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Abstract

An understanding of how fish communities differ among river basin, watershed, and stream reach spatial scales and the factors that influence these differences can help in the design of effective conservation programs and the development of reference models that appropriately represent biota under relatively undisturbed conditions. We assessed the heterogeneity among fish assemblages in first- to fourth-order stream sites from four river basins (Savannah, Chattahoochee, Cape Fear, and Pee Dee rivers) within the Sand Hills ecoregion of the southeastern USA and compared it with the heterogeneity associated with watershed and stream reach spatial scales. Fifty-five species of fish representing 15 families were collected by electrofishing, with the most speciose families being Cyprinidae, Centrarchidae, Percidae, Ictaluridae, and Catostomidae. Constrained ordination identified clearly demarcated species assemblages among river basins as well as subbasin environmental variables that affected fish species

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composition; the amounts of variance attributable to basin, watershed, and stream reach spatial scales were roughly equivalent. Prominent differences occurred between Gulf of Mexico coast and Atlantic coast river basins, but differences among Atlantic coast basins were also apparent. Key variables at the watershed scale included watershed size, relief, extent of anthropogenic disturbance, and forest cover; key variables at the stream reach scale included instream habitat quality, proximity to a larger stream, and stream width. Fish assemblage collective and functional properties were more strongly influenced by variables acting at watershed and stream reach spatial scales than by differences among basins. Species richness peaked at intermediate levels of habitat quality as a likely result of biotic homogenization, indicating that the least disturbed sites within the region do not necessarily possess the highest species richness. Failure to consider this may lead to the overrating of moderately disturbed sites and the underrating of minimally disturbed sites, thus contributing to false conclusions about fish assemblage integrity.

Instream ambient conditions strongly influence stream fish species composition; however, such conditions are affected by factors operating at the watershed scale and larger spatial scales. Thus, the assembly of fish communities has been conceptualized as a series of different processes and events operating at spatially hierarchical scales that sequentially exclude species from the historically available species pool based on ranges of tolerance, habitat requirements, behaviors, and natural history factors (Tonn 1990; Jackson et al. 2001). Each scale is characterized by selective forces that "filter" species based on adaptive functional traits (Poff 1997). The environmental factors that operate at different scales vary in intensity depending on geologic history, dispersion barriers, and other factors that affect colonization; climatic factors; landscape and edaphic factors; and a variety of other influences (Tonn 1990). The extent to which larger-scale factors (e.g., geology, topography, and watershed) influence smaller-scale features (e.g., substrate composition, instream structure, and mesohabitat development) also varies among geographic areas and is further affected by anthropogenic activities that have altered the landscape (Richards et al. 1996).

The recognized importance of local factors as an abiotic habitat template has been the impetus for studies concerning the effects of instream habitat on fish assemblage structure and the development of habitat evaluation protocols for identifying anthropogenic disturbances (Barbour et al. 1999). However, increasing recognition of influences at the watershed scale and larger scales has contributed to a growing number of studies on the effects of landscape and an effort to identify the relative importance of factors operating at different spatial scales. Such studies often compare the influences of multiple-scale factors, including instream habitat, watershed land cover and morphometry, longitudinal position on the stream continuum, river basin, and ecoregion, with differing results. Grubbs et al. (2007) found that watershed area and stream size had greater effects on fish assemblage structure than watershed land use and local water chemistry in the upper Green River basin of Kentucky. Pease et al. (2011), working in two large basins and across three ecoregions in central Texas, detected strong ecoregion effects but weak river basin effects as well as influential local-scale instream factors and watershed-scale

land use factors. In contrast, Kaller et al. (2013) reported that fish assemblages in Louisiana coastal plain streams differed more among basins than among ecoregions. Marsh-Matthews and Matthews (2000) found that fish assemblages in the Midwestern USA were more strongly affected by broad geographic factors than by instream habitat. These and other studies have also observed considerable unexplained variance in community composition, which may be partly attributable to the interdependence between factors operating at different scales. These differing findings may be related to differences in (1) the current and historical operation of factors that interact to affect fish assemblage structure in different study areas; (2) the scales at which the studies were conducted; and (3) the variables that the researchers chose to study and the way in which those variables were measured, organized, and analyzed.

In addition to taxonomic composition, considerable interest has been directed toward the functional and collective properties of fish assemblages (e.g., species richness and trophic guild structure) because they reflect functional evolutionary responses to prevailing adaptive pressures and are sensitive to anthropogenic disturbance. For the latter reason, these variables constitute many of the metrics used in bioassessment protocols. Relative to taxonomic composition, functional and collective properties may be more closely related to environmental factors and less closely related to geography because they are less influenced by historical factors that affect species distributions.

The Sand Hills region is an important ecoregion in the southeastern USA that covers about 20,600 km^2 and consists of the inland coastal plain bordering the Fall Line (Markewich and Markewich 1994; Schmidt 2013). The Sand Hills region is characterized by deep sands with clay and silt and is dominated by longleaf pines *Pinus palustris* and Turkey oaks *Quercus cerris*. Most of the larger streams are "blackwater" streams: low gradient, slow flowing, and fed by water seeping through the sandy soils that underlie floodplains and swamps. The water is stained by decaying organic matter, usually acidic, and contains little sediment (Sabater et al. 1993; Carlough 1994). Snags and other large woody material form debris dams that affect detrital dynamics and geomorphology and provide important aquatic habitat (Benke and Meyer 1988). Blackwater streams are ecologically important and

constitute the most characteristic stream type of the south-eastern coastal plain. Within the Sand Hills are numerous habitat and environmental factors (e.g., stream size, channel morphometry, instream structure, and water chemistry) that influence the composition of stream fish assemblages (Sheldon 1968; Paller 1994).

Here, we present findings from a study on fish assemblage structure in Sand Hills streams. Our first objective was to assess the spatial heterogeneity in species composition among river basins and compare it with the heterogeneity associated with natural and disturbance-related factors acting at the subbasin watershed scale and the stream reach scale. We hypothesized that significant heterogeneity would be asso-ciated with (1) the basin scale because of zoogeographic factors and (2) the subbasin scale because of local habitat gradients, especially those associated with stream size and anthropogenic disturbance. An understanding of how fish communities differ among ecoregion, river basin, and subba-sin scales and the variables that influence these differences can assist in the design of effective conservation programs for Sand Hills fishes. In addition, knowledge of natural gradients and geographic factors that organize species assemblages con-tributes to the establishment of appropriate reference models (Stoddard et al. 2006) representing the biota that would be expected under relatively undisturbed conditions. Such models are needed to define thresholds for biological impairment and to measure the extent to which anthropogenic disturbances cause deviations from a natural state (Bailey et al. 2004). Reference models are often developed on an ecoregion basis in the southeastern USA and elsewhere (Hughes and Larsen 1988; Feminella 2000), but landscape classifications may account for less biological variation than factors acting at smaller spatial scales (Hawkins et al. 2000). Such factors may have to be included as covariates in assessment protocols to avoid conflating natural and disturbance-related variance.

Our second objective was to compare spatial heterogeneity in species composition with spatial heterogeneity in the collec-tive and functional properties of fish assemblages. We hypothe-sized that basin-scale differences in species composition within the Sand Hills would be relatively large due to the influence of historical factors and the relative isolation of river basins within this region but that collective and functional properties would show less basin-scale variation due to regional similarities in habitat and resource availability and the similar adaptive responses of fishes to these conditions. Differences between patterns in species composition and patterns in collective and functional properties can be important for the development of bioassessment protocols that employ fish assemblage metrics. Basin-scale differences in species occurrences may limit the regionwide applicability of metrics based on species composi-tion and may favor the use of collective and functional proper-ties that exhibit greater regional uniformity.

Our third objective was to assess changes in species rich-ness and composition across a gradient of anthropogenic

habitat disturbance. Although severe disturbance often results in the loss of sensitive species, moderate disturbance can increase species richness, as indicated by Connell (1978), who showed that richness is maximized when ecological dis-turbance is neither too rare nor frequent (i.e., the "intermediate disturbance hypothesis") because species of early and late successional stages coexist. Higher numbers of species at moderately disturbed sites could also be a manifestation of biotic homogenization—the increased similarity in biotas caused by the supplementation and eventual replacement of native species with nonindigenous species (Rahel 2000; Scott and Helfman 2001)—which can result in an initial increase in the number of species followed by a decrease as degradation worsens and as endemic species become extirpated. Biotic homogenization can result in greater assemblage similarity among sites and a subsequent decrease in beta diversity (β; McKinney and Lockwood 1999). Therefore, we hypothesized that the relationship between species richness and disturbance in Sand Hills streams would be curvilinear rather than linear, with least disturbed streams that support only endemic species having lower richness than moderately disturbed streams that support endemic species as well as generalist and nonnative species. If so, this can have important consequences for bioas-sessment protocols, which often assume that species richness is directly related to a lack of disturbance.

