RELATION OF ENVIRONMENTAL CHARACTERISTICS TO FISH ASSEMBLAGES IN THE UPPER FRENCH BROAD RIVER BASIN, NORTH CAROLINA

BRENDA RASHLEIGH
U.S. Environmental Protection Agency, Office of Research and Development, National Exposure Research Laboratory, Athens, Georgia, U.S.A
(e-mail: rashleigh.brenda@epa.gov)*

(Received 19 February 2002; accepted 9 May 2003)

Abstract. Fish assemblages at 16 sites in the upper French Broad River basin, North Carolina were related to environmental characteristics using detrended correspondence analysis, principal components analysis, and linear regression. The primary gradient affecting sites in this basin was related to agricultural influence, characterized by high levels of agricultural land cover, nitrate plus nitrite, sulfate, specific conductance, and sediment. Agricultural influence on the fish assemblage was represented as a trophic shift from specialized insectivores to generalized insectivores and an herbivore. A secondary influence on variation among sites was related to urban land cover, population density, increased concentrations of metals, and soil erodibility. This primarily urban gradient was characterized by an increase in the number of native and introduced fish species, particularly sunfish and omnivores species, and a decline in the percent of piscivores. These results support the identification of indicators for different environmental influences, which can improve the ability of resource managers to diagnose impairment in this basin and in similar basins.

Keywords: detrended correspondence analysis, fish assemblage metrics, water quality

1. Introduction

Resource managers are often interested in assessing aquatic resources at the scale of a river basin. This is the spatial scale where management decisions and activities are usually focused, and where restoration activities will most likely occur (Holling, 1992). Within moderately-sized river basins, variation in natural environmental characteristics such as geographic and geologic features (Matthews and Robison, 1988; Whittier *et al.*, 1988; Changeux and Pont, 1995), climate (Lyons, 1996), and the evolutionary history of the drainage basin (Angermeier and Winston, 1999) are reduced. Aquatic communities within a basin may be structured by more local factors such as hydrology, water quality, and habitat (Matthews, 1998). Human activity in a basin can have a significant effect on aquatic communities, and without appropriate management, streams can experience a decline in biological integrity (U.S. Environmental Protection Agency, 2001).

Fish assemblage characteristics are widely used as a measure of biological integrity at the basin scale. A method for assessing biological integrity of streams

Environmental Monitoring and Assessment **93:** 139–156, 2004.

using fish assemblage characteristics (bioassessment) was originally developed for the Midwestern United States but has been modified for application in many geographic regions (Leonard and Orth, 1986; Angermeier and Schlosser, 1987; Miller *et al.*, 1988; Hughes *et al.*, 1998). In bioassessment, changes in fish assemblages are typically quantified by a variety of statistics, termed metrics (Karr, 1981; Hughes *et al.*, 1998). Metrics may be used to summarize species richness and composition, feeding guilds, and reproductive guilds (Karr, 1981). Two underlying assumptions of the bioassessment approach are that fish assemblages within a region are structured primarily by their level of degradation, and that metrics integrate multiple sources of anthropogenic influence such that degradation can be considered in a single dimension.

Because metrics integrate multiple sources of anthropogenic influence, they are useful for tracking and monitoring complex systems (Karr and Chu, 1999), but less useful for identifying specific causes of impairment (Bryce *et al.*, 1999). Multivariate statistical techniques are often used to provide objective methods for identifying the most significant environmental gradients. For example, Waite and Carpenter (2000) found that fish communities in Oregon's Willamette basin were related to stream channel width and concentrations of dissolved oxygen, phosphate, and pesticides. Maret *et al.* (1997) found that distributions of fish species among sites in the upper Snake River Basin in the western U.S.A. were related primarily to stream gradient, watershed size, conductivity, and percent forested land. Zampella and Bunnell (1998) found that anthropogenic influences such as increased pH, conductivity, and agricultural land use were most important for explaining differences among fish assemblages in New Jersey Pineland streams. Basin-scale studies using multivariate statistical analysis can provide insight into the response of stream ecology to environmental variables, and can help identify and diagnose specific causes of impairment.

