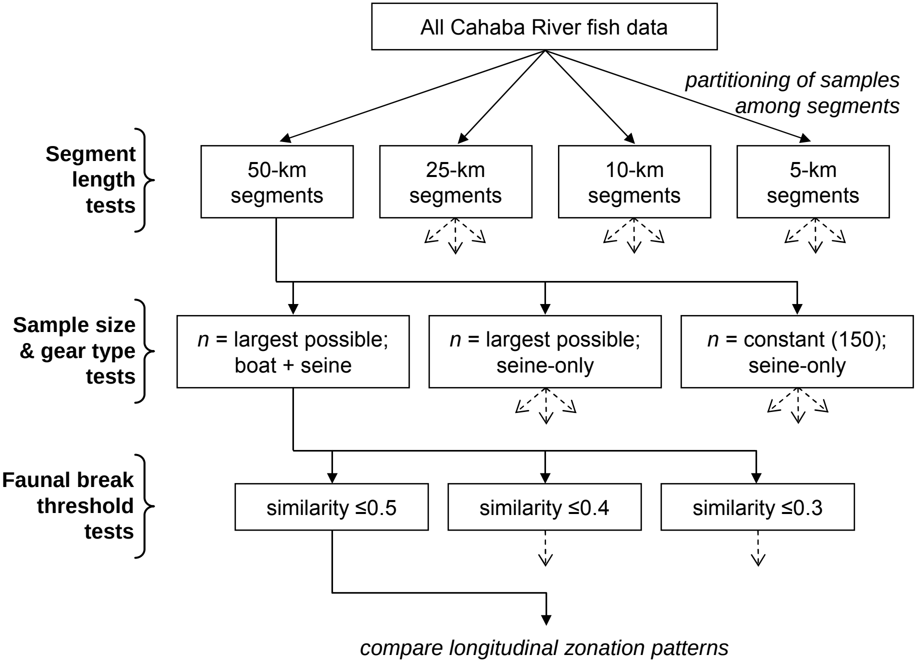
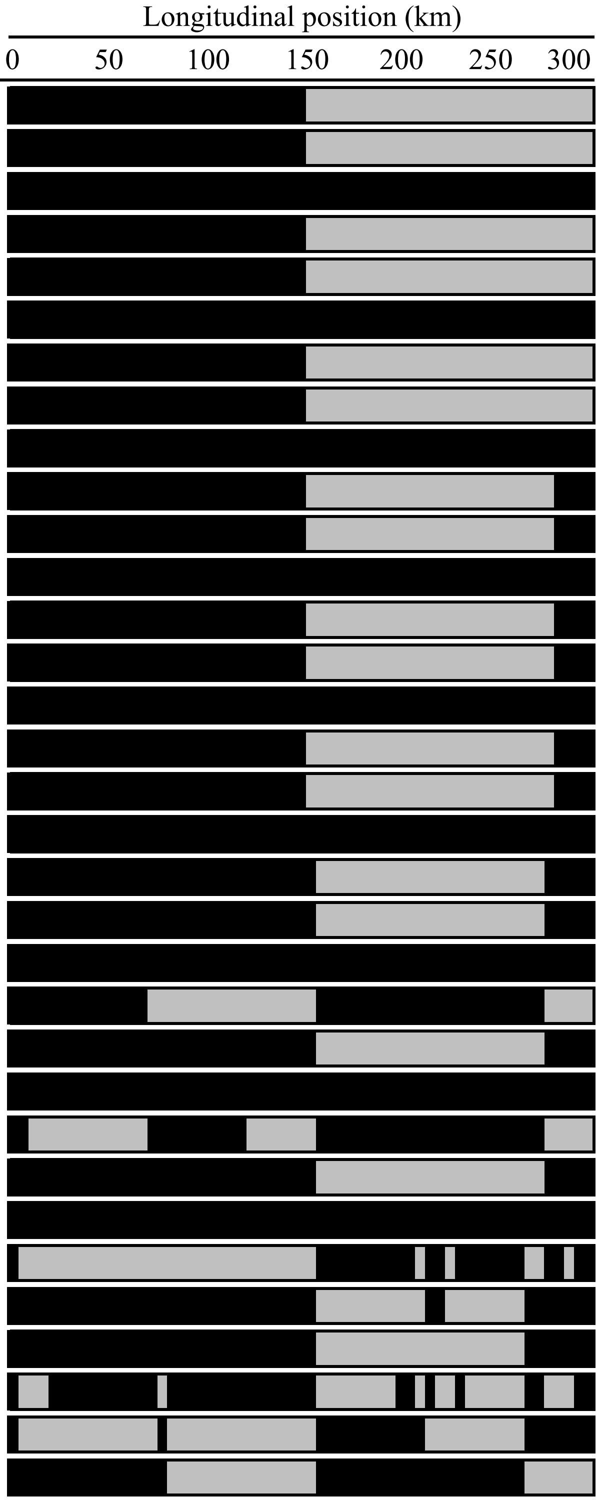


