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Ecological limit functions relating fish community response to hydrologic departures of the ecological flow regime in the Tennessee River basin, United States

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ABSTRACT

Ecological limit functions relating streamflow and aquatic ecosystems remain elusive despite decades of research. We investigated functional relationships between species richness and changes in streamflow characteristics at 662 fish sampling sites in the Tennessee River basin. Our approach included the following: (1) a brief summary of relevant literature on functional relations between fish and streamflow, (2) the development of ecological limit functions that describe the strongest discernible relationships between fish species richness and streamflow characteristics, (3) the evaluation of proposed definitions of hydrologic reference conditions, and (4) an investigation of the internal structures of wedge-shaped distributions underlying ecological limit functions.

Twenty-one ecological limit functions were developed across three ecoregions that relate the species richness of 11 fish groups and departures from hydrologic reference conditions using multivariate and quantile regression methods. Each negatively sloped function is described using up to four streamflow characteristics expressed in terms of cumulative departure from hydrologic reference conditions. Negative slopes indicate increased departure results in decreased species richness.

Sites with the highest measured fish species richness generally had near-reference hydrologic conditions for a given ecoregion. Hydrology did not generally differ between sites with the highest and lowest fish species richness, indicating that other environmental factors likely limit species richness at sites with reference hydrology.

Use of ecological limit functions to make decisions regarding proposed hydrologic regime changes, although commonly presented as a management tool, is not as straightforward or informative as often assumed. We contend that statistical evaluation of the internal wedge structure below limit functions may provide a probabilistic understanding of how aquatic ecology is influenced by altered hydrology and may serve as the basis for evaluating the potential effect of proposed hydrologic changes.
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INTRODUCTION

Despite decades of effort, quantitative models relating streamflow to riverine ecosystems remain elusive (Poff and Zimmerman, 2010; King and Caylor, 2011). One obstacle to developing such models is the scarcity of spatially and temporally concurrent ecological and hydrologic data at the same points across broad regions (Poff and Allan, 1995; Knight *et al.*, 2008; McManamay *et al.*, 2013). More fundamental obstacles, however, are conceptual and methodological issues involving the identification and interpretation of relationships between streamflow regime (Bryan, 1922; Blench, 1957; Langbein and Iseri, 1960) and biota.

Key conceptual papers (Poff *et al.*, 1997; Arthington *et al.*, 2006; Poff *et al.*, 2010) provide a broad and widely accepted framework for ecological flow studies involving definition of a hydrologic reference condition, quantification of actual or hypothetical departures from that condition (hydrologic alteration), and the inference of ecological responses to such departures. The application of this framework presents a number of practical and conceptual challenges, notably (1) the choice of hydrologic models used to characterize flow at ungauged sites, (2) the definition of hydrologic reference conditions, (3) the quantification of departures from those conditions, and (4) the interpretation of apparent relationships between such departures and expressions of ecological health.

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The definition of reference hydrologic conditions and the quantification of hydrologic departure are fundamental to understanding ecological response to changes in streamflow. However, how to define reference hydrologic conditions and departures from those conditions is open to question and defies a 'one-size-fits-all' approach (Kendy *et al*., 2012). Reference hydrology has been described by dividing available data (modelled or measured) into subsets representing 'natural' (reference) and 'developed' (altered) conditions (Sanderson *et al*., 2012) or defined using current hydrologic conditions (Nelson *et al*., 2009; Viger *et al*., 2011). When rainfall–runoff models are applied in this context, natural conditions are typically simulated by first modelling the current condition and then setting model parameters (land cover, water use, etc.) such that human influence is nil. Although rainfall–runoff models have been widely promoted for ecological flow studies, (Richter *et al*., 1997; Arthington *et al*., 2006; Poff *et al*., 2010; Richter, 2010), a number of papers have questioned the practical use of such models to characterize ecological flow regimes (Kendy *et al*., 2009; Murphy *et al*., 2012a; Shrestha *et al*., 2013). Other approaches for establishing reference hydrologic conditions and quantifying hydrologic alteration include the selection of streamflow characteristics (SFCs) and values informed by best professional judgment (Gerritsen *et al*., 1993; Tennessee Wildlife Resources Agency, 2010; Richter *et al*., 2012), and the statistical selection of SFCs (values and ranges) based on measured streamflow for basins with the best obtainable (Stoddard *et al*., 2006) landscape (Carlisle *et al*., 2010; Kennen and Riskin, 2010; Knight *et al*., 2012). Each of these approaches presents difficulties, such as the reproducibility of professional judgment and the temporal disagreement between spatially distributed basin-attribute data (land cover, climate, soil characteristics, etc.), and between those data and the period of observed streamflow records (Koltun and Whitehead, 2002; Hamilton *et al*., 2008; Knight *et al*., 2012).

Quantifiable relationships between biota and hydrologic alteration commonly take the form of wedge-shaped distributions (Terrell *et al*., 1996; Vaz *et al*., 2008; Catalinas and Cantón, 2012; Zorn *et al*., 2012) or, more rarely, tighter linear distributions (Camp Dresser and McKee Inc., 2009; Sanderson *et al*., 2012). Generally, statistically significant bounding envelopes are used to describe wedge-shaped distributions and are commonly interpreted as reflecting limits on ecological potential imposed by hydrologic alteration (Stewart and Anderson, 2007; Kennen and Riskin, 2010). With few exceptions (Booth *et al*., 2004; Carter *et al*., 2009), ecological flow studies have rarely examined the points beneath these proposed ecological limits. However, in other areas of ecology, the internal structure of these wedge-shaped distributions has been explored more thoroughly (Thomson *et al*., 1996; Cade *et al*., 1999; Cade and Noon, 2003; Schmidt *et al*., 2012). Without an examination of the points beneath proposed ecological limits, even highly significant bounding envelopes are difficult to interpret.

This paper builds upon earlier work that statistically related SFCs to fish community structure in the Tennessee River basin (Knight *et al*., 2008), developed predictive equations for SFCs, and proposed hydrologic reference conditions for three ecoregions within the Tennessee–Cumberland River system (Knight *et al*., 2012). In this paper, we apply these earlier results to 662 fish sampling sites in the Tennessee River basin, subdivided by ecoregions. The scope of the paper includes (1) a brief summary of relevant literature on functional relations between fish and streamflow, (2) the development of ecological limit functions that describe the strongest discernible relationships between fish species richness and a refined set of the 17 previously identified SFCs, (3) the evaluation of proposed definitions of hydrologic reference conditions, and (4) an investigation of the internal structures of wedge-shaped distributions underlying ecological limit functions.

STREAMFLOW AS AN ECOLOGICAL LIMIT

Numerous authors have observed that streamflow regime is one of the many factors that help determine the abundance, richness, and composition of riverine ecosystems (Terrell *et al*., 1996; Wilding and Poff, 2008; Armanini *et al*., 2011; Sanderson *et al*., 2012). Other important determinants include water quality and measures of habitat, such as substrate size and type, amount of shade, and water depth and velocity. Streamflow thus acts as one among a suite of ecologically limiting factors. Wedge-shaped distributions have been associated with limiting factors (Figure 1a) in the environmental flow literature (Thomson *et al*., 1996; Cade and Noon, 2003; Armstrong *et al*., 2011), but few papers have examined their internal structure (e.g. Thomson *et al*., 1996; Cade *et al*., 1999; Schmidt *et al*., 2012).

The classic model of multiple limiting factors operating simultaneously, Liebig's law of the minimum (Von Liebig, 1840), assumes that the various factors operate independently of one another and that only one factor actually limits the state of the system at a given point in space and time. The traditional analogue of this model is a barrel with staves of various lengths (Figure 1b). The level of the water in the barrel, representing system state, depends only on the shortest stave and is unaffected by lengthening or shortening of other staves. Applied to the interaction of a controlling variable, such as mean annual streamflow, and a response variable, such as number of fish species, Liebig's law would imply that mean annual streamflow is limiting only along the defining upper bound or 'limit' (Figure 1a). Therefore, the points located below the upper bound of the distribution may not show a measureable ecological response for a given amount of hydrologic alteration until sufficient horizontal movement

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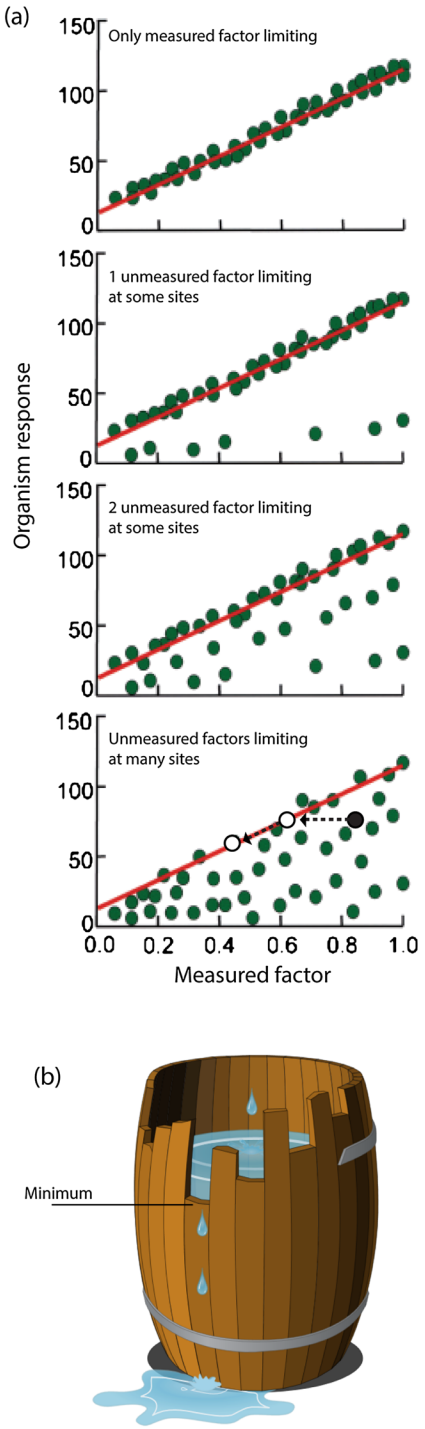