The first objective of this paper is to assess how differences among sites, in terms of their fish assemblages, are related to environmental characteristics in the tributaries to the upper French Broad River in North Carolina. A multivariate statistical approach is used for this assessment. The second objective of this paper is to identify the specific changes in fish assemblage metrics that occur in response to identified gradients. The upper French Broad River provides several advantages for addressing basin-scale variables because of the relative homogeneity of its tributaries with respect to regional-scale characteristics such as geologic setting, climate, and ecoregion. The results should be useful in the development and refinement of environmental management approaches for fishes in the French Broad River basin, and in similar basins where biological criteria are employed.

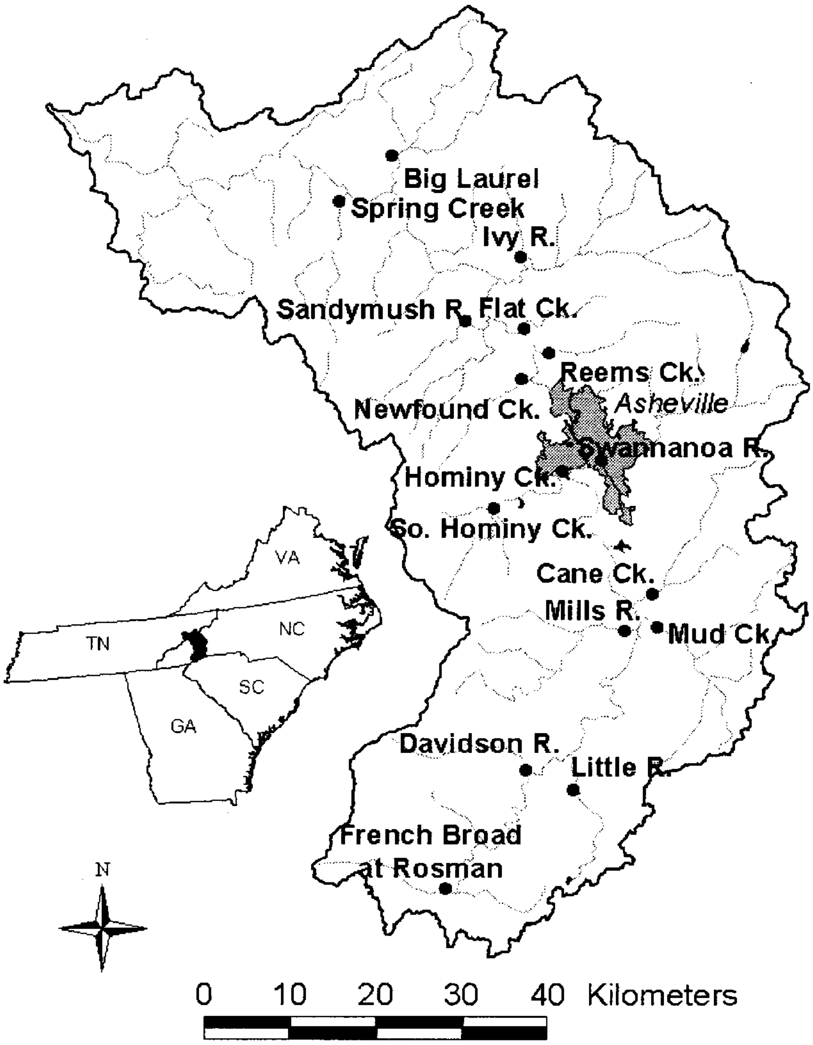

Figure 1. Location of study sites in the upper French Broad River basin, North Carolina.

2. Methods

2.1. Description of the study area

The French Broad River, the largest tributary to the Tennessee River, originates near the North Carolina-South Carolina state line and flows north though the Appalachian mountains into Tennessee (Figure 1). The river crosses the Appalachian divide near the Tennessee-North Carolina state line; it was able to cut into the rock faster than the mountains were being uplifted. The upper French Broad River drains a 4862 km^2 area of the Blue Ridge ecoregion (Omernick, 1987). This basin is mountainous, and is underlain by igneous and metamorphic geology (USGS, 1979). Streams in the upper French Broad are typically clear and are of relatively low productivity, with alternating riffles and pools over bedrock and boulder substrates (Etnier and Starnes, 1993). The majority of the upper French Broad River basin is forested (70%), while agriculture accounts for 21% of the basin's land area. The remaining area of the basin supports mainly industrial and urban land uses, especially in and around Asheville, North Carolina.