METHODS

Study Area

Study sites included first- through fourth-order wadeable streams in the Sand Hills (level IV) ecoregion, extending from west-central Georgia to south-central North Carolina (Griffith et al. 2001). The Sand Hills have been extensively modified since presettlement times (Loehle et al. 2009) and now consist of a mosaic of agricultural lands, urban areas, public natural areas, and military training facilities. Most sample sites were in lightly populated, largely forested, publically owned lands or protected private holdings with a smaller anthropogenic "footprint" than the surrounding land, as shown by satellite photographs. We sampled 69 sites distributed over four Department of Defense installations; one Department of Energy installation; and four state, federal, and private hold-ings, including Fort Bragg and Camp McKall (North Carolina), Fort Benning (Georgia), Fort Gordon (Georgia), the Savannah River site (South Carolina), The Nature Conservancy holdings near Fort Benning, Manchester State Forest (South Carolina), Sand Hills State Forest (South Carolina), Carolina Sand Hills National Wildlife Refuge (South Carolina), and Sand Hills Gamelands (North Carolina; Figure 1). The sites were in four major river basins: the Chattahoochee River (15 sites), Savannah River (25 sites), Pee Dee River (8 sites), and Cape Fear River (21 sites) basins. The Chattahoochee River basin is part of the larger Apalachicola–Chattahoochee–Flint River basin, which drains
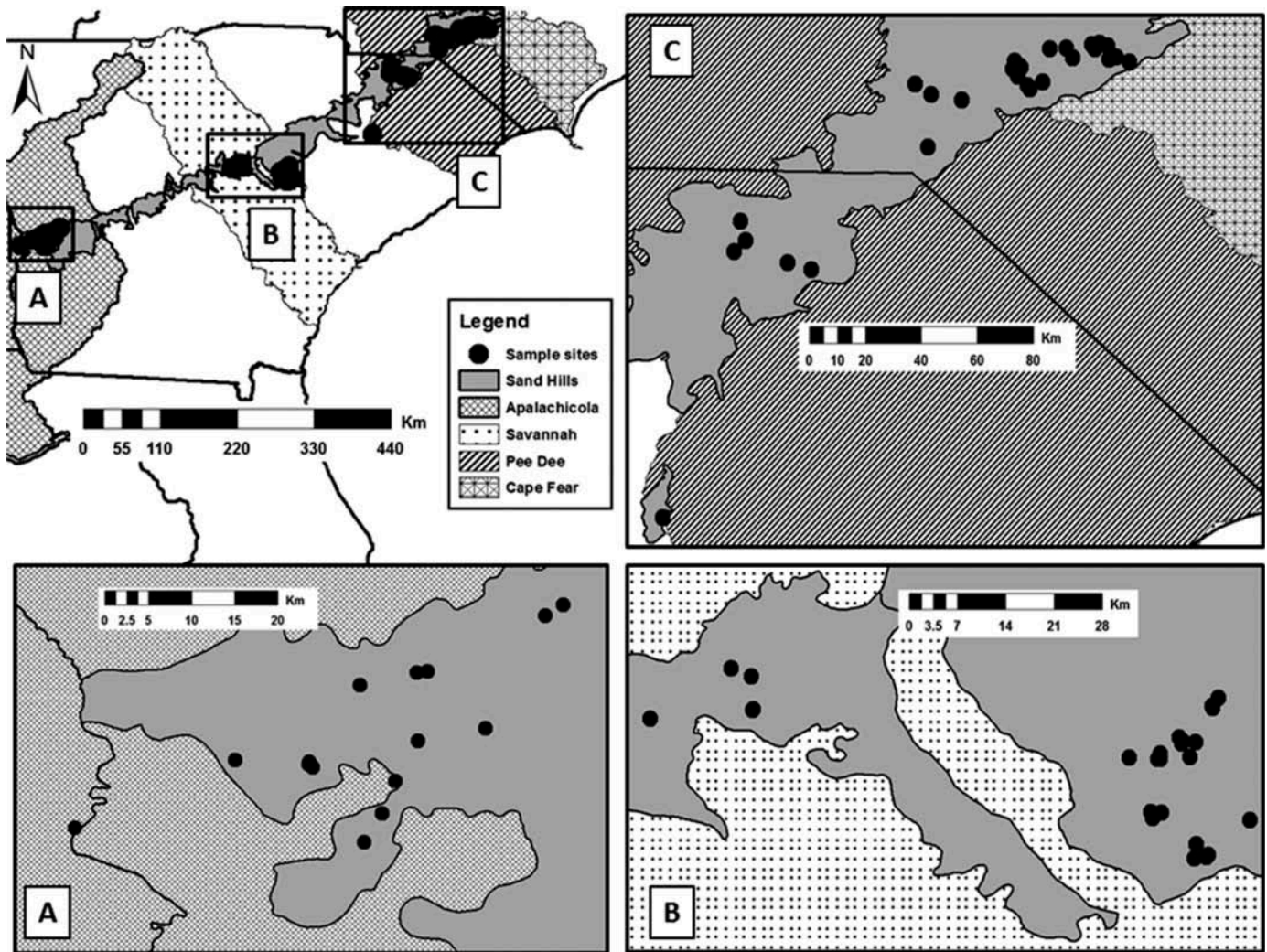
FIGURE 1. Fish assemblage sampling sites located in the Chattahoochee, Savannah, Pee Dee, and Cape Fear River basins within the Sand Hills ecoregion.

into the Gulf of Mexico (hereafter, Gulf), whereas the other three drainages are on the Atlantic slope.

Most of our sample sites represented "least disturbed" conditions within the region, although a lesser number represented disturbed sites. Least disturbed sites were discriminated from other sites based on a disturbance gradient that was derived from a principal components analysis (PCA) of abiotic variables associated with anthropogenic stress, including a habitat quality score, erosion score, channel modification score, bank height, paved road area, unpaved road area, number of road crossings, watershed disturbance, and percentage of bare ground (Kosnicki et al. 2014). The cutoff on the gradient for separating least disturbed sites from other sites was the inflection point derived from the second derivative of a spline function of ranked values. This method indicated that 14 of the 69 sites were disturbed. The disturbance variables and the PCA methodology are described fully by Kosnicki et al. (2014).

Watershed Assessment

"River basin" refers to the large watersheds that constituted each of the four previously mentioned river systems. Within these basins were smaller watersheds and portions of watersheds corresponding to the catchments located upstream of individual sample sites; hereafter, these are referred to as "watersheds." High-resolution (HUC-12) watershed maps generated from digital elevation models (created from light detection and ranging [LIDAR] data) and the National Land Cover Database (U.S. Geological Survey) were used to compute the amount of disturbed land for each watershed, including lands that were characterized by low-, medium-, and high-intensity development plus cultivated, pasture, and bare lands. Other variables computed from the watershed maps included watershed area, watershed perimeter, drainage density, watershed length and maximum elevation, elevation of the stream mouth, basin relief, basin relief ratio, entire stream

gradient, drainage direction, sinuosity, percent land cover types, bifurcation ratio, cumulative stream length, stream order, stream magnitude, and length of main-stem tributaries.

Instream Habitat Assessment

Instream habitat at each site was assessed in two ways. In the first method, variables including epifaunal substrate quality, pool substrate quality, pool variability, sediment deposition, channel flow status, channel alteration, channel sinuosity, bank stability, vegetative protection, and riparian vegetation were each rated on a scale of 1 (poor) to 20 (optimal) for each sample site by the same observer and were summed to provide a summary measure of instream habitat quality (SCDHEC 1998). In the second method, 19 instream habitat attributes were either measured or estimated along a transect that was situated perpendicular to the direction of water flow at the downstream end of each electrofishing sample segment for a total of 14–21 transects/site. Measured variables included maximum depth, stream width, and left- and right-bank heights from the water level to the top of the bank. Estimated variables were assessed visually within a 1.0-m band (0.5 m below and 0.5 m above each transect). The estimated variables included left- and right-bank angles (estimated to the nearest 5°); bank vegetative cover (nearest 5%) within 5 m on either side of the stream; percent areal coverage (nearest 5%) of the substrate by macrophytes, overhanging vegetation, root mats, coarse woody debris (diameter > 3 cm), and small woody debris (diameter < 3 cm); and undercut banks (percentage of the total wetted stream width under a bank; nearest 5%). The percent areal coverage (nearest 5%) of clay, silt/muck, sand, gravel, and rocks (>30 mm) was visually estimated, as was the streambank and riparian zone vegetation coverage (nearest 5%). Riparian zone vegetation was categorized as hardwood, pine, shrub, or herbaceous and was ranked according to dominance. A qualitative score of 0 (none) to 3 (high) was given for bank erosion based on the extent of erosional scarring, bank instability, and bank material entering the stream. Artificial channel modification received a score of 0 (none) to 3 (high). Mesohabitat type was designated as riffle, run, shallow pool, or deep pool.