Appendix 1 Outline of the sampling effect tests. Segment length effects on the longitudinal zonation results were assessed by partitioning the Cahaba fish samples among four different segment configurations: 50-km, 25-km, 10-km, and 5-km. Tests were performed independently, so that the complete Cahaba dataset was applied to each configuration. (Dashed arrows beneath boxes indicate that all subsequent procedures were performed for that configuration.) Rarefied sample size and gear type effects were assessed by examining three configurations: (i) equal numbers of individuals drawn from boat electrofishing (in segments where the river was large enough to accommodate it) and seine samples (i.e. ½ *n* boat samples + ½ *n* seine samples), with the rarefied *n* determined by the segment with the fewest total individuals; (ii) individuals drawn only from seine samples, with the rarefied *n* determined by the segment with the fewest total individuals; and (iii) 150 rarefied individuals drawn only from seine samples. This tiered procedure was used to determine whether boat electrofishing samples were necessary to detect longitudinal zonation, and to evaluate whether zonation patterns were dependent upon rarefied sample sizes. Finally, Bray-Curtis similarity values were calculated for each sampling effect configuration (4 equal-length segment configurations × 3 sample size and gear type configurations = 12 sampling effect configurations), and the resulting longitudinal zonation patterns were examined, using three different faunal break thresholds: ≤0.5, ≤0.4, and ≤0.3.

Appendix 2 Sampling effect test results for the Cahaba River. Segment length, sample size (number of rarefied individuals), gear type (combined boat electrofishing plus seining vs. seining only), and the faunal break threshold (Bray-Curtis similarity) are shown for each test. Longitudinal positions of each faunal break, or zone boundary, are indicated by alternating black and grey rectangles. Tests that detected three zones, with similar boundary locations, are shown in bold text.

Segment length (km)	Sample size & gear type	Faunal break threshold
50	1500; boat+seine	≤0.5
50	1500; boat+seine	≤0.4
50	1500; boat+seine	≤0.3
50	1500; seine only	≤0.5
50	1500; seine only	≤0.4
50	1500; seine only	≤0.3
50	150; seine only	≤0.5
50	150; seine only	≤0.4
50	150; seine only	≤0.3
25	**600; boat+seine**	**≤0.5**
25	**600; boat+seine**	**≤0.4**
25	600; boat+seine	≤0.3
25	**600; seine only**	**≤0.5**
25	**600; seine only**	**≤0.4**
25	600; seine only	≤0.3
25	**150; seine only**	**≤0.5**
25	**150; seine only**	**≤0.4**
25	150; seine only	≤0.3
10	**300; boat+seine**	**≤0.5**
10	**300; boat+seine**	**≤0.4**
10	300; boat+seine	≤0.3
10	300; seine only	≤0.5
10	**300; seine only**	**≤0.4**
10	300; seine only	≤0.3
10	150; seine only	≤0.5
10	**150; seine only**	**≤0.4**
10	150; seine only	≤0.3
5	150; boat+seine	≤0.5
5	150; boat+seine	≤0.4
5	**150; boat+seine**	**≤0.3**
5	150; seine only	≤0.5
5	150; seine only	≤0.4
5	150; seine only	≤0.3



Appendix 3 Species distributions within the lower (Low), middle (Mid), and upper (Up) zones of the Cahaba, Black Warrior, and Tallapoosa Rivers. Species presences within zones are indicated by an 'X'. Species names with an asterisk (*) indicate recognized subspecies (Boschung & Mayden, 2004).

	Cahaba			Black Warrior			Tallapoosa		
Family/species	Low	Mid	Up	Low	Mid	Up	Low	Mid	Up
Acipenseridae									
Scaphirhynchus suttkusi Williams & Clemme	X								
Amiidae									
Amia calva Linnaeus	X			X			X	X	
Anguillidae									
Anguilla rostrata (Lesueur)	X			X	X		X		
Aphredoderidae									
Aphredoderus sayanus (Gilliams)	X			X			X		
Atherinopsidae									
Labidesthes sicculus (Cope)	X			X	X		X		
Belonidae									
Strongylura marina (Walbaum)	X			X	X		X		
Catostomidae									
Carpiodes cyprinus (Lesueur)	X			X	X		X		
C. velifer (Rafinesque)	X			X			X		
Cycleptus meridionalis Burr & Mayden	X						X		
Erimyzon oblongus (Mitchell)	X			X	X		X		
E. sucetta (Lacepède)	X			X					
Hypentelium etowanum (Jordan)	X	X	X	X	X	X	X	X	X
Ictiobus bubalus (Rafinesque)	X			X			X		
Minytrema melanops (Rafinesque)	X	X		X	X		X	X	X
Moxostoma carinatum (Cope)	X			X	X		X	X	
M. duquesnei (Lesueur)	X	X	X	X	X	X	X	X	X
M. erythrurum (Rafinesque)	X	X	X	X	X	X	X	X	X
M. poecilurum Jordan	X	X	X	X	X	X	X	X	X
Centrarchidae									
Ambloplites ariommus Viosca	X	X		X	X	X	X	X	X
Centrarchus macropterus (Lacepède)	X			X			X		
Lepomis auritus (Linnaeus)							X	X	X
L. cyanellus Rafinesque	X	X	X	X	X	X	X	X	X
L. gulosus (Cuvier)	X	X		X	X		X	X	X
L. humilis (Girard)					X		X		
L. macrochirus Rafinesque	X	X	X	X	X	X	X	X	X
L. marginatus (Holbrook)	X			X					
L. megalotis (Rafinesque)	X	X	X	X	X	X	X	X	
L. microlophus (Günther)	X	X	X	X	X		X	X	X
L. miniatus Jordan	X	X		X	X		X	X	
Micropterus coosae Hubbs & Bailey		X	X		X			X	