Water-quality conditions in the French Broad River have varied greatly over time. In 1945, the Tennessee Valley Authority (TVA) reported that severe industrial pollution from tannery and textile wastes was a particular problem, and that bacterial contamination was widespread at that time because most municipal sewage systems discharged raw sewage directly into streams (TVA, 1945). Water-quality monitoring data from the U.S. Geological Survey (USGS) station on the French Broad at Marshall, North Carolina, 33 river kilometers downstream of Asheville, suggests that water quality first declined but subsequently improved during the period 1958–1977 (USGS, 1979). By 1977, improved industrial technology and implementation of waste-water treatment had combined to reverse earlier trends and improve overall water-quality conditions at least to the levels observed in 1958. This occurred in spite of increased population and industrial growth in the areas upstream of the monitoring site. In a more recent study, agricultural runoff, urban runoff, and construction have been identified as the major sources of water-quality impairment in the upper French Broad River basin (North Carolina Department of Environment and Natural Resources, 1998).

2.2. Data collection

Quantitative fish assemblage data were collected at 16 sites in the upper French Broad River basin by the USGS National Water-Quality Assessment (NAWQA) Program (Figure 1, Table I). Sites were selected to be representative of the stream, to avoid discontinuities in riparian or instream characteristics, and to minimize the impact of impoundments. Fish data were collected during low- and stable-flow conditions between 7 April and 13 August 1997. Fish samples at each site were collected using NAWQA Program electrofishing and seining protocols (Meador *et al.*, 1993). Backpack electrofishing gear was used for one-pass sampling in an upstream direction, and seining was used to complement electrofishing, particularly for deep pools. Samples were collected in a reach length equivalent to 20 times the channel width that included at least two units of pools and riffles (Table I). Fish collected across all habitats were combined in a single sample, and specimens were identified and counted in the field. Species were grouped by family and trophic group. The trophic groups used (insectivores, specialized insectivores, omnivores, herbivores, and piscivores) corresponded to those used by TVA (Saylor and Scott, 1987). Species were also identified as native or introduced and tolerant or intolerant to environmental degradation based on TVA data.

TABLE I

Name, code, and watershed characteristics of sampling sites in the upper French Broad River basin, North Carolina

Site name	Site code	River (km)	Area sampled sampled (m^2)	Drainage area (km^2)	Site elevation (m)	Land cover (percentage)		
						Forest	Agriculture	Urban
Big Laurel Creek near Stackhouse	Laurel	6.0	640	332	1600	93.83	6.17	0
Cane Creek at US 25 at Fletcher	Cane	3.7	754	212	2060	77.71	20.23	1.73
Davidson River at Brevard	David	0.3	1172	124	2110	97.53	0.94	1.18
Flat Creek near Weaverville	Flat	0.6	692	65	1780	68.54	30.77	0.69
French Broad River at Rosman	FBRos	348.2	1088	176	2174	94.96	4.27	0.10
Hominy Creek near W. Asheville	Hominy	1.3	866	272	1960	71.79	23.51	4.57
Ivy River at Marshall	Ivy	19.6	811	409	1700	80.38	19.30	0.23
Little River near Little River	Little	5.5	838	111	2110	96.09	1.94	0.32
Mills River at Mills River	Mills	1.5	641	187	2060	93.13	6.22	0.22
Mud Creek at Naples	Mud	2.7	552	285	2050	59.42	32.31	7.48
Newfound Creek near Alexander	New	1.5	780	88	1940	52.61	46.71	0.66
Reems Creek near Weaverville	Reems	4.0	587	86	1940	81.95	15.73	2.29
Sandymush Creek near Volga	Sandy	5.5	751	122	1840	75.10	24.90	0
South Hominy Creek at Candler	SoHom	0.3	641	98	2100	82.95	16.99	0
Spring Creek near Hot Springs	Spring	3.1	614	184	1400	91.83	8.16	0
Swannanoa River at Biltmore	Swann	2.6	782	337	1977	86.16	6.43	6.56