Fish Assemblage Assessment

Electrofishing was conducted during the late spring, summer, and fall of 2009–2012. A representative stream reach averaging about 200 m and varying from 140 m for the smallest stream to 285 m for the largest stream was electrofished at each sample site. Longer reaches were sampled in larger streams to better represent all habitats and to increase the likelihood of capturing all species. Each reach was divided into contiguous 10–15-m segments (with longer segments in longer reaches) to quantify species–reach length relationships (described later). Block nets were not used because of the habitat disturbance associated with setting them and the difficulty of keeping them in place. Field observations indicated that most fish exhibited little upstream or downstream movement during sampling. Fish were collected by using one or two Smith-Root LR-24 backpack electrofishers that were calibrated with the "Quick Setup" function, sometimes followed by adjustments to optimize collection efficiency and to minimize mortality. Two crew members with one electrofisher sampled streams with widths less than about 3.5 m; three crew members and two electrofishers were used to sample streams that were about 3.5–6.0 m wide; and four crew members and two electrofishers were employed for sampling larger streams. Effort was increased with stream size to maintain sampling efficiency. All microhabitats and macrohabitats were sampled, including riffles, pools, runs, snags, logs, root mats, and undercut banks. All fish that were collected from each segment were identified and counted. Fish that were not readily identifiable in the field were preserved in a 10% solution of formalin and were later identified at the Georgia Museum of Natural History, Athens. Total shock time (duration for which the current was directed into the water) was commensurate with stream size and averaged 75 min (SD = 40; range = 20–224 min) for the first pass and 56 min (SD = 29; range = 12–163 min) for the second pass. Both passes were made by crews moving in an upstream direction.

Statistical Methods

The statistical methods were designed to address our three objectives: (1) to assess spatial heterogeneity in species composition; (2) to assess spatial heterogeneity in collective and functional properties; and (3) to assess changes in species richness and composition across a gradient of habitat disturbance.

Spatial heterogeneity in species composition.—Relationships between fish assemblage composition and environmental variables measured at different spatial scales were analyzed with canonical correspondence analysis (CCA), a constrained ordination technique that elucidates the relationships between species scores and explanatory environmental variables (conducted with CANOCO version 4.5; Ter Braak and Šmilauer 2002). Before conducting the CCA, we used detrended correspondence analyses (DCAs) to estimate gradient lengths in the fish assemblage data and to determine whether CCA was appropriate. The length of the longest DCA axis provides an estimate of species turnover within the study area (Lepš and Šmilauer 2007). Unimodal ordination methods like CCA are appropriate when environmental gradients are relatively long and when species turnover is high, as indicated by a first DCA axis length greater than 4.0. The length of the first DCA axis in the fish assemblage data was 4.65. The abundance × species data matrix used for CCA was centered and $\log(x + 1)$ transformed prior to analysis to de-emphasize the influence of highly abundant taxa; species that appeared only once were eliminated because they contributed little to the analysis.

Statistically significant ($P \leq 0.05$) PCA axis scores were used as predictive environmental variables in the CCA. The PCA axes summarized information from related environmental variables and were assigned meaning based on variable loadings (Pearson's product-moment correlations between the axis scores and individual environmental variables). We used this approach because the number of unsummarized environmental variables was large relative to the number of sample sites—a situation that can lead to model overfitting and multicollinearity. The significance of the PCA axes was assessed by comparing the PCA eigenvalues with eigenvalues that were generated by null models (McCune and Grace 2002). The habitat variables were divided into three groups for separate PCAs: watershed size and shape, watershed land cover, and instream habitat. Differently scaled environmental variables were normalized by analyzing correlation matrices. Other variables in the CCA (in addition to PCA-derived variables) included basin identity (the four basins under study, represented by dummy variables), the instream habitat quality score derived from the South Carolina Department of Health and Environmental Control (SCDHEC 1998) protocol, stream width, and connectivity with a larger stream. The latter variable was binary and was based on proximity to a confluence with a larger stream that could serve as a source of colonists. A sample site located in a small stream (average width < 1.5 m) was considered connected with a larger stream (i.e., at least one stream order larger) if it was less than 250 m from the confluence with the larger stream; a sample site located in a large stream (average width > 1.5 m) was considered connected if it was located within 750 m of a larger stream. Rationales for these distances will be presented later. Stream width (a surrogate for stream size) and stream connectivity variables were explicitly included in the CCA because of their influence on fish assemblage structure (Horwitz 1978; Paller 1994; Hitt and Angermeier 2011). The highest Pearson's r-value among the preceding variables was 0.71, indicating that all variables contributed unique variance to the analysis.

The CCA was first conducted by using an automated forward selection procedure to rank the individual variables in order of importance and then by using variance partitioning methods (Jongman et al. 1995; Lepš and Šmilauer 2007) to assess the amount of unique variance associated with basin, watershed, and instream habitat variables (or groups of variables). Monte Carlo permutation tests determined the significance of individual variance components, making it possible to compare the importance of basins with subbasin environmental factors.

Spatial heterogeneity in collective and functional properties.—We calculated 22 collective and functional properties that have been used in bioassessment frameworks developed for the Sand Hills (Paller et al. 1996; Barbour et al. 1999; Schleiger 2000; Morris et al. 2007; Hain et al. 2012). These properties assessed numerical and proportional abundances and species richness:

1. Numerical abundance (all normalized to 200 m): total number of fish (N_{tot}), number of centrarchid individuals (N_{cen}), number of cyprinid individuals (N_{cyp}), number of insectivorous fish (N_{ins}), and number of tolerant fish (N_{tol}).
2. Proportional abundance: percent benthic fluvial specialists (P_{bfs}), percent generalized insectivores and herbivores (P_{ghr}), percent insectivorous cyprinid individuals (P_{inc}), percent cyprinid individuals (P_{cyp}), percent sunfish *Lepomis* spp. individuals (P_{sf}), percent lithophilic spawners (P_{lth}), percent tolerant fish (P_{tol}) and percent omnivores (P_{omn}).
3. Species richness: number of darter species (R_{drt}), number of insectivorous cyprinid species (R_{inc}), number of sensitive species (R_{sen}), number of sucker species (R_{suc}), number of sunfish species (R_{sf}), number of cyprinid species (R_{cyp}), number of tolerant species (R_{tol}), and total number of species (R_{tot}).

The data on collective and functional properties were analyzed using redundancy analysis (RA), a constrained ordination technique similar in purpose to CCA. We avoided the use of CCA because it is inappropriate when response variables have different units (see Lepš and Šmilauer 2007). The collective and functional property variables were centered and standardized before RA because they were scaled differently. The RA employed the same explanatory environmental variables and same approach used in the CCA of fish assemblage composition.

Changes in species richness and composition across a gradient of habitat disturbance.—The division of sample sites into segments permitted us to estimate true species richness at each site from the patterns of species accumulation with the addition of sample segments. (Colwell and Coddington 1994; Colwell et al. 2004). Estimated richness more accurately approximates true richness and is typically higher than observed richness, which is usually an underestimate due to the dependency of richness on sample area and other factors. We used a first-order jackknife procedure (McCune and Grace 2002) for estimation because it is appropriate when the number of sample subunits is comparatively small and because preliminary work showed that it produced accurate results. The number of species in each segment at each site was the sum of species collected from the segment during both passes.

We used general linear models (GLMs; SYSTAT version 12, SYSTAT Software 2007) to assess the effects of disturbance and other environmental factors on estimated species richness. Independent variables included measures of stream size (average width, depth, and cross-sectional area; and Strahler stream order), basin identity (a categorical variable that represented each basin), the PCA-derived summary variables, connectivity, and instream habitat quality. We also included a squared instream habitat quality term to test whether the relationship between species richness and instream habitat quality was curvilinear, as might be expected

if moderate levels of disturbance were associated with an increase in the number of species. We relied on theoretical considerations rather than automated procedures to select variables for inclusion in the final model based on their statistical significance at $P \leq 0.05$. Proportions of the variance in species richness explained by individual independent variables were computed by leaving each variable out of the model and noting the resulting change in the R^2 value.

For each site, we computed β in accordance with Whittaker (1960): β = γ/α, where γ = total species diversity for all sites within each basin; and α = the species diversity at each site. We divided the sample sites into three groups of equal size corresponding to high, medium, and low levels of instream habitat quality. We then tested for differences in β among groups with one-way ANOVA followed by pairwise Holm–Sidak tests. This allowed us to determine whether higher species richness at sites of moderate habitat quality was associated with lower β and, by inference, biotic homogenization. Data were $\log_{10}$ transformed to meet the assumptions of normality and homoscedasticity.