Figure 1. (a) Example distributions showing ecological response to single and multiple unmeasured factors (modified with permission from Cade and Noon, 2003) and (b) example of a barrel with different length staves representing Liebig's law of the minimum.

shifts that point to intersect with the ecological limit function. From this intersection, increased alteration would cause the number of fish species to respond according to the ecological limit function (see dashed line on Figure 1a). The location of a point below the limit function implies the influence of other limiting, although not necessarily interacting, factors (Hiddink and Kaiser, 2005).

The emphasis on limit functions in the ecological flow literature and their interpretation as straightforward expressions of ecological potential (Knight *et al.*, 2008; Kennen and Riskin, 2010; Murphy *et al.*, 2012a; Sanderson *et al.*, 2012) implicitly suggest a model in which multiple limiting factors operate independently. Ecological limit functions typically take the form of a linear equation relating a single environmental driver, such as hydrologic alteration, to a single ecological response variable, such as fish species richness (Sanderson *et al.*, 2012). However, there are numerous well-known examples of interactions between streamflow and other ecologically limiting factors, including turbidity, suspended sediment concentration, temperature, dissolved oxygen, and general chemical constituents (Gilbert, 1914; Vanoni, 1946; Leopold and Maddock, 1954; Porterfield, 1972; Walling, 1977; Walling and Webb, 1980; Dowling and Wiley, 1986; Constantz, 1998; Evans and Davies, 1998; House and Warwick, 1998; Chanat *et al.*, 2002; Ulrich and Bragg, 2003; Bonta, 2004, 2005; Rice *et al.*, 2004; Godsey *et al.*, 2009; Rasmussen *et al.*, 2009; Siegel, 2009; Ali *et al.*, 2010; Murphy *et al.*, 2012b). Without accounting for other ecologically limiting factors, bivariate models may only be of limited use in a management context.

Quantile regression has emerged as a tool for exploring the interactions of ecological limits beyond the simple definition of a distribution's upper boundary. Thomson *et al.* (1996) and Cade *et al.* (1999) contended that the interior of wedge-shaped distributions contains discernible information about ecological limits imposed by multiple environmental factors. Schmidt *et al.* (2012) examined these concepts in detail. Comparable analyses applied to ecological flows will require a stronger empirical and conceptual foundation than is currently available. This paper seeks to contribute to that foundation by examining relations between streamflow and fish communities in the Tennessee River basin.

STUDY AREA

The Tennessee River basin (Figure 2) drains approximately 106 000 km^2 of the Southeastern United States and is one of the most diverse temperate freshwater ecosystems in the world (Etnier and Starnes, 1993; Olson and Dinerstein, 1998; Abell *et al.*, 2000). More than 230 fish species, 141 freshwater mussels species, 160 aquatic snail species, 115 crayfish species, and North America's largest salamander, the hellbender (*Cryptobranchus alleganiensis*) live within the basin (Abell *et al.*, 2000). This diverse aquatic fauna is distributed across approximately 64 000 km of streams

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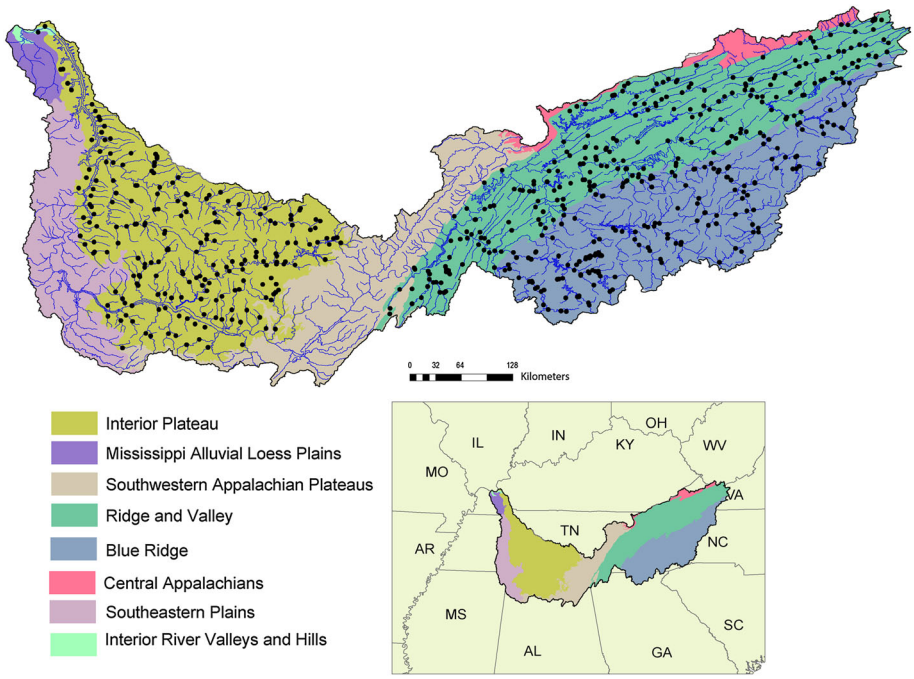

Figure 2. Distribution of fish sampling sites in the Blue Ridge, Ridge and Valley, and Interior Plateau ecoregions of the Tennessee River basin, 2000–2005.

(1:100 000 scale, National Hydrography Dataset; http://nhd.usgs.gov/). Approximately 67 of the fish species are endemic to the region, and assemblages of mussels, crayfish, and salamanders are considered to be globally outstanding (Abell *et al.*, 2000). In fact, the Tennessee–Cumberland River basin has more endemic aquatic species than any other river basin in North America (Stein *et al.*, 2000).

The Tennessee River basin is divided into Level III Ecoregions (Omernik, 1987), which roughly align with similarly named physiographic provinces (Fenneman, 1938): Mississippi Alluvial Loess Plains, Interior Plateau, Interior River Valleys and Hills, Southeastern Plains, Cumberland Mountain, Southwestern Appalachian Plateaus, Ridge and Valley, and Blue Ridge (Figure 2). Three Level III Ecoregions – Interior Plateau, Ridge and Valley, and Blue Ridge – comprise approximately 87% of the Tennessee River basin.

DATA AND METHODS

Data analysis consisted of three main components. First, we identified suites of SFCs related to fish species richness for the three primary ecoregions in the basin. Next, ecological limit functions were developed using quantile regression models that related changes in the potential fish species richness to departures from hydrologic reference conditions. Finally, we evaluated the ecoregion-specific reference hydrologic profiles published by Knight *et al.* (2012) by comparing alternative profiles.

Ecologic, hydrologic, and ancillary datasets

The analysis dataset was composed of three types of information: fish species richness, hydrologic data, and ancillary data. Fish species richness data were collected by the Tennessee Valley Authority (TVA) from 2000 to 2005 using consistent protocols (TVA, 1997). Of 860 TVA fish sampling sites, 662 sites were retained for analysis; sites draining basins with less than 50% of their area in one of the three primary ecoregions were excluded. Although many sites were sampled multiple times, only the first sample in the 5-year period was included. For each site, species richness was determined for the fish community as a whole and for ten general fish groups defined by habitat preference, environmental tolerance, reproductive mode, and trophic class (Table I). Knight *et al.* (2008) identified 17 SFCs (calculated from measured data) as ecologically relevant to fish community data in the Tennessee River basin. Estimates for these 17 and 2 additional SFCs (Table II) were developed using published regional regression equations (Knight *et al.*, 2012). These equations were developed using regional basin characteristics as explanatory variables. Four of these SFCs were excluded from this analysis either because of a strong correlation with another SFC (Sep_med was removed because it was well correlated with e85; see Table II for SFC definitions) or because their respective equations had *r*-squared values that were less than 0·3 (TH1, RA8, and RA5; see Table III in Knight *et al.*, 2012 for model diagnostics). SFCs were also compiled from observed daily streamflow data for the

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Table I. Fish category definitions based on trophic, habitat, and tolerance characteristics.