Basin-scale habitat variables were obtained for each of the 16 sites sampled, mostly using Geographic Information System (GIS) methods. Delineation of the drainage basins above each of the sampling sites was accomplished by merging estimated drainage divides taken from USGS 1:24 000-scale topographic maps with existing digitized hydrologic unit delineations from the U.S. Department of Agriculture Natural Resources Conservation Service. The resulting drainage delineations were used to calculate drainage area, mean basin elevation, mean rainfall, soil erodibility, and population density. Mean basin elevations for each watershed were taken from USGS digital elevation models at 30 m resolution. Mean rainfall was calculated from 1997 TVA data; Theissen polygons were used to estimate between 18 rainfall stations for the upper French Broad basin and an area-weighted average rainfall was calculated for the watershed of each site. Soil erodibility, which indicates how likely soil is to erode based on its physical and chemical properties, was calculated as an area-weighted average using the U.S. Department of Agriculture State Soil Geographic Database. Population density was estimated as an area-weighted average from 1990 census data. Percentages for forested, agricultural, and urban land cover were taken from 1990 TVA Landsat data at the scale of 50 m cells. Stream gradient, site elevation, and sinuosity were measured on USGS 1:24 000-scale topographic maps. Sinuosity was measured as river distance divided by the straightline distance between the upstream and downstream ends of a segment of stream (minimum length of 2 km) containing the sample site.

Hydrologic and water-quality data were collected once at each of the 16 sites between 8 April and 9 July 1997, in most cases (11 of 16 sites) on the same day as the collection of fish samples. All fish and water-quality sampling sites coincided except at the Ivy River where water-quality data was collected at river km 8.7 and fish samples were collected at river km 1.5. This difference was considered minor because the drainage areas of the two sites differed by less than 3%. Stream flow measurements and field measurements for water temperature and specific conductance were collected on-site. Water samples were collected at all sites and shipped to the USGS National Water Quality Laboratory in Arvada, Colorado for analysis of multiple water quality parameters (suspended sediment, ammonia, nitrate plus nitrite, total phosphorus, iron, manganese, calcium, magnesium, and sulfate). Samples for analysis of atrazine and metolachlor, the most commonly detected herbicides in the basin, and total coliform bacteria were also taken. All water-quality data were collected and recorded according to USGS NAWQA protocols to insure consistency (Shelton, 1994).

2.3. Data analysis

Detrended correspondence analysis (DCA) was used to analyze fish assemblage data that were converted to relative abundances (percent of the total abundance at each site). DCA is a unimodal ordination technique that can be used to derive important environmental gradients from species composition data. Correspondence analysis arranges species and sites across a set of orthogonal axes so that sites that are similar in terms of their species compositions are closer to one another in ordination space, and species with similar distributions across sites tend to group together in ordination space. Species are located at the mode of the unimodal distribution of the individual species' relative abundance along that axis. Sites are located along the DCA axes based on a weighted average of species scores. Analyses were conducted using PC-ORD software (McCune and Mefford, 1999). Detrending by segments, using 26 segments, was applied to remove the arch effect (Gauch, 1982). Rare species were censured at 0.03% total abundance because multivariate analysis can be overly sensitive to rare species (Ter Braak and Smilauer, 1998). The analysis was also run with rare species retained, because their omission has recently been criticized (Karr and Chu, 1999, and references therein). Initial DCA analyses indicated that one site (Mud Creek) was an extreme outlier. The degree of separation between Mud Creek and all other sites along the first DCA axis was so great that it obscured the identification of differences among other sites (Gauch, 1982). For this reason, Mud Creek was dropped from further analyses.