Lastly, we used indicator species analysis to identify species that were characteristic of disturbed conditions by comparing fish assemblage composition between the 14 disturbed sites (identified by the previously described PCA gradient approach) and the other sites. Indicator species analysis assigns indicator values to species based on differences in relative abundance and frequency of occurrence and tests their significance by using a Monte Carlo procedure (McCune and Grace 2002).

RESULTS

Habitat Principal Components Analysis

The first and second axes of the watershed morphometry PCA were significant ($P = 0.001$) and explained 38.0% and 21.9%, respectively, of the variance in the watershed morphometry variables. Variables with the strongest influence on axis 1 were related to watershed size and stream length, and variables with the strongest influence on axis 2 were related to watershed elevation and relief (Table 1). The first three axes of the land cover PCA were significant ($P < 0.001$) and explained 25.3, 16.9, and 14.2%, respectively, of the variance in the land cover environmental variables. The first axis reflected anthropogenic development in the watershed; the second axis represented a gradient of vegetation type, with pine forests on one end and deciduous trees, scrubs/shrubs, and other vegetation on the other; and the third axis was correlated with the number of unpaved roads and the Maloney disturbance index (MDI; composite of unpaved roads and bare ground; Maloney et al. 2005; Table 2). The instream habitat PCA had three significant ($P < 0.001$) axes that explained 19.1, 13.0, and 11.0%, respectively, of the variance in the instream habitat environmental variables. The first axis represented a mesohabitat and substrate gradient extending from a greater frequency of runs with

TABLE 1. Variable loadings (Pearson's product-moment correlation coefficient r) on axes 1 and 2 of the principal components analysis (PCA) of sample sites based on variables representing basin size, shape, and relief in the Sand Hills ecoregion.

Variable	PCA axis 1	PCA axis 2
Cumulative stream length (km)	−0.96	−0.04
Stream length (km)	−0.90	−0.02
Drainage area (km²)	−0.89	0.28
Basin length (km)	−0.88	0.23
Drainage perimeter (km)	−0.65	0.32
Basin relief (m)	−0.63	−0.54
Basin high point (m)	−0.37	−0.85
Drainage density (m⁻¹)	−0.19	−0.56
Stream mouth elevation (m)	−0.05	−0.79
Tributary length (km)	0.00	−0.27
Sinuosity	0.06	−0.04
Stream gradient (m/km)	0.21	−0.55
Basin relief ratio	0.49	−0.66
Drainage shape	0.86	0.28

sand bottoms to a greater frequency of riffles and deep pools with more varied substrates, including gravel, silt, and small woody debris; the second axis represented a gradient of erosion, bank height (an indication of channel incision), and channel modification (with higher scores indicating a greater

TABLE 2. Variable loadings (Pearson's product-moment correlation coefficient r) on axes 1, 2, and 3 of the principal components analysis (PCA) of sample sites based on land cover variables in the Sand Hills ecoregion.

Land cover type	PCA axis 1	PCA axis 2	PCA axis 3
Developed, high intensity (%)	−0.89	0.08	0.08
Developed, medium intensity (%)	−0.96	0.06	0.04
Developed, low intensity (%)	−0.91	−0.12	−0.08
Developed, open space (%)	−0.96	−0.05	0.05
Cultivated (%)	−0.08	−0.64	−0.18
Pasture (%)	0.00	−0.62	−0.06
Grassland/herbaceous (%)	0.46	−0.17	−0.45
Deciduous trees (%)	0.07	−0.65	0.46
Evergreen forest (%)	0.40	0.80	−0.23
Mixed forest (%)	0.32	−0.47	0.29
Shrubs/scrubs (%)	0.09	−0.62	0.21
Palustrine (%)	0.25	−0.32	0.53
Water (%)	0.08	0.00	−0.39
Bare ground (%)	0.08	0.17	−0.36
Paved roads (%)	−0.87	0.09	−0.05
Unpaved roads (%)	−0.07	−0.52	−0.72
Road crossings	−0.02	0.04	−0.49
Maloney disturbance index (%)	−0.07	−0.52	−0.72

TABLE 3. Variable loadings (Pearson's product-moment correlation coefficient r) on axes 1, 2, and 3 of the principal components analysis (PCA) of sample sites based on variables representing instream habitat in the Sand Hills ecoregion.

Variable	PCA axis 1	PCA axis 2	PCA axis 3
Sand substrate (%)	0.78	−0.24	−0.05
Number of runs	0.64	−0.17	−0.53
Depth (m)	0.59	−0.18	0.10
Bank vegetative cover (%)	0.54	0.38	−0.20
Overhanging vegetation (%)	0.41	0.28	0.13
Large woody debris (%)	0.37	0.14	0.58
Macrophytes (%)	0.36	0.19	−0.33
Riparian shrubs (%)	0.36	−0.19	0.07
Root masses (%)	0.34	−0.06	0.39
Undercut banks (%)	0.30	−0.16	0.72
Bank angle (degrees)	0.17	−0.12	0.75
Number of shallow pools	−0.03	−0.06	0.38
Riparian pines (%)	−0.04	−0.09	0.24
Riparian hardwoods (%)	−0.06	−0.29	0.21
Channel modification (scale of 0–4)	−0.07	−0.61	−0.05
Clay substrate (%)	−0.10	−0.57	0.07
Erosion (scale of 0–4)	−0.39	−0.67	−0.30
Number of deep pools	−0.39	0.25	0.49
Bank height (m)	−0.42	−0.72	−0.04
Riparian vegetation, herbaceous (%)	−0.42	0.49	−0.28
Small woody debris (%)	−0.45	0.55	0.18
Silt/muck substrate (%)	−0.56	0.50	0.03
Rock substrate (%)	−0.56	−0.34	0.08
Number of riffles	−0.58	0.10	0.06
Gravel substrate (%)	−0.69	−0.18	0.10

prevalence of these features); and the third axis was strongly influenced by variables that reflected the occurrence of steep and undercut banks (Table 3).

Based on the preceding PCA axes, the following variables were used in the CCA of fish assemblage structure:

1. Watershed size (W_{size}): drainage basin size and stream length based on axis 1 of the watershed morphometry PCA (Table 1).
2. Watershed relief (W_{rel}): watershed elevation and stream gradient based on axis 2 of the watershed morphometry PCA (Table 1).
3. Watershed disturbance (W_{dist}): percentage of anthropogenically developed land in the watershed based on axis 1 of the land cover PCA (Table 2).

4. Watershed forest type (W_{pine}): from coniferous forest to deciduous forest and shrubs/scrubs, represented by axis 2 of the land cover PCA (Table 2).
5. Watershed MDI and road coverage (W_{rds}): the extent of roads and bare ground, represented by axis 3 of the land cover PCA (Table 2).
6. Stream substrate and mesohabitat (S_{drn}): from deep, sandy-bottom runs to shallower riffles with more diverse bottom types, represented by axis 1 of the instream habitat PCA (Table 3).
7. Stream erosion and incision (S_{eros}): the extent of erosion, channel incision, and channel modification, represented by axis 2 of the instream habitat PCA (Table 3).
8. Stream undercut banks (S_{unbk}): the extent of steep, undercut banks, represented by axis 3 of the instream habitat PCA (Table 3).

Spatial Heterogeneity in Species Composition

In total, 55 fish species representing 15 families were collected from the study area, with the most speciose families being Cyprinidae, Centrarchidae, Percidae, Ictaluridae, and Catostomidae (Table 4). Several species were ubiquitous, including the Yellow Bullhead, Redfin Pickerel, and Pirate Perch. Other species were more localized, as shown in a biplot derived from the CCA, which indicated distinct geographic differences in species composition: the Savannah and Chattahoochee River basins differed from each other and from the Cape Fear and Pee Dee River basins, which clustered together (Figure 2). The latter two basins lie in relatively close proximity on the Atlantic slope and occupied the northeastern part of the study area; the Savannah River basin, also on the Atlantic slope, occupied the central portion; and the Chattahoochee River basin, which drains into the Gulf, occupied the southwestern portion (Figure 1). The Gulf and Atlantic slope basins were partitioned on the x-axis, whereas the Savannah River basin was separated from the other Atlantic slope basins on the y-axis.