Appendix 3 (*continued*)

	Cahaba			Black Warrior			Tallapoosa		
Family/species	Low	Mid	Up	Low	Mid	Up	Low	Mid	Up
M. punctulatus (Rafinesque)	X	X	X	X	X	X	X	X	X
M. salmoides (Lacepède)	X	X	X	X	X	X	X	X	X
Pomoxis annularis Rafinesque	X			X	X		X	X	
P. nigromaculatus (Lesueur)	X	X		X			X	X	X
Clupeidae									
Alosa alabamae Jordan & Evermann	X	X		X					
A. chrysochloris (Rafinesque)	X	X		X	X		X		
Dorosoma cepedianum (Lesueur)	X	X		X	X		X	X	
D. petenense (Günther)	X	X		X	X		X	X	
Cottidae									
Cottus carolinae (Gill)	X	X	X				X	X	X
Cyprinidae									
Campostoma oligolepis Hubbs & Greene	X	X	X	X	X	X	X	X	X
Ctenopharyngodon idella (Valenciennes)	X			X			X	X	
Cyprinella caerulea (Jordan)	X	X	X						
C. callistia (Jordan)	X	X	X	X	X	X		X	X
C. gibbsi (Howell & Williams)								X	X
C. trichroistia (Jordan & Gilbert)		X	X		X	X			
C. venusta Girard	X	X	X	X	X	X	X	X	X
C. whipplei Girard				X	X				
Cyprinus carpio Linnaeus	X	X		X	X		X	X	X
Hybognathus hayi Jordan	X			X			X		
H. nuchalis Agassiz	X			X					
Hybopsis lineapunctata Clemmer & Suttkus							X	X	X
H. winchelli Girard	X	X		X	X		X		
Luxilus chrysocephalus Rafinesque	X	X	X	X	X	X	X	X	X
L. zonistius Jordan								X	
Lythrurus bellus (Hay)	X	X		X	X		X	X	X
L. fasciolaris (Gilbert)						X			
L. lirus (Jordan)		X							
Macrhybopsis sp. cf. *M. aestivalis* "A"*	X			X			X		
Macrhybopsis sp. cf. *M. aestivalis* "B"*								X	X
M. storeriana (Kirtland)	X			X	X		X		
Nocomis leptocephalus (Girard)	X		X	X			X	X	X
Notemigonus crysoleucas (Mitchill)	X	X		X	X	X	X	X	X
Notropis ammophilus Suttkus & Boschung	X			X			X		
N. asperifrons Suttkus & Raney		X	X	X	X	X			
N. atherinoides Rafinesque	X	X		X	X		X		
N. baileyi Suttkus & Raney	X			X			X	X	
N. buccatus (Cope)	X						X	X	
N. cahabae Mayden & Kuhajda	X	X			X				

Appendix 3 (*continued*)