The initial set of 26 environmental variables was examined for variability, normality, and multicolinearity, then a subset of these variables was used in a Principal Components Analysis (PCA). Variables were first tested to insure sufficient variability (Coefficient of Variation >10); mean basin elevation did not meet this criterion and was deleted. The Shapiro-Wilk test was then used to examine normality of variables (SAS Institute, 1988). Non-normally distributed variables were transformed to improve normality and homoscedasity (Gauch, 1982); those that could not be transformed to meet the normality criterion ($p > 0.05$, ammonia, atrazine, and metolachlor) were dropped from further analysis. Spearman rank correlations were used to examine remaining environmental variables for multicolinearity (SAS Institute, 1988). When a set of highly correlated variables ($r > |0.95|$) was identified, the most easily interpreted variable was chosen to represent the set (Glantz and Slinker, 1990). Using this approach, percent agricultural land was used to represent percent forested land, and specific conductance was selected to represent calcium and magnesium. The remaining 19 environmental variables were analyzed using PCA (SAS Institute, 1988). Site scores for PCA axes that explained at least 10% of the variation among sites were related to DCA site scores using linear regression analysis (SAS Institute, 1988).

Nine established fish assemblage metrics developed by TVA for use in the upper Tennessee River basin were calculated based on the raw fish data from each site. The metrics represented fish species composition (numbers of native species, sunfish species less *Micropterus*, darter species, sucker species, and intolerant species, and percent of individuals as tolerant species) and trophic groupings (percent of individuals as specialized insectivores, omnivores plus herbivores less *Ichthyomyzon*, and piscivores) (Saylor and Scott, 1987). Most of these metrics were expected to decrease with increasing anthropogenic impact. Only two metrics, percent omnivores plus herbivores and percent tolerant species, were expected to increase with increasing impairment. Metrics were related to DCA species axis scores using Pearson correlation analysis (SAS Institute, 1988). All statistical tests were considered significant at $p < 0.05$.

3. Results

A total of 46 fish species were collected and 38 of these were retained for analysis (Table II). Five of the 38 species were introduced. Based upon past sampling by North Carolina Wildlife Resources Commission and TVA, one species, *Lepomis cyanellus* (green sunfish), was a new report for the basin, representing a range extension (C. Saylor, TVA, pers. comm. 1998). Eight Families were collected, with the dominant Family being Cyprinidae (minnows, 18 of the 38 species) followed by the Centrarchidae (sunfish) and Percidae (perches and darters) with 6 species each (Table II). Species richness ranged from 9 at Reems Creek to 27 at Davidson River. Comparisons with earlier studies indicate that fish species richness and composition found during this survey were typical for the upper French Broad River basin (Harned, 1979).

The eigenvalues for the first three DCA axes were 0.473, 0.262, and 0.072, and only the first two were retained for further analysis. Results from the DCA analysis of fish assemblage data are shown for the case where rare species are censured (Figure 2); results from this analysis did not differ significantly from results where species were retained. Generally, the groupings of sites in the ordination diagram were closely related to their longitudinal position in the basin. The most upstream sites in the upper French Broad basin (the French Broad River at Rosman, South Hominy Creek, and Little, Mills, and Davidson Rivers) were located at the left of Figure 2. Sites near Asheville (Swannanoa River, Cane Creek, and Hominy Creek) were located at the bottom of Figure 2. Sites directly downstream of Asheville (Flat, Reems, Newfound, and Sandymush Creeks) were located on the right of Figure 2, and sites located furthest downstream in the upper French Broad basin, (Big Laurel Creek, Spring Creek, and Ivy River) plotted at the top of Figure 2.

The first three PCA axes explained a total of 73% of the variance in environmental data (Table III). Environmental variables with the highest absolute loadings on PCA Axis 1 were sulfate, rainfall, specific conductance, nitrate plus nitrite, percent agricultural land cover, and suspended sediment. The environmental variables with the highest absolute loadings on PCA Axis 2 were population density, manganese, percent urban land cover, soil erodibility, iron, stream gradient, and site elevation. For PCA Axis 3, drainage area and flow had the highest factor loadings. Linear regression analysis of the first two DCA axes to the first three PCA axes gave the following results: DCA Axis 1 was significantly related only to PCA Axis 1 (Figure 3a); and DCA Axis 2 was significantly related only to PCA Axis 2 (Figure 3b).