To describe these patterns further, the Chattahoochee River basin was distinguished by species that were restricted to the Gulf drainages, including the Broadstripe Shiner, Dixie Chub, and Southern Brook Lamprey. Seven of the eight species that clustered with the Chattahoochee River basin were not found on the Atlantic slope (Figure 2). The one exception, the Goldstripe Darter, occurs only as far north on the Atlantic slope as the Altamaha River drainage, adjacent to the Flint River (Gulf coast). The Savannah River basin was distinguished by the Yellowfin Shiner, Bluehead Chub, and Creek Chub. The Cape Fear River and Pee Dee River basins were distinguished by the Dusky Shiner, Margined Madtom, and Sandhills Chub. The sharing of species among the Atlantic slope basins was indicated by the overlap of those basins on the y-axis.
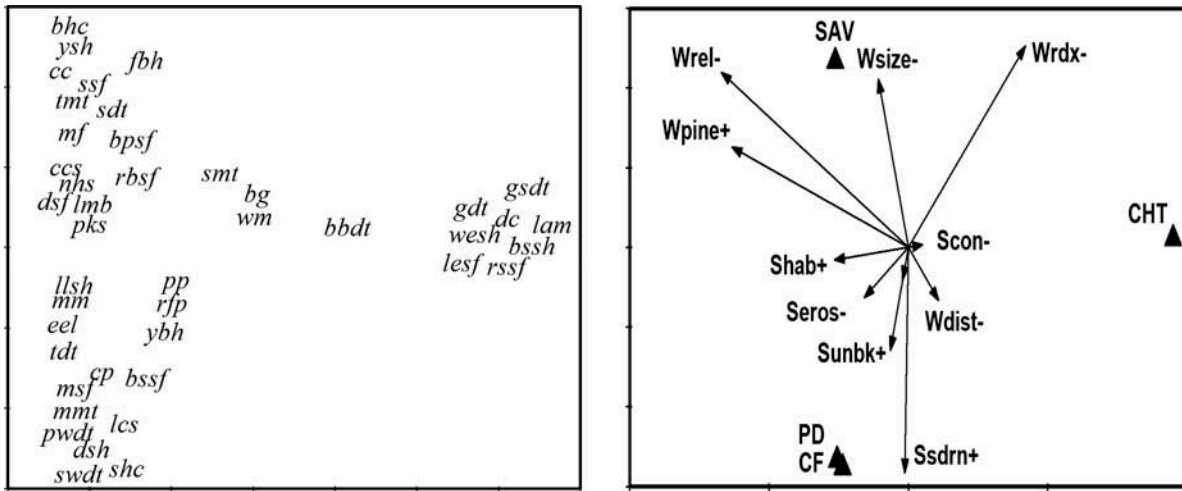
FIGURE 2. Canonical correspondence analysis plots of species based on fish assemblage data from the Sand Hills ecoregion: species occurrences (left panel; species codes are defined in Table 4) and environmental variables (right panel). Triangles represent the centroids for the four river basins (SAV = Savannah River; CF = Cape Fear River; PD = Pee Dee River; CHT = Chattahoochee River). Watershed-scale variables include size (W_{size}), relief (W_{rel}), disturbance (W_{dist}), pine forest (W_{pine}), and roads and bare ground (W_{rdx}). Stream-scale variables include deep, sandy runs (S_{drn}); erosion (S_{eros}); undercut banks (S_{unbk}); instream habitat quality (S_{hab}); and proximity/connectivity to a larger stream (S_{con}). Plus and minus signs indicate directions of the environmental variables (+ = increase; – = decrease).

Lower on the hierarchical scale of spatial factors that influenced species composition were several watershed variables (W_{size}, W_{rel}, W_{rds}, and W_{pine}) as well as stream reach variables (S_{drn}; Figure 2). Marginal effects calculated by the stepwise forward selection procedure ranked the environmental variables in order of explained variance. Differences among basins had the greatest marginal effect on fish assemblage composition, followed by the watershed descriptors W_{rel} and W_{pine} (Table 5). Marginal effects do not account for the covariance shared among variables, whereas conditional effects show the unique independent effect of each variable. Eight of the environmental variables had significant ($P < 0.05$) conditional effects, including the basin identity variables as well as watershed variables (W_{dist}, W_{pine}, W_{size}, and W_{rel}) and stream reach variables (S_{hab} and stream connectivity S_{con}).

Variance partitioning showed that all environmental variables collectively accounted for 42% ($P < 0.002$) of the variance in fish assemblage composition, with the remaining variance being unexplained. The independent effects of the basin variables (i.e., after fitting the other variables as covariables) accounted for 24.0% of the explained variance; the independent effects of the watershed-scale variables (W_{size}, W_{rel}, W_{dist}, W_{pine}, and W_{rds}) accounted for 16.7%; and the independent effects of the stream-reach-scale variables (S_{drn}, S_{eros}, S_{unbk}, S_{hab}, and S_{con}) accounted for 26%. The remaining 33.3% of the explained variance in species composition was shared among hierarchical scales. These analyses showed that basin-related differences as well as variables attributable to watershed and stream reach spatial scales significantly affected species composition. However, variables acting at different scales were interrelated; for example, W_{dist} was inversely associated with S_{hab} ($r = –0.69$, $P < 0.001$). Of the variance explained by the environmental variables, 18.3% ($P = 0.002$) was attributable to variables that could be clearly linked to disturbance or habitat quality at both the watershed scale and the stream reach scale (W_{dist}, W_{rds}, S_{eros}, and S_{hab}).

Spatial Heterogeneity in Collective and Functional Properties

The RA biplots showed that most of the species richness variables increased with a combination of stream width (S_{width}), W_{size}, and S_{hab} and with decreasing W_{dist} (Figure 3). In contrast, P_{tol}, N_{tol}, and N_{cen} (primarily sunfish) increased with decreasing S_{hab} and with increasing W_{dist}. A stepwise forward selection procedure showed that several watershed-scale and stream-reach-scale environmental variables had greater marginal effects than the basin identity variables. However, analysis of conditional effects showed that the basin identity variables were statistically significant ($P \leq 0.05$), along with W_{dist}, W_{rel}, and S_{width} (Table 6).

Variance partitioning showed that all environmental variables together explained 42.5% of the variance in the collective and functional properties. Of the explained variance, 9.9% was attributable to basin, 24.7% was attributable to watershed-scale variables, and 24.0% was attributable to instream-scale variables. These results differed from the results for species composition, which was influenced more strongly by basin. Twenty-five percent of the explained variance was attributable to disturbance-related watershed and stream reach variables (W_{dist}, W_{rds}, S_{eros}, and S_{hab}).

TABLE 4. Fish collected by backpack electrofishing from four river basins in the Sand Hills ecoregion.

Species name	Code	Family	River basin			
			Cape Fear ($n = 20$)	Chattahoochee ($n = 15$)	Pee Dee ($n = 9$)	Savannah ($n = 25$)
American Eel *Anguilla rostrata*	eel	Anguillidae	14		11	22
Banded Pygmy Sunfish *Elassoma zonatum*	bpsf	Elassomatidae			1	6
Blackbanded Darter *Percina nigrofasciata*	bbdt	Percidae	2	54		18
Blackbanded Sunfish *Enneacanthus chaetodon*	bbsf	Centrarchidae			1	2
Blackspotted Topminnow *Fundulus olivaceus*	bstm	Fundulidae		4		
Bluespotted Sunfish *Enneacanthus gloriosus*	bssf	Centrarchidae	10		4	3
Bluefin Stoneroller *Campostoma pauciradii*	bfst	Cyprinidae		2		
Bluegill *Lepomis macrochirus*	bg	Centrarchidae	11	8		12
Bluehead Chub *Nocomis leptocephalus*	bhc	Cyprinidae				155
Broadstripe Shiner *Pteronotropis euryzonus*	bssh	Cyprinidae		287		
Brook Silverside *Labidesthes sicculus*	bss	Atherinopsidae				1
Chain Pickerel *Esox niger*	cp	Esocidae	5		5	5
Coastal Shiner *Notropis petersoni*	csh	Cyprinidae	2			
Creek Chub *Semotilus atromaculatus*	cc	Cyprinidae			5	169
Eastern Creek Chubsucker *Erimyzon oblongus*	ccs	Catostomidae	6	1	12	38
Dixie Chub *Semotilus thoreauianus*	dc	Cyprinidae		218		
Dollar Sunfish *Lepomis marginatus*	dsf	Centrarchidae	21	2	10	60
Dusky Shiner *Notropis cummingsae*	dsh	Cyprinidae	194		62	20
Eastern Mosquitofish *Gambusia holbrooki*	mf	Poeciliidae	95			32
Eastern Mudminnow *Umbra pygmaea*	mm	Umbridae	26		5	4
Flat Bullhead *Ameiurus platycephalus*	fbh	Ictaluridae				18
Flier *Centrarchus macropterus*	fl	Centrarchidae		1	1	
Golden Shiner *Notemigonus crysoleucas*	gsh	Cyprinidae			1	2
Goldstripe Darter *Etheostoma parvipinne*	gsdt	Percidae		30		
Green Sunfish *Lepomis cyanellus*	gsf	Centrarchidae		2		
Gulf Darter *Etheostoma swaini*	gdt	Percidae		3		
Lake Chubsucker *Erimyzon sucetta*	lcs	Catostomidae	16	1	11	2
Largemouth Bass *Micropterus salmoides*	lmb	Centrarchidae	6		2	6
Lined Topminnow *Fundulus lineolatus*	ltm	Fundulidae		1		
Longear Sunfish *Lepomis megalotis*	lesf	Centrarchidae		7		
Lowland Shiner *Pteronotropis stonei*	llsh	Cyprinidae			97	95
Margined Madtom *Noturus insignis*	mmt	Ictaluridae	121		71	20
Mud Sunfish *Acantharchus pomotis*	msf	Centrarchidae	34		35	12
Northern Hog Sucker *Hypentelium nigricans*	nhs	Catostomidae				4
Pinewoods Darter *Etheostoma mariae*	pwdt	Percidae			34	
Pirate Perch *Aphredoderus sayanus*	pp	Aphredoderidae	197	124	129	245
Pumpkinseed *Lepomis gibbosus*	pks	Centrarchidae	5			

TABLE 4. Continued.