	Cahaba			Black Warrior			Tallapoosa		
Family/species	Low	Mid	Up	Low	Mid	Up	Low	Mid	Up
N. candidus Suttkus	X			X			X		
N. chrosomus (Jordan)	X	X	X						
N. edwardraneyi Suttkus & Clemmer	X			X			X		
N. maculatus (Hay)				X					
N. stilbius Jordan	X	X	X	X	X	X	X	X	X
N. texanus (Girard)	X			X	X		X	X	
N. uranoscopus Suttkus	X						X		
N. volucellus (Cope)	X	X	X	X	X		X		
N. xaenocephalus (Jordan)								X	X
Opsopoeodus emiliae Hay	X			X			X	X	
Phenacobius catostomus Jordan	X	X	X		X			X	X
Pimephales notatus (Rafinesque)	X			X		X	X		
P. promelas Rafinesque	X				X		X	X	X
P. vigilax (Baird & Girard)	X	X	X	X	X	X	X	X	X
Pteronotropis welaka (Evermann & Kendall)	X								
Semotilus atromaculatus (Mitchill)	X	X	X	X	X	X	X	X	X
S. thoreauianus Jordan				X				X	X
Elassomatidae									
Elassoma zonatum Jordan	X			X			X		
Engraulidae									
Anchoa mitchilli (Valenciennes)				X					
Esocidae									
Esox americanus Gmelin	X	X		X	X		X		
E. niger Lesueur	X	X	X	X	X		X		
Fundulidae									
Fundulus bifax Cashner & Rogers								X	
F. dispar (Agassiz)	X			X					
F. nottii (Agassiz)				X					
F. olivaceus (Storer)	X	X	X	X	X		X	X	
F. stellifer (Jordan)	X	X							
Hiodontidae									
Hiodon tergisus Lesueur	X			X			X		
Ictaluridae									
Ameiurus melas (Rafinesque)	X	X		X	X			X	X
A. natalis (Lesueur)	X	X		X	X		X	X	X
A. nebulosus (Lesueur)	X						X	X	
Ictalurus furcatus (Lesueur)	X			X	X		X		
I. punctatus (Rafinesque)	X	X	X	X	X		X	X	X
Noturus funebris Gilbert & Swain	X			X			X	X	X
N. gyrinus (Mitchill)	X			X					
N. leptacanthus Jordan	X	X		X	X		X	X	X

Appendix 3 (*continued*)

	Cahaba			Black Warrior			Tallapoosa		
Family/species	Low	Mid	Up	Low	Mid	Up	Low	Mid	Up
N. munitus Suttkus & Taylor	X								
N. nocturnus Jordan & Gilbert	X			X					
Pylodictis olivaris (Rafinesque)	X	X		X	X		X	X	
Lepisosteidae									
Lepisosteus oculatus Winchell	X	X		X	X		X		
L. osseus (Linnaeus)	X	X		X	X		X		
Moronidae									
Morone chrysops (Rafinesque)	X			X	X		X	X	
*M. chrysops x M. saxatili**	X			X			X	X	
M. saxatilis (Walbaum)	X			X			X	X	
Mugilidae									
Mugil cephalus Linnaeus	X			X					
Percidae									
Ammocrypta beanii Jordan	X			X			X		
A. meridiana Williams	X			X					
Crystallaria asprella (Jordan)	X						X		
Etheostoma bellator Suttkus & Bailey						X			
E. chlorosoma (Hay)	X			X					
E. chuckwachatte Mayden & Wood								X	X
E. douglasi Wood & Mayden					X				
E. histrio Jordan & Gilbert	X						X		
E. jordani Gilbert	X	X					X		
E. lachneri Suttkus & Bailey				X					
E. nigrum Rafinesque	X			X			X		
E. parvipinne Gilbert & Swain	X			X					
E. proeliare (Hay)				X					
E. ramseyi Suttkus & Bailey	X	X	X						
E. rupestre Gilbert & Swain	X	X	X	X	X		X		
E. stigmaeum (Jordan)	X	X	X	X	X	X	X	X	X
E. swaini (Jordan)	X	X		X			X		
E. tallapoosae Suttkus & Etnier								X	X
E. whipplei (Girard)	X	X	X	X	X	X			
E. zonifer (Hubbs & Cannon)	X						X		
Percina aurolineata Suttkus & Ramsey	X	X							
P. brevicauda Suttkus & Bart	X	X	X	X	X				
P. kathae Thompson	X	X	X	X	X	X	X	X	X
P. lenticula Richards & Knapp	X						X		
P. sp. cf. *P. macrocephala* "B"*								X	X
P. maculata (Girard)					X				
P. nigrofasciata (Agassiz)	X	X	X	X	X	X	X		
P. palmaris (Bailey)								X	X

Appendix 3 (*continued*)

	Cahaba			Black Warrior			Tallapoosa		
Family/species	Low	Mid	Up	Low	Mid	Up	Low	Mid	Up
P. sciera (Swain)				X					
P. shumardi (Girard)	X			X	X		X		
P. suttkusi Thompson	X			X					
P. vigil (Hay)	X			X			X		
Sander vitreus (Mitchill)	X			X	X		X		
Petromyzontidae									
Ichthyomyzon castaneus Girard	X				X		X		
I. gagei Hubbs & Trautman	X						X	X	X
Lampetra aepyptera (Abbott)	X								
Poeciliidae									
Gambusia affinis (Baird & Girard)	X	X	X	X	X	X	X	X	X
Polyodontidae									
Polyodon spathula (Walbaum)	X			X	X		X		
Sciaenidae									
Aplodinotus grunniens Rafinesque	X	X		X	X		X		