TABLE II

Names, feeding groups, and tolerance ratings of fish species collected in the Upper French Broad River basin, North Carolina

Family	Scientific name (I = Introduced)	Common name	Feeding[a]	Tolerance
Petromyzontidae	*Ichthyomyzon greeleyi*	mountain brook lamprey	H	
Cyprinidae	*Campostoma anomalum*	central stoneroller	H	
	Cyprinella galactura	whitetail shiner	I	
	Cyprinella spiloptera	spotfin shiner	I	Tolerant
	Erimystax insignis	blotched chub	O	
	Luxilus coccogenis	warpaint shiner	S	
	Nocomis micropogon	river chub	O	
	Notemigonus crysoleucas	golden shiner	O	Tolerant
	Notropis amblops	bigeye chub	S	
	Notropis leuciodus	Tennessee shiner	S	
	Notropis photogenis	silver shiner	S	
	Notropis rubricroceus	saffron shiner	S	
	Notropis spectrunculus	mirror shiner	S	
	Notropis telescopus	telescope shiner	S	Intolerant
	Phenacobius crassilabrum	fatlips minnow	S	
	Pimephales promelas (I)	fathead minnow	O	
	Rhinichthys atratulus	blacknose dace	I	
	Rhinichthys cataractae	longnose dace	S	
	Semotilus atromaculatus	creek chub	I	Tolerant
Catostomidae	*Catostomus commersoni*	white sucker	O	Tolerant
	Hypentelium nigricans	northern hogsucker	I	
	Moxostoma duquesnei	black redhorse	I	Intolerant
Ictaluridae	*Ameiurus platycephalus* (I)	flat bullhead	I	
Salmonidae	*Oncorhynchus mykiss* (I)	rainbow trout	I	
	Salmo trutta (I)	brown trout	P	
Cottidae	*Cottus bairdi*	mottled sculpin	I	
Centrarchidae	*Ambloplites rupestris*	rock bass	P	Intolerant
	Lepomis auritus (I)	redbreast sunfish	I	
	Lepomis cyanellus	green sunfish	I	Tolerant
	Lepomis macrochirus	bluegill	I	
	Micropterus dolomieu	smallmouth bass	P	
	Micropterus salmoides	largemouth bass	P	
Percidae	*Etheostoma blennioides*	greenside darter	S	
	Etheostoma chlorobranchium	greenfin darter	S	
	Etheostoma flabellare	fantail darter	S	Intolerant
	Etheostoma rufilineatum	redline darter	S	
	Etheostoma swannanoa	Swannanoa darter	S	
	Percina evides	gilt darter	S	Intolerant

[a] H = Herbivore, O = Omnivore, I = Insectivore, S = Specialized insectivore, P = Piscivore.