Species name	Code	Family	River basin			
			Cape Fear ($n = 20$)	Chattahoochee ($n = 15$)	Pee Dee ($n = 9$)	Savannah ($n = 25$)
Redspotted Sunfish *Lepomis miniatus* × Spotted Sunfish *L. punctatus* hybrid	rssf	Centrarchidae		12		
Redbreast Sunfish *Lepomis auritus*	rbsf	Centrarchidae	34	8		43
Redfin Pickerel *Esox americanus americanus*	rfp	Esocidae	37	40	45	71
Sandhills Chub *Semotilus lumbee*	shc	Cyprinidae	144		108	
Savannah Darter *Etheostoma fricksium*	sdt	Percidae				65
Sawcheek Darter *Etheostoma serrifer*	swdt	Percidae	1		16	
Snail Bullhead *Ameiurus brunneus*	sbh	Ictaluridae				4
Southern Brook Lamprey *Ichthyomyzon gagei*	lam	Petromyzontidae		212		
Speckled Madtom *Noturus leptacanthus*	smt	Ictaluridae		23		52
Spotted Sucker *Minytrema melanops*	ss	Catostomidae				4
Spotted Sunfish *Lepomis punctatus*	ssf	Centrarchidae		1		55
Swampfish *Chologaster cornuta*	swf	Amblyopsidae			3	
Tadpole Madtom *Noturus gyrinus*	tmt	Ictaluridae			4	26
Tessellated Darter *Etheostoma olmstedi*	tdt	Percidae	35		5	18
Warmouth *Lepomis gulosus*	wm	Centrarchidae	31	15	2	12
Weed Shiner *Notropis texanus*	wesh	Cyprinidae		14		
Yellow Bullhead *Ameiurus natalis*	ybh	Ictaluridae	106	43	77	57
Yellowfin Shiner *Notropis lutipinnis*	ysh	Cyprinidae				337

Changes in Species Richness and Composition across a Gradient of Habitat Disturbance

Observed species richness ranged from 1 to 20. Estimated species richness (first-order jackknife) ranged from 1.0 to 27.4. The difference between observed and estimated richness averaged 1.8 and was greater in large streams (S_{width} versus difference: Pearson's $r = 0.52$, $P < 0.001$). This pattern was likely attributable to (1) a decline in sampling efficiency with stream size due to smaller electrical field size relative to the area sampled and (2) the greater difficulty in seeing and netting fish in deeper water. However, sample reach lengths may have been insufficient to permit inclusion of all habitat types in larger streams, where habitats (e.g., pools and runs) occurred on a larger scale. The use of estimated species richness rather than observed species richness in the GLMs helped to reduce the bias caused by the underestimation of richness, especially in larger streams.

After examining several GLMs, we selected a definitive model that had an R^2 of 0.76, with the following independent variables that were significantly ($P < 0.05$) related to estimated richness: basin identity, S_{width}, W_{size}, S_{con}, S_{hab}, and the square of S_{hab} ($[S_{hab}]^2$; Table 7). Interactions were not significant. The least-squares mean number of species (i.e., means computed at the means of the continuous covariables) was highest for the Savannah River basin (13.4, compared with 8.8–10.1 for the other basins). Basin accounted for more of the variance in species richness (11.0%) than any other individual variable, followed by S_{width} (10.2%), W_{size} (8.5%), S_{hab} (including $[S_{hab}]^2$, 6.5%), and S_{con} (2.9%).

The increase in species richness with S_{width} was mainly the result of species additions rather than the replacement of species that were characteristic of small streams by different species that were representative of larger streams. Most species in the smallest streams were also in all but the largest streams, albeit often in smaller numbers: for example, the Sandhills Chub in Cape Fear River basin streams; Dixie Chub in Chattahoochee River basin streams; Creek Chub, Bluehead Chub, and Yellowfin Shiner in Savannah River basin streams; and Pirate Perch in all streams (Table 8). Proximity to a larger stream increased average species richness by 2.0 compared with sites that were more distant from larger streams.

The relationship between species richness and S_{hab} was curvilinear, as indicated by a significant $(S_{hab})^2$ term ($P < 0.03$; Table 7). Residuals were calculated from a reduced model with estimated species richness as the dependent variable and with basin, S_{width}, W_{size}, and S_{con} as independent variables to eliminate the variance associated with watershed size, stream

TABLE 5. Permutation test results for environmental variables included in the canonical correspondence analysis of fish species composition in Sand Hills streams (MDI = Maloney disturbance index). See Methods and Results text for a description of the variables.

Variable and symbol	Marginal effect	Conditional effect		
	Lambda	Lambda	F	P
Chattahoochee River basin (CHT)	0.60	0.60	9.39	0.002
Watershed disturbance (W_{dist})	0.24	0.25	4.45	0.002
Savannah River basin (SAV)	0.41	0.36	6.24	0.002
Watershed forest type (W_{pine})	0.32	0.14	2.51	0.014
Watershed size (W_{size})	0.12	0.10	1.76	0.018
Watershed relief (W_{rel})	0.36	0.09	1.81	0.020
Connection with a larger stream (S_{con})	0.11	0.11	2.04	0.036
Instream habitat quality (S_{hab})	0.23	0.08	1.55	0.046
Stream erosion and incision (S_{eros})	0.16	0.08	1.56	0.080
Stream undercut banks (S_{unbk})	0.12	0.05	1.10	0.360
Cape Fear River basin (CF)	0.27	0.06	1.02	0.440
Stream substrate and mesohabitat (S_{drn})	0.21	0.05	0.99	0.486
Stream width (S_{width})	0.05	0.04	0.87	0.584
Watershed MDI and road coverage (W_{rds})	0.24	0.04	0.82	0.672

size, and stream connectivity. A plot of these residuals against S_{hab} showed that richness was low when S_{hab} was low, peaked at intermediate to moderately high levels of S_{hab}, and decreased at very high levels of S_{hab} (Figure 4). Instream habitat quality was related to W_{dist} (Pearson's $r = -0.69$), W_{rds} (Pearson's $r = -0.58$), and S_{eros} (Pearson's $r = -0.63$) and can be considered a measure of relative disturbance.

Average β was highest (11.0) at sites with the highest S_{hab} (habitat scores = 168–183), lowest (β = 5.2) at sites with moderate S_{hab} (habitat scores = 167–152), and intermediate (β = 8.8) at sites with the lowest S_{hab} (habitat scores ≤ 151). Differences between sites with the highest habitat quality and sites with moderate quality were statistically significant ($P < 0.05$), but differences between sites with the lowest habitat quality and the other sites were not significant.

Indicator species analysis examining differences in species composition between the 14 disturbed sites and the remaining sites showed that five species occurred in significantly ($P < 0.05$)

greater relative abundance and with greater frequency at disturbed sites: the Warmouth, Bluegill, Lake Chubsucker, Largemouth Bass, and Eastern Mosquitofish. We divided our sample sites into three equal-sized groups based on instream habitat quality scores to further investigate the relationship between these species and disturbance. The five species occurred at 57% of the lowest quality sites, 43% of the intermediate-quality sites, and 21% of the highest quality sites.

DISCUSSION

Spatial scales ranging from ecoregion to instream habitat have been studied by various researchers. Ecoregion effects on taxonomic composition are prominent when there are large differences in climate, topography, and vegetation, with corresponding effects on instream habitat (Pease et al. 2015). Basin effects are prominent when fish distribution is limited by barriers to dispersion (Kaller et al. 2013), whereas such effects are comparatively weak when barriers are lacking and when endemism is low (Pease et al. 2015). Much research (Grubbs et al. 2007; D'Ambrosio et al. 2009; Paller 2014; Pease et al. 2015) indicates the importance of environmental factors acting at multiple spatial scales. Similarly, our results show that stream fish assemblages within the Sand Hills ecoregion were influenced by naturally occurring and disturbance-related habitat gradients manifested at basin, watershed, and stream reach scales, although the relative magnitudes of these effects differed between taxonomic properties and collective and functional properties. The present results also demonstrate that collective properties based on species richness do not necessarily increase with habitat quality. These results contribute to a better understanding of factors affecting the distribution of stream fishes, provide basic information necessary for the conservation of Sand Hills fishes, and have consequences for the development of bioassessment protocols and associated reference models for the Sand Hills region.