Category	Definition	Citations
Pool dwellers	Species that require pool habitat (both shallow pools and deep pools) and feed on drifting and surface invertebrates; most are active swimmers	US EPA (1999)
Riffle dwellers	Species that feed and reproduce in benthic habitats (in riffles); these species are sensitive to degradation resulting from siltation and benthic oxygen depletion (darters, sculpins, and madtoms)	US EPA (1999)
Natives	Total number of resident native fish species	Lee *et al*. (1980), Karr (1981), Etnier and Starnes (1993), US EPA (1999)
Specialized insectivores	Species that feed predominately on insects found in benthic habitat; these species are highly susceptible to habitat degradation	US EPA (1999)
Invertivores	Species that are invertivores, primarily insectivores (excluding generalized insectivores and opportunistic feeders such as western blacknose dace or creek chub.) As invertebrate food source decreases in diversity and abundance as a result of habitat degradation, there is a shift from insectivorous to omnivorous fish	US EPA (1999)
Omnivores	Species that consistently feed on substantial proportions of plant and animal material	US EPA (1999)
Top predators	Species that feed, as adults, predominately on fish, other vertebrates, or crayfish	US EPA (1999)
Lithophilic spawners	Species that spawn where their eggs can develop in the interstices of sand, gravel, and cobble substrates without parental care	Balon (1975), US EPA (1999)
Headwater intolerants	Headwaters are somewhat harsh environments, and those pioneering species that thrive to maturity there are considered headwater-intolerant taxa	US EPA (1999)
Intolerants	Species that are intolerant of various chemical and physical perturbations; typically, the first species to disappear after a disturbance	US EPA (1999)

20 most forested sites in each of the three primary ecoregions in the Tennessee River basin following Knight *et al*. (2012). All SFCs, estimated or observed, are in standardized units (mean of 0 and standard deviation of 1). Ancillary data include information that may be related to fish community structure, such as human population data (http://census.gov) and modelled nutrient concentrations (Hoos *et al*., 2008; Hoos and McMahon, 2009; García *et al*., 2011).

Departure from hydrologic reference conditions

Three ecoregion-specific hydrologic reference profiles (Interior Plateau, Ridge and Valley, and Blue Ridge; Figure 3; Knight *et al*., 2012) were defined using SFCs found to be statistically related to the fish community in the Tennessee River basin (Knight *et al*., 2008). The reference range for each SFC was defined by the 25th and 75th percentiles (Table III) of the 20 most forested sites in each ecoregion. SFCs were calculated using at least 10 years of daily mean streamflow data (Knight *et al*., 2012). For the purposes of our study, basins with the highest percentage of forest cover were assumed to represent the least disturbed landscape conditions. The interquartile range (IQR) for SFCs eliminates extreme values while capturing the natural variability.

To quantify the hydrologic departure for each site, each estimated SFC was subtracted from the reference range with respect to region (Knight *et al*., 2012). These differences (departures) are quantified in absolute terms and describe the distance outside (above or below) the range. Although fish communities may have different sensitivities to positive or negative departures from reference conditions, representing departure in absolute terms is a useful simplification to support a general analysis. Absolute departures can be summed across relevant SFCs to arrive at the cumulative departure for a site. All analyses in this study were completed using departure values of the SFCs from reference conditions.

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Table II. Definitions of streamflow characteristics predicted using regression analysis (adapted and modified from Knight *et al*., 2012).

	Streamflow characteristic	Definition (units)
Magnitude	MA41: mean annual runoff	Compute the annual mean daily streamflow and divide by the drainage area (cubic feet per second (cfs) per square mile (cfsm))
	AMH10: maximum October streamflow	Maximum October streamflow across the period of record divided by watershed area (cfsm)
	e85: streamflow value exceeded 85% of time	85% exceedance of daily mean streamflow for the period of record normalized by the watershed area (cfsm)
	Sep_med: median September daily flow	Calculate the median of daily mean streamflow values for the period of record that occurred in the month of September normalized by watershed area (cfsm)
	LRA7: rate of streamflow recession	Log transform of the median change in log of flow for days in which the change is negative across the entire flow record (flow units per day)
Ratio	LDH13: average 30-day maximum	Log transform of the average over the period of record of the annual maximum of 30-day moving average flows divided by the median for the entire record (dimensionless)
	ML20: base flow	Divide the daily flow record into 5-day blocks. Assign the minimum flow for the block as a base flow for that block if 90% of that minimum flow is less than the minimum flows for the blocks on either side. Otherwise, set it to zero. Fill in the zero values using linear interpolation. Compute the total flow for the entire record and the total base flow for the entire record. ML20 is the ratio of total flow to total base flow (dimensionless)
	TA1: constancy	Measure the stability of flow regimes by dividing daily flows into predetermined flow classes (dimensionless)
	RA5: number of day rises	Compute the number of days in which the flow is greater than the previous day divided by the total number of days in the flow record (dimensionless)
Frequency	FH6: frequency of moderate flooding (three times the median annual flow)	Average number of high-flow events per year that are equal to or greater than three times the median annual flow for the period of record (number per year)
	LFH7: frequency of moderate flooding (seven times median annual flow)	Log transform of the average number of high-flow events per year that are equal to or greater than seven times the median annual flow for the period of record (number per year)
Variability	MA26: variability of March streamflow	Compute the standard deviation for March streamflow and divide by the mean streamflow for March (%)
	LML18: Variability in base flow	Log transform of the standard deviation of the ratios of 7-day moving average flows to mean annual flows for each year multiplied by 100 (%)
	LDL6: variability of annual minimum daily average streamflow	Log transform of the standard deviation for the minimum daily average streamflow. Multiply by 100 and divide by the mean streamflow for the period (%)
	LDH16: variability in high-pulse duration	Log transform of the standard deviation for the yearly average high-flow pulse durations (daily flow greater than the 75th percentile) (%)
	FL2: variability in low-pulse count	Coefficient of variation for the number of annual occurrences of daily flows less than the 25th percentile (dimensionless)
Date	TL1: annual minimum flow	Julian date of annual minimum flow occurrence (Julian day)
	TH1: annual maximum flow	Julian date of annual maximum flow occurrence (Julian day)
	RA8: flow direction reversals	Average number of days per year when flow changes from rising to falling (or from falling to rising) (number per year)

Data analysis

Hydrologic departure and fish species richness. Multivariate correlation was used to parse SFCs in terms of their relation to specific fish groups within each ecoregion. Species richness for each fish group (all species plus 10 subgroups, Table I) was used as the response variable, and all 15 SFCs, in terms of departure, were potential explanatory variables. Species counts were transformed using a Jaccard transform (Jaccard, 1901) prior to analysis. Maximized Spearman rank correlation was completed using the BEST procedure within

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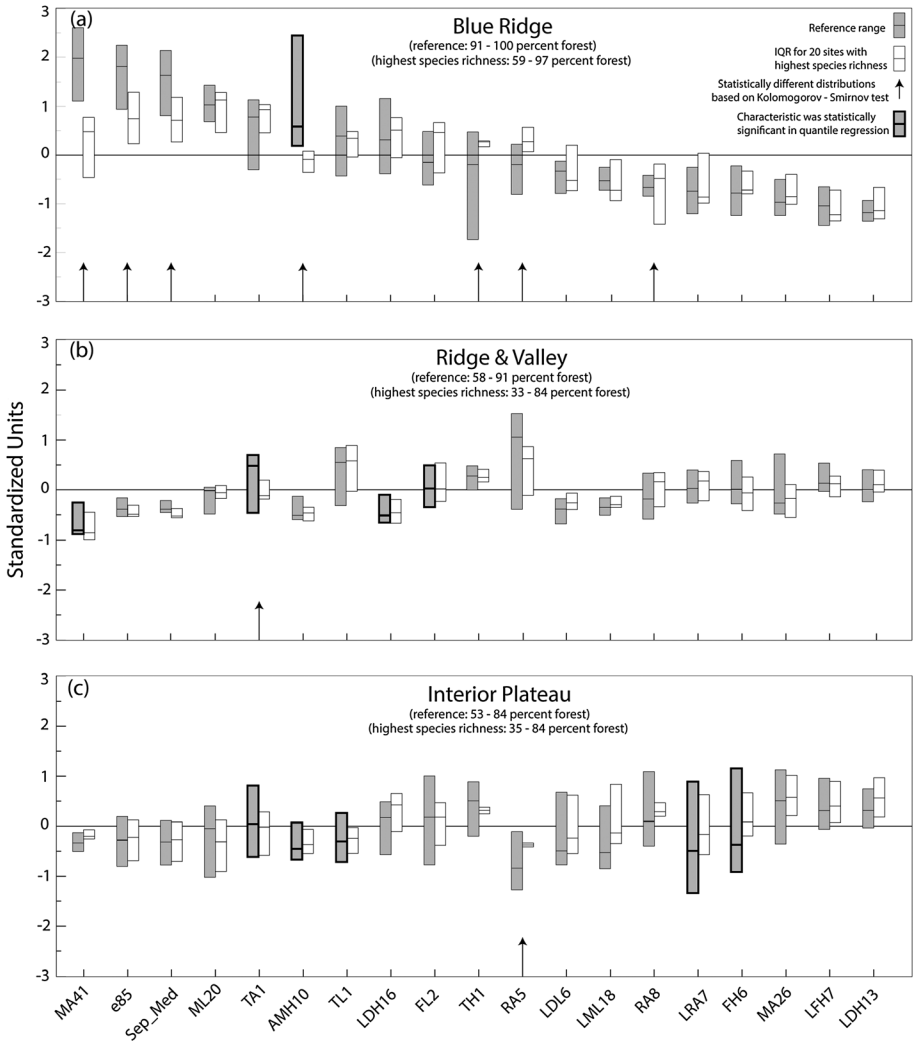