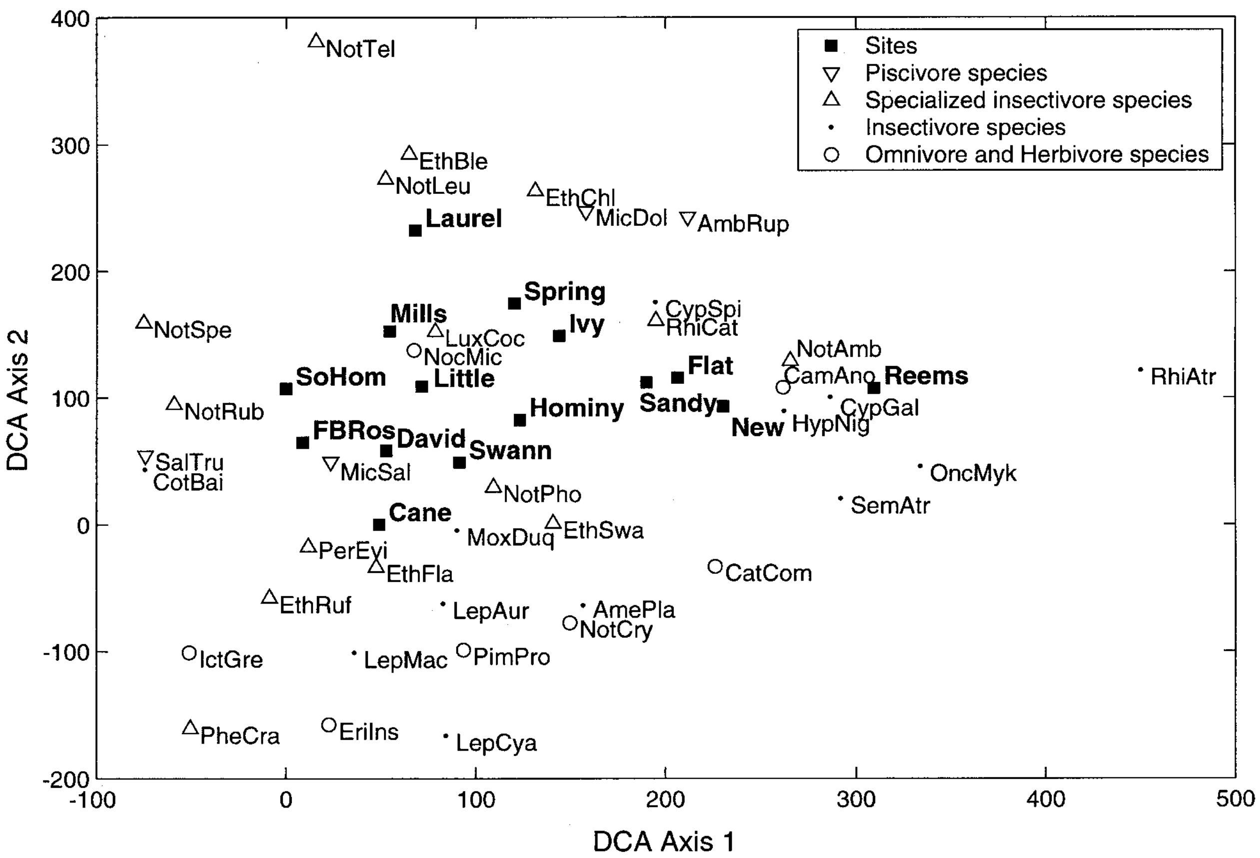

Figure 2. Results from detrended correspondence analysis (DCA) based on fish assemblages from 15 sites in the upper French Broad River basin. Full site names and descriptions are given in Table I. Species are plotted according to trophic group and coded by the first three letters of their genus and species names (Table II).

TABLE III

Factor loadings of environmental variables on the first three principal components

Environmental variable	Principal component		
	1	2	3
Agricultural land cover (%)	0.843	0.345	– 0.086
Drainage area (km^2)	– 0.093	– 0.088	0.759
Flow (cubic feet per second)	– 0.416	– 0.580	0.752
Iron ($\mu g\ L^{-1}$ as Fe)[a]	– 0.578	0.674	– 0.010
Manganese ($\mu g\ L^{-1}$ as Mn)[a]	0.321	0.839	– 0.062
Nitrate plus nitrite (mg L^{-1} as N)	0.877	0.183	0.183
Population density[b]	0.319	0.848	0.032
Rainfall (cm yr^{-1})	– 0.906	0.233	– 0.144
Site elevation (m)	– 0.563	0.625	– 0.089
Soil erodibility	0.072	0.790	– 0.326
Specific conductance ($\mu S\ cm^{-1}$)	0.887	0.232	– 0.105
Stream gradient (%)[b]	0.524	– 0.661	– 0.358
Sinuosity[a]	0.322	0.065	0.491
Sulfate (mg L^{-1} as SO_4)	0.908	0.168	– 0.052
Suspended sediment (mg L^{-1})	0.828	– 0.132	0.268
Temperature (C)[b]	– 0.699	– 0.043	– 0.109
Total coliform (colonies per 100 mL)	0.628	– 0.206	– 0.446
Total phosphorous (mg L^{-1} as P)[b]	0.554	0.077	0.526
Urban land cover (%)[b]	0.008	0.794	0.188
Percent of variance explained	38.0	22.8	12.1

[a] Log transformed.
[b] Square-root transformed.