Effects of River Basin

The significance of differences in fish assemblage composition among basins reflected the high level of endemism that is characteristic of fish faunas in the southeastern USA. Rivers within the study area were not subject to Pleistocene glaciation, resulting in a relatively long history of environmental stability. This stability—combined with geographic isolation and relatively high habitat diversity—has contributed to the development of regionally distinct faunas (Swift et al. 1986; Warren et al. 2000), which was particularly evident in the comparisons of Gulf versus Atlantic slope drainages (Figure 2). Differences among basins indicate that broad-scale historical zoogeography in the Sand Hills region influenced the species pools that were available for local factors to act upon.

The effect of river basin on collective and functional properties was weaker than the effect on species composition, indicating that the former were more uniform across the ecoregion. Species richness, which was significantly greater in the Savannah River
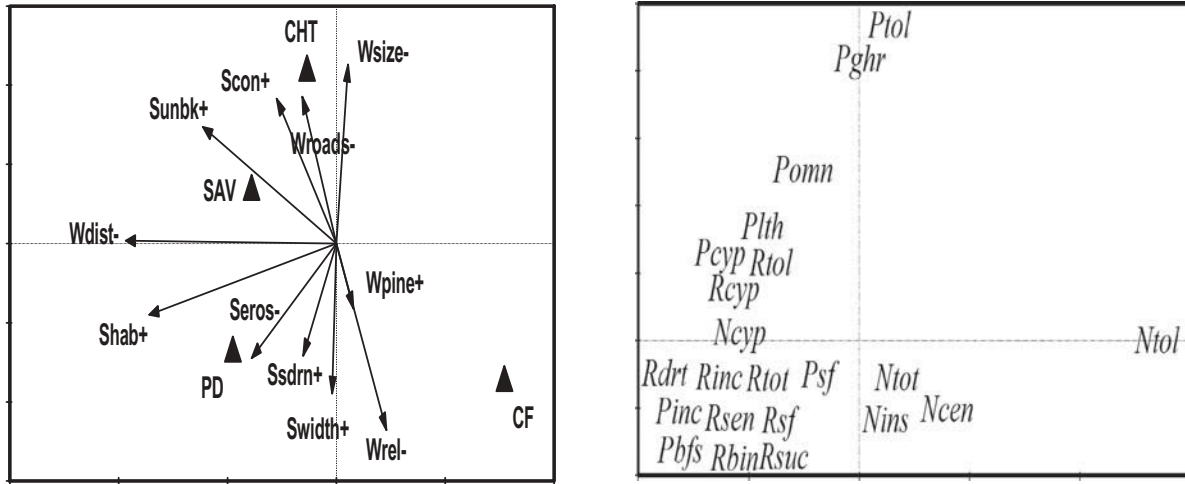
FIGURE 3. Redundancy analysis plots of species based on fish collective and functional properties in the Sand Hills ecoregion: environmental variables (left panel; triangles = river basin centroids [SAV = Savannah River; CF = Cape Fear River; PD = Pee Dee River; CHT = Chattahoochee River]; other symbols are defined in Figure 2) and collective and functional properties (right panel; N = numerical abundance, P = percent abundance, R = species richness; see Methods for additional description of symbols).

basin, was an exception. The relative lack of a river basin influence on collective and functional properties may reflect a general similarity in regional ecological conditions and the adaptive responses of Sand Hills fishes to these conditions. Furthermore, historical factors are less likely to influence collective and functional properties than taxonomic composition. Similar findings were reported by Hoeinghaus et al. (2007), who indicated that analyses based on taxonomy reflected patterns of species distribution, whereas analyses based on functional groups reflected the habitat template.

TABLE 6. Permutation test results for environmental variables included in the redundancy analysis of fish collective and functional properties in Sand Hills streams. See Methods and Results text for a description of the variables (symbols for variables are defined in Table 5).

Variable	Marginal effect	Conditional effect		
	Lambda	Lambda	F	P
CHT	0.04	0.05	4.27	0.002
W_{dist}	0.09	0.09	6.86	0.002
W_{rel}	0.07	0.05	4.27	0.002
S_{width}	0.09	0.09	7.2	0.002
SAV	0.03	0.03	2.63	0.008
S_{eros}	0.04	0.03	2.57	0.014
S_{hab}	0.07	0.02	1.75	0.094
S_{unbk}	0.04	0.01	1.62	0.124
W_{size}	0.06	0.02	1.43	0.208
S_{drn}	0.04	0.01	1.15	0.346
CF	0.04	0.01	0.87	0.508
S_{con}	0.01	0.01	0.9	0.532
W_{pine}	0.01	0.00	0.39	0.972
W_{rds}	0.04	0.00	0.29	0.992

Because collective and functional properties constitute many of the metrics that are used for bioassessment, comparatively small basin-related effects suggest that a single bioassessment protocol may be acceptable for the region, perhaps with basin- and watershed/stream-size-related modifications of metrics related to species richness.

Effects of Watershed and Stream Reach

Factors acting at smaller spatial scales tend to be complex and location specific, and their relative importance may depend partly on the variables that are measured and their range of variation. For example, Wang et al. (2003) observed that watershed-scale variables were more influential as landscape disturbance increased and that reach-scale variables were more influential in nondegraded landscapes. Grubbs et al. (2007) found that land use was less important than other factors in a watershed without large urban and

TABLE 7. Factors influencing fish species richness in the Sand Hills ecoregion, as indicated by a general linear model (SS = sum of squares; species richness values were estimates of true species richness derived from a first-order jackknife estimator). Symbols for variables are defined in Table 5.

Source	Type III SS	df	Mean square	F	P
Basin	229.8	3	76.6	6.9	<0.001
W_{size}	183.3	1	183.3	16.6	<0.001
S_{width}	205.9	1	205.9	18.7	<0.001
S_{con}	60.6	1	60.6	5.5	0.022
S_{hab}	120.9	1	120.9	11.0	0.002
$(S_{hab})^2$	114.9	1	114.9	10.4	0.002
Error	661.9	60	11.0		

TABLE 8. Changes in fish numbers with stream width in the Savannah River, Cape Fear River, and Chattahoochee River basins (species codes are defined in Table 4).

Species code	<1.5 m	1.5–1.9 m	2.0–3.0 m	>3.0 m
Savannah River basin				
cc	91	61	17	0
mm	1	3	0	0
bhc	38	41	71	5
ysh	70	123	119	25
tmt	11	10	2	3
pp	23	102	89	32
ssf	0	20	27	8
mf	3	16	11	2
sdt	0	8	44	13
lmb	0	0	3	4
msf	0	8	3	1
ccs	1	5	26	6
ybh	6	7	34	11
rbsf	5	9	22	7
dsf	0	15	37	8
rfp	0	13	38	22
bg	0	0	10	11
llsh	0	14	51	30
mmt	3	3	11	3
nhs	0	0	0	4
dsh	0	7	2	11
wm	0	0	6	7
tdt	0	2	11	5
eel	1	2	5	14
smt	0	7	20	25
bbdt	0	0	6	12
Cape Fear River basin				
mm	0	19	7	0
lcs	0	15	1	0
shc	47	8	88	1
pp	13	68	113	12
mf	0	0	78	17
lmb	0	0	1	5
msf	0	12	21	3
ccs	0	0	6	3
ybh	5	12	73	19
rbsf	0	3	31	0
dsf	0	4	15	4
rfp	1	8	23	13
bg	0	3	0	8
mmt	0	29	86	10
dsh	0	43	140	37
cp	0	3	0	2
wm	0	0	2	30
tdt	0	2	27	7

TABLE 8. Continued.