Figure 3. Comparison of reference profiles (defined by Knight *et al*., 2012, as the 20 sites with the highest percentage of forest cover) and profiles developed using 20 Tennessee Valley Authority fish sampling sites with the highest species richness, according to the (a) Blue Ridge, (b) Ridge and Valley, and (c) Interior Plateau ecoregions (modified from Knight *et al*., 2012). Statistically different distributions for a given streamflow characteristic, as determined by Kolmogorov–Smirnov tests, are noted with arrows. Streamflow characteristics used in final quantile regression analyses are indicated using bold boundaries on the interquartile range (IQR).

the Plymouth Routines in the Multivariate Ecological Research (PRIMER-E) software package (Clarke and Gorley, 2006). The BEST procedure uses a Bray–Curtis similarity matrix of the species richness and conducts a stepwise search across the standardized hydrologic dataset, which consists of SFC values in terms of departure, to search for the appropriate combination of SFCs that maximize the correlation coefficient between species richness and hydrologic departure matrices (Clarke and Ainsworth, 1993). The BEST procedure identified a preliminary subset of SFCs that best explained the structure of the data for each fish group and ecoregion. Only statistically significant ($p < 0{\cdot}05$) SFCs were retained in the analysis.

Quantile regression (Cade *et al*., 1999; Cade and Noon, 2003; Vaz *et al*., 2008) was used to further constrain these subsets of SFCs (in terms of departure). Quantile regression identifies and quantifies upper or lower bounds of wedge-shaped point distributions and, by extension, the limiting influence of an explanatory variable to a response variable (Knight *et al*., 2008; Konrad *et al*., 2008). Proc Quantreg (SAS, version 9.2; http://www.sas.com) was used to perform the analyses. Within the preliminary subsets identified by the BEST procedure, each SFC, in terms of departure, was individually tested for significance to its corresponding fish group by developing quantile regression models for the 85th, 90th, and 95th quantiles. SFCs that had at least one significant ($p < 0{\cdot}05$) quantile were retained for further analysis and comprised the final suites of ecologically relevant SFCs by fish group and ecoregion. SFCs without a significant quantile were dropped from the analysis. In aggregate, these procedures led to the progressive reduction of SFCs considered as explanatory

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Table III. Interquartile ranges of the ecoregion-specific hydrologic reference profiles.

	Interquartile ranges (standardized values)					
	Blue Ridge		Ridge and Valley		Interior Plateau	
Streamflow characteristic	25th	75th	25th	75th	25th	75th
Mean annual runoff (MA41)	1·0815	2·6190	−0·8863	−0·2524	−0·5073	−0·1307
Lowest 15% of daily flow (e85)	0·9088	2·1282	−0·4989	−0·1764	−0·7975	0·1893
Median September daily flow (Sep_med)[a]	0·7695	2·1321	−0·4557	−0·2215	−0·7811	0·1070
Base flow (ML20)	0·6841	1·4280	−0·4843	0·0473	−1·0245	0·3925
Stability of flow (TA1)	−0·3056	1·1152	−0·4669	0·6942	−0·6146	0·8062
Maximum October flow (AMH10)	0·1874	2·4486	−0·6047	−0·1350	−0·6642	0·0705
Timing of annual lowest flow (TL1)	−0·4306	1·0017	−0·3132	0·8419	−0·7178	0·2449
Variability in high-pulse duration (LDH16)	−0·3906	1·1526	−0·6599	−0·1007	−0·5771	0·4790
Variability of flow pulses less than the 25th percentile (FL2)	−0·6162	0·4632	−0·3434	0·4928	−0·7718	1·0080
Timing of annual highest flow (TH1)[a]	−1·7445	0·4567	−0·0190	0·4783	−0·2124	0·8831
Number of day rises (RA5)[a]	−0·8037	0·2207	−0·3924	1·5167	−1·2538	−0·1053
Variability of annual minimum daily flow (LDL6)	−0·7891	−0·1342	−0·6901	−0·1856	−0·7705	0·6688
Variability of baseflow (LML18)	−0·7254	−0·2588	−0·5054	−0·1656	−0·8584	0·4017
Flow direction reversals (RA8)[a]	−0·8582	−0·4322	−0·5918	0·3199	−0·4135	1·0792
Rate of recession (LRA7)	−1·2041	−0·2860	−0·2515	0·3813	−1·3412	0·8901
Frequency of moderate flow (FH6)	−1·2420	−0·2222	−0·2922	0·5766	−0·9036	1·1414
Variability of March flow (MA26)	−1·2374	−0·5116	−0·4951	0·7137	−0·3816	1·0956
Frequency of moderate flow (LFH7)	−1·4548	−0·6584	−0·0383	0·5300	−0·0714	0·9510
Ratio of annual 30-day maximum to median annual flow (LDH13)	−1·3689	−0·9454	−0·2339	0·3888	−0·0431	0·7417

Streamflow characteristics from Knight *et al*. (2008, Table I); *r*-squared and root mean square error and information on converting to unstandardized units can be found in Knight *et al*., (2012, Table III).
[a] Characteristics used to define the profile but were not used in analysis either because of low prediction accuracy (<0.3 *r*-squared) or redundancy (Sep_med).

variables for each combination of ecoregion and fish group (Table IV).

Departures for retained SFCs were summed according to fish group and ecoregion to create a new explanatory variable, referred to hereafter as cumulative departure. Quantile regression was then repeated by ecoregion using species richness by fish group and cumulative departures. The resulting quantile regression models describe the probability (dependent on the quantile) of species richness along a gradient of increasing cumulative departure. This statistically discernible upper bound represents an ecological limit function specific to a given fish group and ecoregion.

Hydrologic profile comparisons. The utility of hydrologic reference profiles defined by Knight *et al*. (2012) for predicting SFCs at sites with the best fish was evaluated using four alternative sets of hydrologic profiles. Alternative hydrologic profiles were developed for each ecoregion using suites of 20 TVA fish sites with the (1) highest species richness, (2) lowest species richness, (3) largest percentage of basin area covered in forest, and (4) smallest percentage of basin area covered in forest. Most TVA fish sites do not have measured streamflow data (Knight *et al*., 2012); therefore, predicted SFCs (nondeparture form) were used to calculate the IQRs that define these profiles. Statistical differences between profiles were identified using the nonparametric Kolmogorov–Smirnov (K–S) test, which determines the likelihood that the two distributions are drawn from the same population (Davis, 2002). K–S tests have been used in hydrology (Fleming, 2005) and ecology (Li *et al*., 2011) to identify differences between sites or groups of sites. Because the hydrologic profiles are defined by IQRs, K–S tests were performed using only the data contained within the IQRs. Three profile comparisons were completed by ecoregion: (1) hydrologic reference and highest species richness profiles, (2) highest and lowest species richness profiles, and (3) most and least forested profiles.

RESULTS AND DISCUSSION

Hydrologic departure and fish species richness

Multivariate correlation (BEST procedure in PRIMER-E) and quantile regression analyses reduced the number of SFCs considered from 15 to between one and four, according to fish group and ecoregion. Initially, subsets containing one to seven SFCs were retained on the basis of the BEST procedure. Subsets of SFCs were statistically

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Table IV. Streamflow characteristics influential to fish species richness in the Tennessee River basin (upper table) and final quantile regression models (lower table) ($p < 0{\cdot}05$).

Streamflow characteristics[a]	Fish groups										
	All Species	Pool dwellers	Riffle dwellers	Natives	Specialized insectivores	Invertivores	Omnivores	Top predators	Lithophilic spawners	Headwater intolerants	Intolerants
Magnitude											
Mean annual flow (MA41)		I				**R** I				I	
Lowest 15% of daily flow (e85)	B		B	B	B	B	B		B		
Maximum October flow (AMH10)	**B** **I**	**I**	B I	**I**	B **I**	**B** **I**		**I**	**B**	I	**I**
Rate of streamflow recession (LRA7)						**I**					
Ratio											
Stability of flow (TA1)	R I	**I**	**I**	R **I**	B **R** **I**	R I		I	R **I**	R **I**	I
Average 30-day maximum flow (LDH13)							B				
Frequency											
Frequency of moderate floods[b] (LFH7)	R	R		R	R		R		R		
Frequency of moderate floods[c] (FH6)						**I**				**I**	**I**
Variability											
Variability of flow less than 25% (FL2)	B **R**	**R**		**R**	B		**R**	R	B **R**		R
Variability of March flow (MA26)	R I	R	R I	R I	R	R I	R	R I	R		R I
Variability in high-pulse duration (LDH16)					**R**			R			**R**
Date											
Timing of annual lowest flow (TL1)	**I**	**I**	**I**	**I**		R **I**		**I**		I	
Final quantile regression models											
Blue Ridge (B): quantile	0.90					0.95			0.85		
Slope	-8.9					-3.3			-4.3		
Intercept	37.1					14.8			18.2		
Ridge and Valley (R): quantile	0.90	0.95		0.95	0.95	0.85	0.85		0.85		0.90
Slope	-10.9	-10.1		-13.3	-15.4	-4.7	-0.9		-3.9		-3.1
Intercept	33.3	25.4		35.1	18.7	12.5	5.2		13.5		5.1
Interior Plateau (I): quantile	0.95	0.85	0.90	0.90	0.85	0.95		0.90	0.95	0.95	0.90
Slope	-9.9	-6.0	-4.9	-8.4	-5.8*	-2.4		-1.9	-32.9	-3.8	-2.3
Intercept	57.1	32.3	14.1	49.6	19.6	19.8		6.6	33.1	9.0	7.8

Bold/boxed cells represent streamflow characteristics comprising the final suites of streamflow characteristics by fish group and ecoregion and used in final quantile regression models; non-bold cells were only significant in the BEST procedure; blank cells represent no significant correlation B, Blue Ridge; R, Ridge and Valley; I, Interior plateau.
[a]Identified in Knight et al. (2008, 2012).
[b]Events greater than seven times the median annual flow.
[c]Events greater than three times the median annual flow.
*Significant at p < 0·055.

related to most of the 11 fish groups, but the numbers varied among ecoregions. In the Blue Ridge, no SFCs were statistically related to four fish groups: pool dwellers, top predators, intolerants, and headwater intolerants. In the Interior Plateau, no SFCs were statistically related to omnivorous fish (Table IV).

Quantile regression relating the departures of individual SFCs to species richness further reduced the number of SFCs in each subset to those that had a significant ($p < 0{\cdot}05$) 85th, 90th, or 95th regression quantile. At least one SFC was dropped from most subsets, and seven of the preliminary subsets identified by the BEST procedure were eliminated completely because none of the SFCs composing these subsets had a significant 85th, 90th, or 95th quantile (Table IV). The eliminated fish groups include riffle dwellers, natives, specialized insectivores, omnivores, and headwater intolerants in the Blue Ridge and riffle dwellers, top predators, and headwater intolerants in the Ridge and Valley. Final suites contain one to four SFCs, depending on ecoregion and fish group (Table IV). In the Blue Ridge, AMH10 (see Table II for SFC definitions) was the only significant SFC, defining each of the three fish groups (Table IV). FL2 was frequently significant in the Ridge and Valley, occurring in five of the eight fish groups. Other influential SFCs identified in the Ridge and Valley include TA1, MA41, and LDH16. Interior Plateau fish

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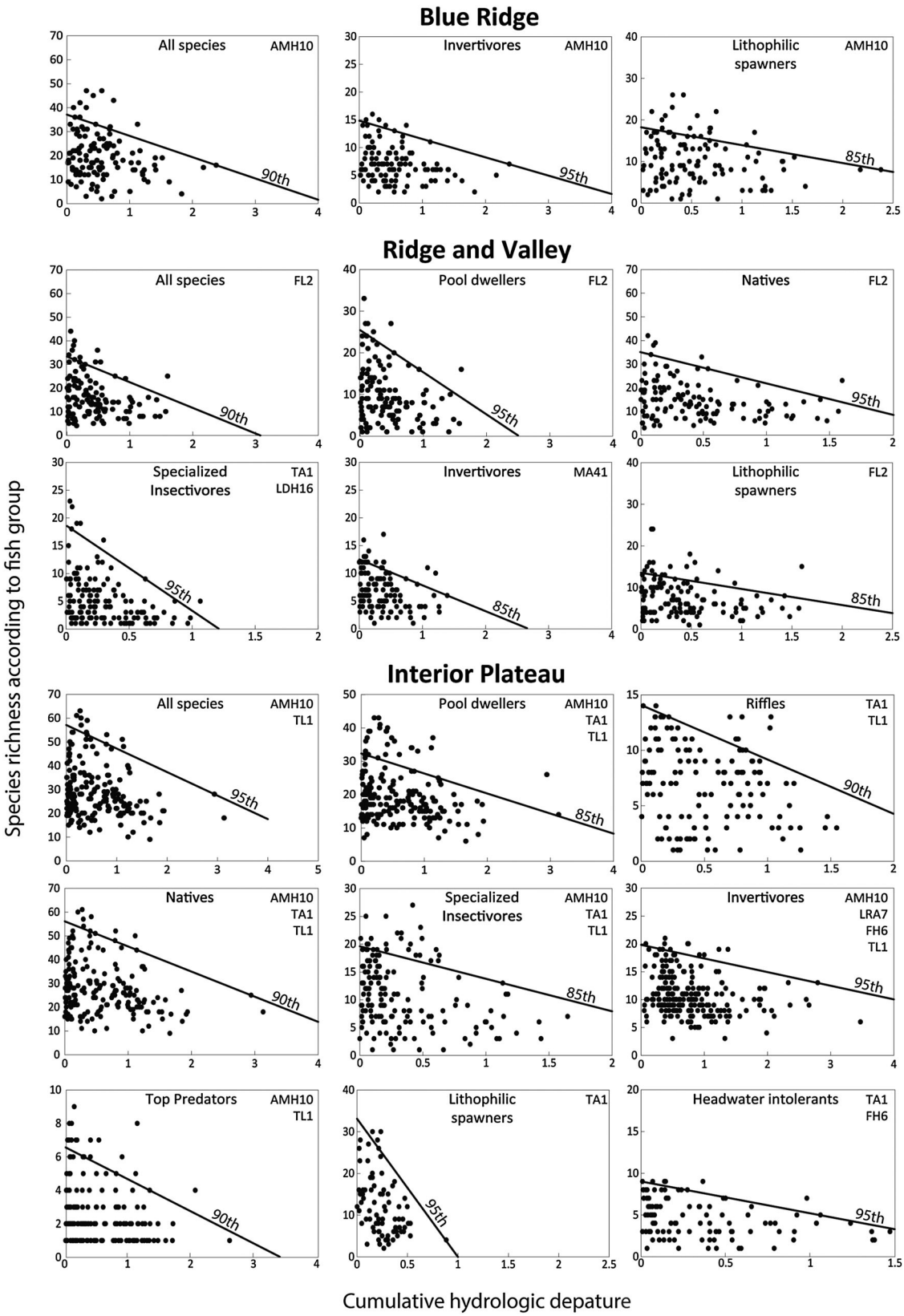

Figure 4. Wedge-shaped distributions and ecological limit functions (solid line, regression quantile noted on line) by ecoregion for select fish groups. Streamflow characteristics used to calculate cumulative departure are listed in the upper right corner of each plot. See Table IV for slopes and intercepts of the limit functions shown.

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groups tended to include more SFCs, between one and four, compared with the Blue Ridge (one SFC per fish group) and the Ridge and Valley (one to two SFCs per fish group). AMH10, TA1, and TL1 are influential in many of the fish groups for the Interior Plateau (Table IV).

Significant departures were summed to represent cumulative departure by fish group and ecoregion and related to species richness using quantile regression. Significant quantile regression lines ($p < 0{\cdot}05$) are assumed to represent ecological limit functions, described by the 85th, 90th, or 95th quantile (Table IV). All limit functions have negative slopes (Figure 4). The intercepts for overall species richness (all species) for the Blue Ridge (37 species), Ridge and Valley (33 species), and Interior Plateau (57 species) highlight the difference in overall species richness between ecoregions. The Interior Plateau has greater species richness than either the Blue Ridge or Ridge and Valley; however, the rate of potential species loss (the slope of the quantile regression) is similar across ecoregions, ranging from 8·9 to 10·9 potential species loss for each unit increase in cumulative departure (Figure 4 and Table IV). Potential species loss as a function of departure varies by both fish group and ecoregion (Table IV). For example, lithophilic spawners are abundant in the Interior Plateau (intercept = 33 species) as compared with the Blue Ridge (18 species) and Ridge and Valley (13 species), but potential species loss for lithophilic spawners is much greater in the Interior Plateau (32·9 potential species loss per unit of departure) than in the Blue Ridge (slope = −4·3) or Ridge and Valley (slope = −3·9). Several fish groups such as pool dwellers (slope = −10.·1), natives (slope = −13·3), and specialized insectivores (slope = −15·4) show a greater rate of potential species loss in the Ridge and Valley, as compared with the Interior Plateau or the Blue Ridge (Table IV).

Hydrologic profile comparison

According to K–S tests, the highest-species-richness profiles were in general agreement with reference profiles (Figure 3) defined by Knight *et al.* (2012), indicating that sites with high species richness generally have reference hydrology. For the Ridge and Valley and Interior Plateau, the IQRs of only one SFC, TA1 and RA5, respectively, were statistically different between these profiles. For the Blue Ridge, IQRs of seven SFCs were different between profiles (Figure 3). This general agreement between reference hydrology and highest-species-richness profiles in the Ridge and Valley and Interior Plateau, and lack of agreement in the Blue Ridge, is illustrated by comparing species richness with SFCs in standardized units (Figure 3).

Sites with maximum species richness were contained within the reference range for the Ridge and Valley and Interior Plateau, whereas maximum species richness for Blue Ridge sites peaked outside of the reference range (Figure 5 and Table III).