Species code	<1.5 m	1.5–1.9 m	2.0–3.0 m	>3.0 m
eel	0	4	9	1
Chattahoochee River basin				
gsdt	14	16	0	0
dc	86	55	76	1
pp	24	50	42	8
bssh	77	128	44	38
lam	62	50	77	23
wesh	0	4	10	0
ybh	7	16	19	1
rbsf	0	4	4	0
rfp	1	12	24	3
bg	0	1	7	0
wm	0	4	10	1
smt	0	6	3	14
bbdt	0	10	36	8
rssf	0	5	2	5

agricultural gradients. Troia and Gido (2013) found that models incorporating local habitat and longitudinal network position performed better in subbasins with more environmental variation. Habitat at the stream reach scale is affected by watershed-scale processes, as was reflected by the observed
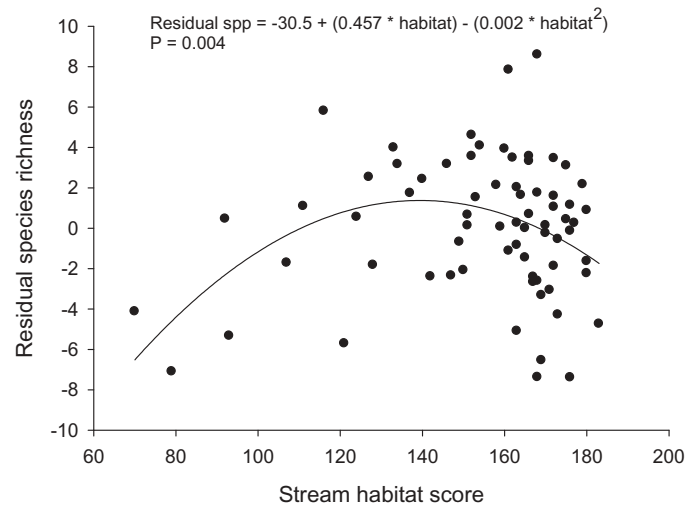

FIGURE 4. Relationship between fish species richness residuals (residual spp) at sample sites and instream habitat quality (habitat) in the Sand Hills ecoregion. The residuals represent species richness after the statistical removal of variance associated with stream width, basin size, basin identity, and connectivity with larger streams.

correlation between watershed disturbance and instream habitat quality. Together, local-, watershed-, and regional-scale processes impose sequential filters that determine local species composition and richness, as was theorized by Tonn et al. (1990) and observed in the present study and other studies (e.g., Angermeier and Winston 1998).

Research shows that stream size and related measures (e.g., stream order) affect fish assemblage richness and composition (Sheldon 1968; Paller 1994), especially in basins with substantial stream size gradients (Troia and Gido 2013). The importance of stream network longitudinal position in the Sand Hills was reflected by the significant effects of watershed size and stream width on fish assemblage composition and species richness. These effects are expected consequences of (1) longitudinal habitat changes in lotic ecosystems, as summarized by Vannote et al. (1980); and (2) dispersal barriers that can limit the upstream movement of some fishes (Grossman et al. 2010). Such barriers might have been imposed by small waterfalls that were formed by tree roots above plunge pools in our smaller study streams. Fish assemblage composition can change with stream size through the addition and replacement of species. Downstream increases in richness via species additions tend to be greater in streams and rivers that possess a strong longitudinal gradient of decreasing environmental variability, whereas species replacements are more common in streams with large longitudinal changes in thermal regime, habitat, or geomorphology (Horwitz 1978; Rahel and Hubert 1991). In our study, additions were more prominent than replacements, suggesting the occurrence of longitudinal increases in habitat stability and diversity. However, sampling of a fairly narrow range of stream sizes (average width was mostly between 1 and 4 m) was likely also a factor, and the inclusion of a greater number of large streams in our study might have resulted in more species replacements (Paller 1994).

Fish assemblage composition was also affected by connectivity (proximity to a larger stream), which can influence resource availability (e.g., habitats and food), the likelihood of immigration, and the risk of extinction. Connectivity can increase richness for taxa such as catostomids, cyprinids, and darters due to immigration from larger streams (Osborne and Wiley 1992; Osborne et al. 1992). Immigration may be strongest when the connection is to a relatively species-rich stream of significantly larger size (Hitt and Angermeier 2011).

A site was considered to be connected if it was near the confluence with a larger stream. "Near" was defined differently for relatively small streams (average width ≤ 1.5 m) and large streams (average width > 1.5 m), and these definitions of proximity (250 and 750 m, respectively) were less than seen elsewhere in large streams where proximity-related effects occurred up to 20 km from the confluence with main-stem rivers (Thornbrugh and Gido 2010). Our distances were based on research showing that many stream fishes are relatively sedentary and characterized by movements of several-hundred

meters or less (Hill and Grossman 1987; Rodríguez 2002). We used a shorter cutoff distance in small streams than in large streams because the small streams often had shallow riffles and runs, log/brush/root jams, and plunge pools that could serve as barriers to upstream movement of fish (especially large fish) from higher-order streams. Such barriers were less common in larger streams, which possessed habitats that were more similar to the habitats in the higher-order streams to which they were joined and that were less likely to act as barriers.

Effects of Disturbance

Variables that were explicitly related to anthropogenic disturbance, regardless of scale, accounted for significant amounts of variance in species composition and collective and functional properties. However, these variables provided minimal indications of disturbance effects on Sand Hills fish assemblages because other variables that were not explicitly measuring disturbance (e.g., forest cover and type) were likely affected by disturbance. Furthermore, the amount of disturbance-related variance was influenced by the proportion of disturbed sites in the study and their degree of disturbance. Inclusion of a greater number of disturbed sites would likely have increased the amount of disturbance-related variance.

Assuming that instream habitat quality was a general indicator of disturbance, the curvilinear relationship between instream habitat quality and species richness demonstrated that richness peaked at intermediate levels of disturbance and declined somewhat at the lowest level of disturbance (Figure 4). Increased species number at sites of slight to moderate disturbance was likely a manifestation of biotic homogenization. Homogenization of fish assemblages in the southeastern USA can result from the replacement of endemic species that are characteristic of small, undisturbed highland streams by generalist species that are characteristic of lowland areas. Endemic highland species, such as some darters and shiners, are relatively specialized and are typically associated with hard bottoms, which are used for feeding and spawning. Generalists are usually widespread native taxa that are characteristic of downstream reaches and that are able to utilize a variety of foods and spawn in a range of habitats (Scott and Helfman 2001). Homogenization occurs in the southern Appalachian highlands, which support comparatively large numbers of endemic fishes. Although Sand Hills streams support fewer endemic taxa (Warren et al. 2000), the headwater fish assemblages are species rich and distinctive (Paller 1994), making them susceptible to homogenization; however, the process may be more subtle than in regions with a greater number of endemic species.

It can be difficult to detect the early stages of homogenization, in which assemblages consist of endemic species plus a small number of downstream generalists. However, sensitive endemic species are more likely to be scarce and generalist species are more likely to be numerically dominant at highly degraded sites. We found that the Bluegill, Largemouth Bass,

Warmouth, Eastern Mosquitofish, and Lake Chubsucker occurred frequently at highly disturbed sites but were scarce at sites with the highest instream habitat quality. Although native to the Sand Hills, most of these species are more typical of large streams or lentic habitats than of small, relatively undisturbed streams. Two of the species, the Largemouth Bass and Bluegill, are often stocked in manmade impoundments from which they may invade contiguous stream reaches. The occurrence of these species at moderately disturbed sites, which also supported relatively sensitive endemic species, was a likely manifestation of biotic homogenization that inflated species richness above levels that were typical of least disturbed sites. This was accompanied by a 50% decrease in β, reflecting the fact that moderately disturbed sites supported a larger proportion of the Sand Hills species assemblage than did higher quality sites, which supported less-speciose but more distinctive assemblages that were typical of undisturbed headwater streams.

Conclusions

Differences in taxonomic composition among river basins showed the significant influence of historical factors on Sand Hills fish assemblages. Conservation programs must account for these differences to avoid the loss of β within the region. However, factors acting at the smaller spatial scales (i.e., watersheds within basins; stream reaches within watersheds) were also influential, especially watershed size, relief, forest cover, and disturbance; and stream size, proximity to a larger stream (connectivity), and habitat quality. Habitat differences associated with these factors contribute to biodiversity, and some of them must be recognized as covariables when assessing anthropogenic disturbance. The collective and functional properties of Sand Hills fish assemblages were affected by many of the same factors as taxonomic composition, although differences among basins were less prominent, indicating the potential usefulness of these properties in regionwide bioassessment protocols.

Stream fish assemblages within the Sand Hills were clearly affected by anthropogenic disturbance through interrelated influences that were manifested at watershed and stream reach scales. Due to the pervasiveness of anthropogenic disturbance within the region, even the best sites should be regarded as least disturbed rather than minimally disturbed. Least disturbed sites within the Sand Hills do not necessarily possess the highest fish species richness—an implicit assumption of many bioassessment protocols. Moderately disturbed sites may exhibit greater richness as a subtle manifestation of faunal homogenization, which is caused by changes in the distribution of native species due to habitat degradation. Failure to consider this may cause moderately disturbed sites to be overrated and minimally disturbed sites to be underrated, thus contributing to false conclusions about the integrity of fish assemblages. This is probably also the case in other regions that have been settled for long periods and are subject to pervasive historical and contemporary disturbances.

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