The most-forested and least-forested hydrologic profiles generally disagreed, indicating that hydrology varies according to percentage of forest, particularly for the Ridge and Valley and Interior Plateau (Figure 6). For the Blue Ridge, only 5 of 19 IQRs were statistically different,

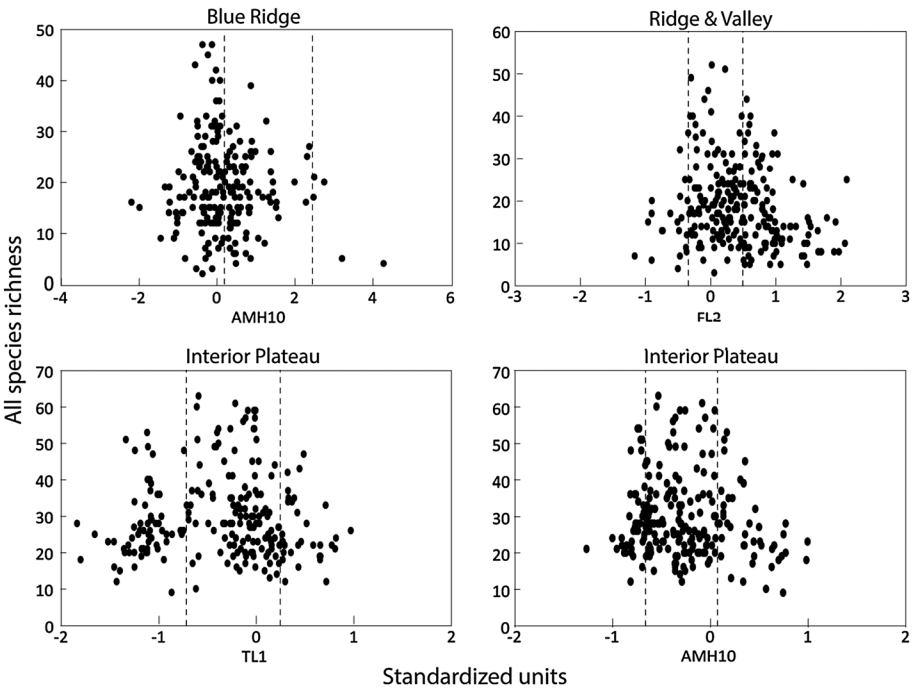

Figure 5. Relationships between all fish species richness and select streamflow characteristics (SFCs; actual values) in the Blue Ridge, Ridge and Valley, and Interior Plateau. Dashed lines represent the reference range for each SFC (see Table III for reference ranges of all SFCs).

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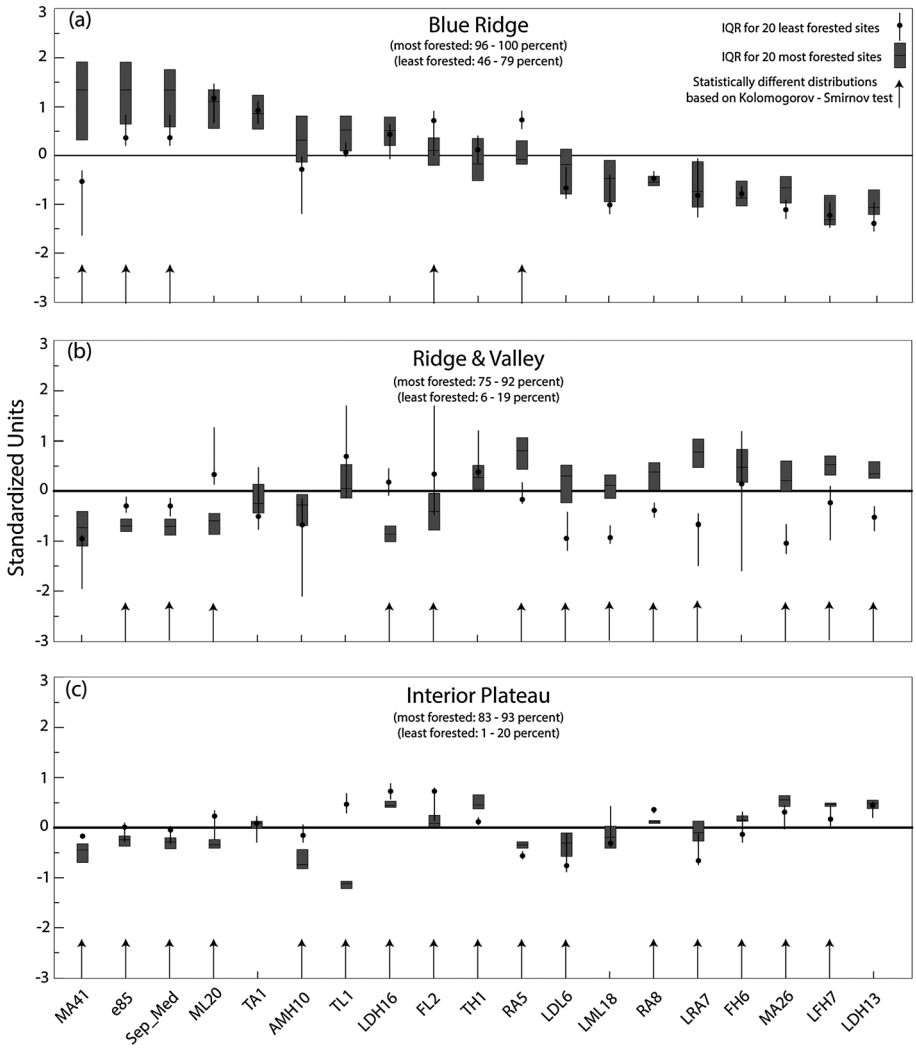

Figure 6. Comparison of profiles based on 20 Tennessee Valley Authority fish sampling sites with highest or lowest percentage of forest cover according to (a) Blue Ridge, (b) Ridge and Valley, and (c) Interior Plateau ecoregions. Statistically different distributions for a given streamflow characteristic, as determined by Kolmogorov–Smirnov tests, are noted with arrows. IQR, interquartile range.

indicating a hydrologic similarity between most-forested and least-forested sites. The IQRs of 13 and 16 SFCs were statistically different for the Ridge and Valley and Interior Plateau, respectively, showing a discernible relation between forest cover and hydrologic regime.

Finally, K–S tests indicated that the highest-species-richness and lowest-species-richness profiles were more similar than anticipated (Figure 7). For the Blue Ridge and Interior Plateau, the IQR for one SFC (RA5) was statistically different between profiles. For the Ridge and Valley, six of the IQRs were statistically different. The lack of hydrologic distinctiveness between highest-species-richness and lowest-species-richness profiles suggests that other influences, such as nutrient concentration, human population density, or water temperature, may be degrading species richness at sites that have otherwise reference hydrology.

Investigation of ecological limit functions and wedge-shaped distributions

The internal structures of wedge-shaped distributions were evaluated to determine if the observations comprising these distributions form groups (clusters) within the analytical space that reflect factors (clustering variables) other than the streamflow regime. Observations comprising the wedge-shaped distributions must be largely unaffected by clustering variables for ecological limit functions to be applicable across the regions for which they were derived. For example, if the observations from specific subregional areas, such as Level IV Ecoregion (US Environmental Protection Agency, 2013), are clustered together in distinct areas of the wedge-shaped distribution, then the ecological limit function would not solely describe the relationship between hydrologic departure and species richness across

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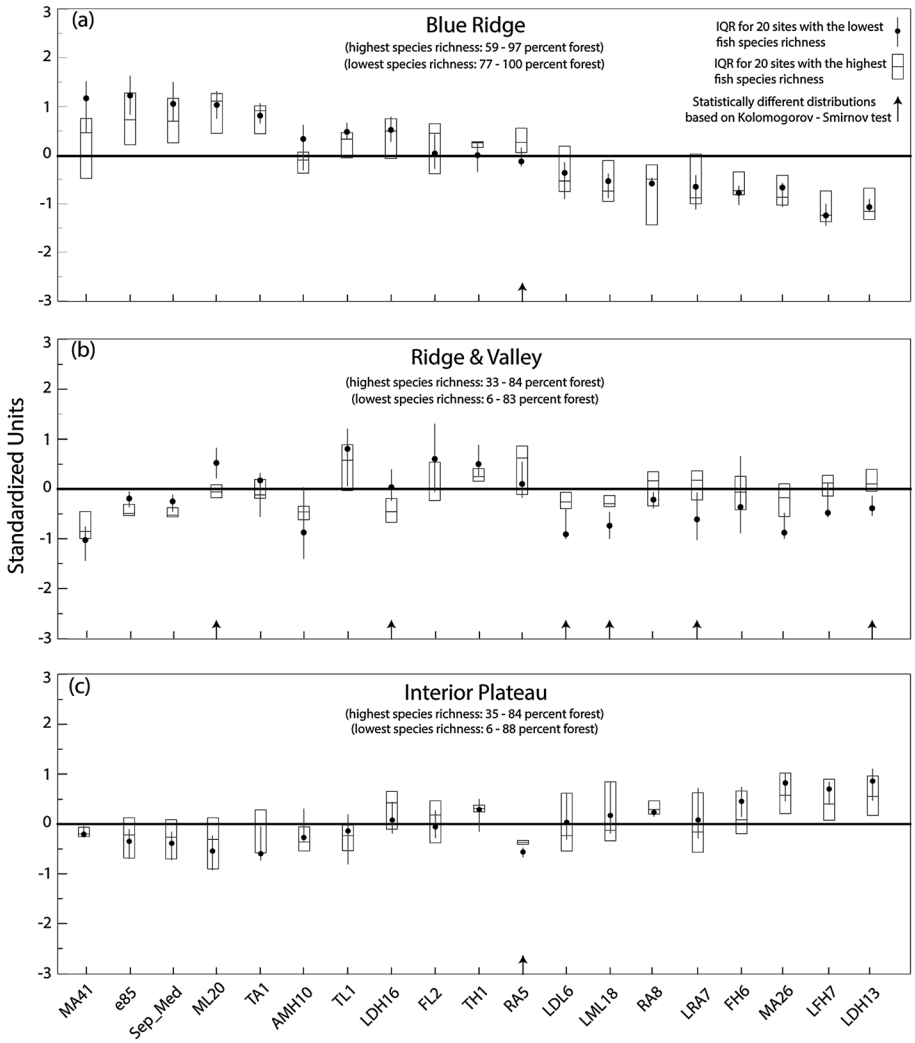

Figure 7. Comparison of profiles based on 20 Tennessee Valley Authority fish sampling sites with the highest or lowest fish species richness according to the (a) Blue Ridge, (b) Ridge and Valley, and (c) Interior Plateau ecoregions. Statistically different distributions for a given streamflow characteristic, as determined by Kolmogorov–Smirnov tests, are noted with arrows. IQR, interquartile range.

the entire ecoregion. Instead, this apparent relationship would be an artefact of the cluster organization within the wedge that would need to be accounted for in the analyses. Thus, subsamples of the measured data, sampled either randomly or according to a subregional characteristic, should exhibit the same general shape and slope of the entire wedge if the ecological limit function is applicable across the entire region. If internal clustering is present, ecological limit functions should be developed at a finer scale (perhaps subdivided according to smaller regions), or the clustering variable should be incorporated into the quantile regression analysis, possibly using a Bayesian approach (Reich *et al.*, 2010).

We visually assessed the internal structure of wedge-shaped distributions for each ecoregion considering the all-species fish group to determine if the ecological limit functions identified in this study could be applied across the three ecoregions. Clustering of observations within the distributions based on Level IV Ecoregions or individual river systems was not evident. In all ecoregions, subsamples based on Level IV Ecoregions have ranges, shapes, and slopes similar to those of the wedge. In the Interior Plateau, subsamples based on individual river systems (Duck River, Buffalo River, and the tributaries to the main stem of the western Tennessee River) show linear relations, with ranges and slopes similar to the entire wedge for all species, riffle dwellers, and native fish groups (Figure 8). From these observations, it appears that the ecological limit functions described in this paper are applicable across their respective ecoregions.

Ecological limit functions typically account for only one aspect of the environment capable of limiting fish species richness, whereas other aspects of the environment may be used to explain some portion of the underlying complexity of wedge distributions (e.g. bottom plot of Figure 1a; Vaz *et al.*, 2008). Depending on the location of an observation

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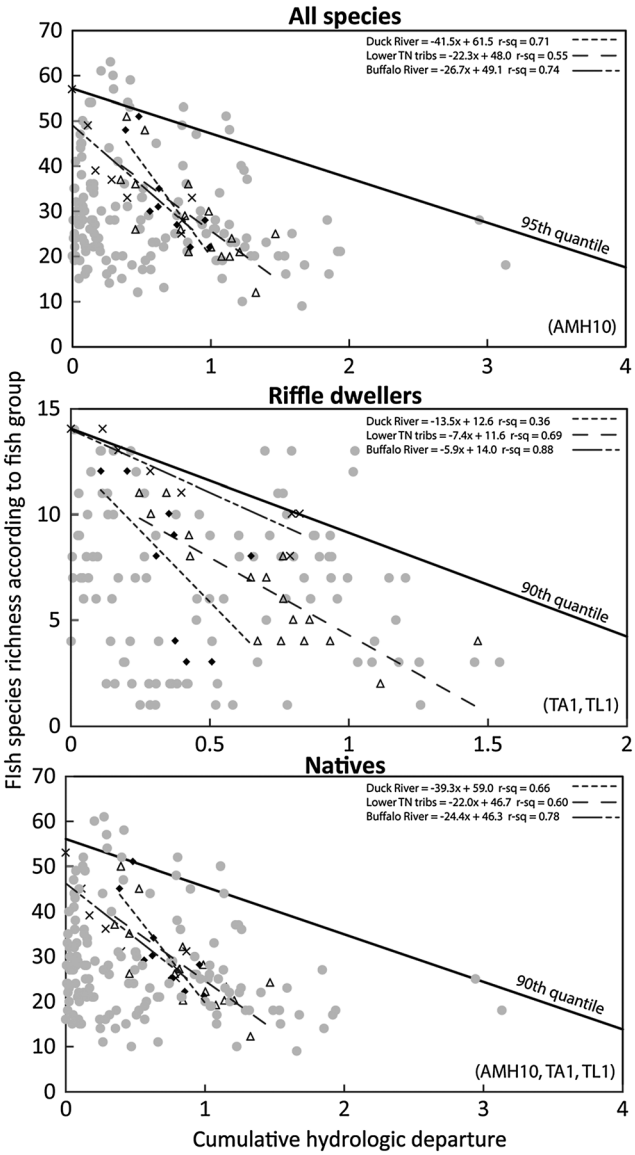

Figure 8. Wedge-shaped distributions and ecological limit functions (solid line) for all species, riffle dwellers, and natives in the Interior Plateau, and examples of internal structure based on specific river systems in the Interior Plateau, including the Duck River (diamonds), Buffalo River (X), and tributaries to the lower Tennessee (TN) River (triangles). Streamflow characteristics used in cumulative hydrologic departure, as per Table IV, listed in the lower right corner of each plot. See Table IV for slopes and intercepts of the ecological limit functions (regression quantiles) shown.

in the wedge distribution, other unmeasured environmental factors, such as water quality or habitat disturbance, may influence fish richness as much or more than hydrology. We explored such influences by statistically comparing environmental factors (physical basin attributes and indicators of human influence such as increased nutrient concentrations and population density) for 20 sites having the highest and lowest species richness and reference hydrology (hydrologic departure either zero or near zero) in each of the three ecoregions (Table V). In the Interior Plateau, total nitrogen concentration was higher at sites with lower species richness (Table V), indicating that increased nitrogen concentration may be a primary significant limiting influence on fish species richness at these sites. Eutrophication of streams indirectly affects fish communities via mechanisms including excessive algal and plant growth, decreased dissolved oxygen, and increased fine-grain sediment deposition (Dubrovsky *et al*., 2010). In the Ridge and Valley, human population density is elevated at sites with low species richness and otherwise reference hydrology. Shifts in human population densities could cause changes in water quality (temperature, bacteria, sediment, and nitrogen input) that affect aquatic ecosystem health. In all ecoregions, the basin area was statistically larger for sites having the highest species richness as compared with sites with lower species richness (Table V). The relationship between basin area and species richness is not surprising and is well documented in field studies (Foltz, 1982).

We accounted for basin area during the development of the regional regression equations used for predicting SFCs because, similar to ecological processes, many hydrologic processes are also scaled by basin area. Nevertheless, we examined the internal structure of wedge-shaped distributions for clustering according to basin area. In general, wedge-shaped distributions relating all-species richness to hydrologic departure did not display distinct clustering according to basin area. However, in the Ridge and Valley and Blue Ridge, the sites with the highest species richness were typically larger basins, although low and moderate species richness was observed in other large basins. In contrast, within the Interior Plateau, all of the sites with drainage areas greater than 2500 km^2 ($n = 6$) showed high species richness and minimal hydrologic departure (upper left portion of wedge). If this cluster were removed from the analysis, the apparent effect on the ecological limit function would be minimal. In general, small basins in both the Interior Plateau and Ridge and Valley appear to be constrained by an upper bound in species richness, with a few outliers, across the range of hydrologic departure. In the Ridge and Valley for example, sites with basin areas less than 26 km^2 typically have fewer than 15 species, but several sites with reference or near-reference hydrology have 40 species (the maximum species richness for basins of any size in the Ridge and Valley was 52 species). All sites in the Interior Plateau with basin areas less than 100 km^2 generally had fewer than 40 species, although species richness for basins of any size in the Interior Plateau was as great as 63 species.

INTEGRATING FLOW ECOLOGY RESPONSE WITH MANAGEMENT ACTIONS

A central challenge for scientists and water resource managers is how to apply scientific understanding of ecological flows to

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Table V. Wilcoxon rank sum test between 20 sites with most and least species richness, with otherwise reference hydrology, according to province.

Environmental factors	Environmental factor (median value)		Wilcoxon rank sum test (*p*-value)
	Lowest species count	Highest species count	
	Blue Ridge		
Nitrogen (mg l^{-1})	0·44	0·53	0·1484
Phosphorus (mg l^{-1})	0·03	0·04	0·0687
Road crossing density (crossings km^{-2})	0·44	0·93	0·0538
Population density (persons km^{-2})	16	23	0·1042
Mean elevation (m)	805	750	0·1972
Drainage area (km^2)	**88**	**808**	**0·0008**
	Ridge and Valley		
Nitrogen (mg l^{-1})	1·07	0·95	0·2159
Phosphorus (mg l^{-1})	0·06	0·07	0·697
Road crossing density (crossings km^{-2})	1·01	1·15	0·925
Population density (persons km^{-2})	**79**	**21**	**0·0014**
Mean elevation (m)	424	376	0·2465
Drainage area (km^2)	**91**	**308**	**0·0101**
	Interior Plateau		
Nitrogen (mg l^{-1})	**1·15**	**0·95**	**0·0074**
Phosphorus (mg l^{-1})	0·27	0·44	0·629
Road crossing density (crossings km^{-2})	0·57	0·72	0·1216
Population density (persons km^{-2})	22	15	0·1413
Mean elevation (m)	239	265	0·1156
Drainage area (km^2)	**278**	**386**	**0·0009**

Values in bold are significant at the indicated *p*-value.

the development of practical resource management tools. Ecological limit functions, such as the ones developed in this paper, are commonly presented as such a tool; however, using ecological limit functions to make decisions about proposed hydrologic changes to a stream or watershed is not straightforward and may not be as informative as assumed. The prevailing hydrologic conditions of a gauged or ungauged location must first be determined, and the proposed change in hydrology must be quantified in terms of alteration to the flow regime. Furthermore, the ecological consequences of proposed hydrologic alterations need to be addressed not only for sites with optimal ecology (high species richness) near the upper bound (85th, 90th, or 95th quantile) of ecological limit functions but also for sites in the interior of the wedge distribution. This need presents a practical question: If ecological conditions are suboptimal (low species richness) for a given hydrologic condition, how will the fish community respond to further hydrologic alteration? Addressing this question requires that hydrologic alteration first be quantified. Within the analytical framework proposed in this paper, such quantification means determining the difference in hydrologic departure between current and proposed conditions.

Once hydrologic alteration is adequately quantified, the question of how to estimate the resulting response in fish species richness remains, particularly for previously altered sites with already diminished fish species richness or for those lacking ecological observations. As demonstrated in this paper, ecological limit functions are typically developed using wedge-shaped distributions. For observations on or near the upper bound of such a distribution, the expected ecological response to a given hydrologic alteration would be to simply decrease species richness according to the slope of that upper bound (the 85th, 90th, or 95th quantile in this study). When ecological observations indicate suboptimal species richness and the observation falls below the upper bound, the expected ecological response to altered hydrology is less obvious. Furthermore, if no current ecological data exist for a given site, understanding the probability of possible ecological responses to hydrologic alteration is not possible by interpreting the upper bound only.

Interpreting interior quantiles of wedge distributions for use in a management context has received relatively little attention in the flow ecology literature, but such interpretation is important to the practical application of ecological limit functions to resource management. The statistical determination of additional, interior quantiles moves beyond an upper bound relationship to provide a more detailed and potentially more useful approach to interpreting wedge distributions. Wedge distributions occur in part because, although increases in hydrologic departure decreases fish species richness to some extent, other unmeasured environmental influences (limiting factors), such as nutrient

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concentrations, drainage area, water temperature, and habitat availability, also potentially limit species richness. These other limiting factors may or may not interact with streamflow (Thomson *et al*., 1996; Cade *et al*., 1999; Schmidt *et al*., 2012). In an examination of multiple limiting factors on aquatic invertebrates, Schmidt *et al*. (2012) argued that quantile lines with statistically significant slopes define an analytical space where the explanatory variable in the quantile regression equation can be considered a primary limiting factor. Thus, the response variable reacts to changes in this factor if it lies between the highest and lowest statistically discernible quantile slopes.

Additionally, as the magnitude of a quantile slope decreases, other unmeasured environmental factors have an increasing influence on the response variable (Cade *et al*., 1999; Schmidt *et al*., 2012); if a quantile line has a zero or near-zero slope, the explanatory variable has little or no influence on the response. The response variable is influenced by the interaction between the explanatory variable and other limiting factors when successive quantile lines with heterogeneous slopes are present (Qian and Cuffney, 2012; Schmidt *et al*., 2012). Near-parallel quantile lines indicate no interaction, although other limiting factors may still have an additive effect on the response (Cade *et al*., 1999). We demonstrate the application of these concepts using as examples the ecological limit functions for omnivorous fish in the Ridge and Valley and all species in the Interior Plateau (Figure 9).

Interpretation of the relationship between omnivorous fish and hydrologic departure (Figure 9) might only consider the highest quantile with the most statistical significance. For omnivorous fish in the Ridge and Valley, this upper bound (85th quantile) indicates that for a given site with zero hydrologic departure, there is an 85% probability that five or fewer omnivorous species are present. As hydrologic departure increases, the upper bound shows that the likelihood of maintaining five species decreases. The exclusive use of the upper bound limit function provides information about the potential species richness for a given site, essentially giving a 'best possible' scenario, which may not provide resource managers with the information needed to make a decision about a proposed change in the watershed or stream. Therefore, in addition to the upper quantile (85th), interior quantiles (10th–80th, in intervals of 10%) for this distribution were also determined. Although only two additional quantiles (30th and 80th) were statistically significant for omnivorous fish in the Ridge and Valley, these interior quantiles provide some information about the influence of hydrologic departure on species richness for sites with suboptimal richness. The near-parallel slopes of the significant quantile lines suggest no interaction with other environmental factors although an additive effect is possible (Cade *et al*., 1999). For a site with no measured

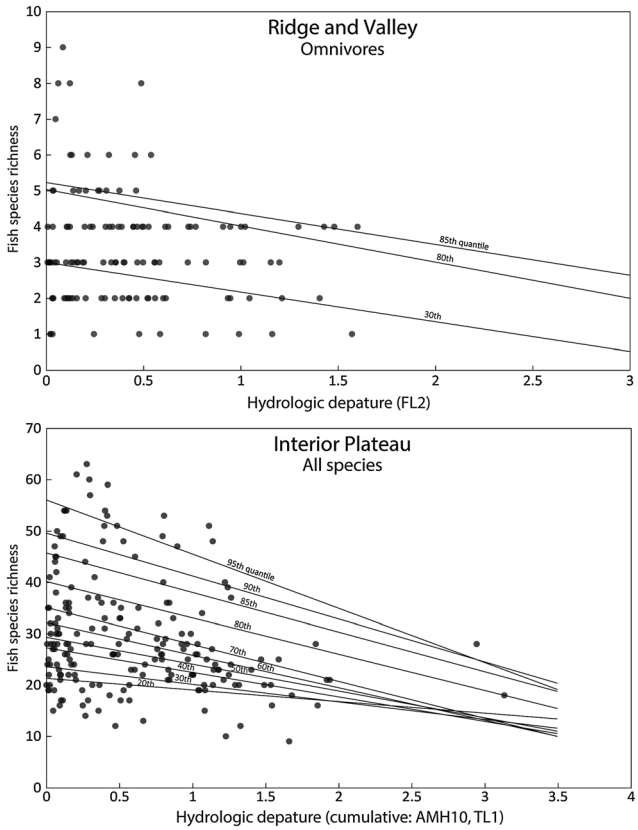

Figure 9. Comparison of statistically significant quantile regression lines ($p < 0{\cdot}05$) for cumulative departure versus (a) omnivore species richness in the Ridge and Valley and (b) all species richness in the Interior Plateau.

ecological data, the interior quantile lines probabilistically describe a range of possible ecological outcomes for a given hydrologic departure. When measured ecological data are available under current hydrologic conditions, this information can serve as a starting point for management decisions, and the potential ecological response due to hydrologic alteration can be estimated by interpolation between statistically significant interior quantile lines.

For total fish species richness in the Interior Plateau, upper bound and interior quantiles provide information about the influence of hydrologic alteration on sites with optimal or suboptimal fish species richness (Figure 9). Hydrologic departure was statistically significant for total species richness for all of the estimated interior quantiles, except the lowest quantile (10th). The greater number of significant interior quantiles provides additional structure to the wedge distribution and allows for greater interpolation accuracy. Quantile lines for total species richness in the Interior Plateau are not parallel and generally converge with increasing hydrologic departure (Figure 9), suggesting that other environmental factors are interacting with hydrology to influence total species richness. In the Interior Plateau, nitrogen concentration may be one environmental factor interacting with hydrology to decrease species richness (Table V). The low

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slopes (20th and 30th quantiles) may reflect an increased influence of nitrogen concentration (or another unmeasured limiting factor) on low species richness.

CONCLUSIONS

The analyses presented in this paper lead to the following conclusions:

- Hydrologic reference conditions can be defined using landscape attributes, particularly when the attribute exhibits a variance such that reference sites are distinct from other sites.
- Statistically discernible functions relating fish species richness and hydrologic departure can be defined using one to four SFCs at the scale of Level III Ecoregions.
- Wedge-shaped distributions underlying ecological limit functions identified in this study are not the result of internal structure, indicating regional applicability.
- Ultimately, characterizing the unique role of hydrology in the management of aquatic ecosystems will require an approach that accounts for multiple limiting influences and interactions among hydrology, water quality, and physical basin attributes.

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