Six of the nine original metrics were significantly correlated with site scores for DCA axes 1 and 2. The metrics with significant correlations to DCA Axis 1 were percent specialized insectivores (r = –0.77, $p = 0.0008$), number of darters (r = –0.73, $p = 0.0019$), number of intolerant species (r = –0.56, $p = 0.0295$), and percent omnivores plus herbivores (r = 0.52, $p = 0.0461$) (Figure 3a). Percent specialized insectivores and numbers of darter and intolerant species decreased with increased agricultural land use. However, all darter species and most intolerant species are specialized insectivores, so these metrics are not independent. The left side of the ordination diagram (Figure 2) was characterized by specialized insectivore minnow and darter species such as saffron shiner, mirror shiner, gilt darter, and redline darter, and also by fish species known to occur at higher elevations, including mottled sculpin, rainbow trout, and brown trout. The more agricultural

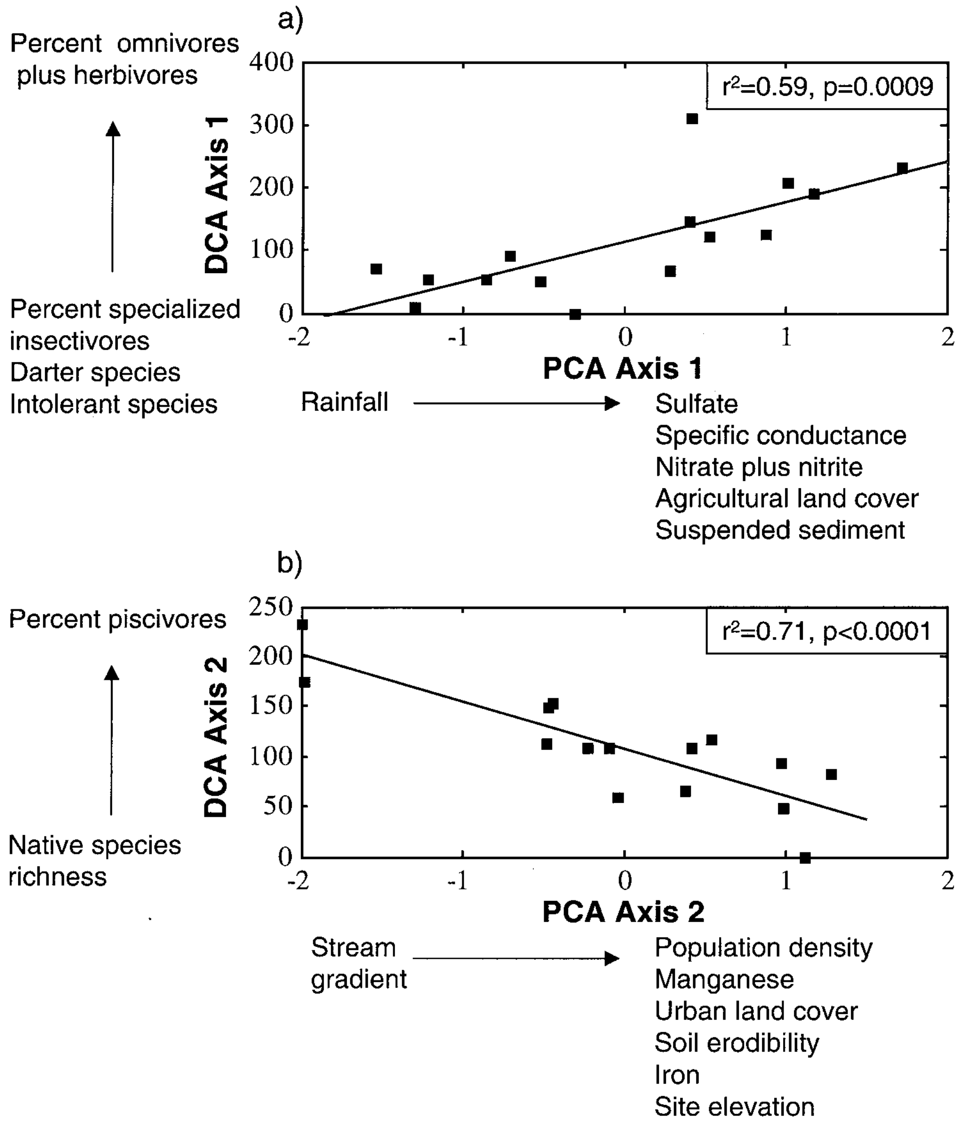

Figure 3. Significant linear regression results from (a) first axes and (b) second axes of principal components analysis (PCA) and detrended correspondence analysis (DCA). Also shown are the environmental variables with the highest absolute loadings on the PCA axes and the bioassessment metrics with significant relations to the DCA axes.

sites at the far right of Figure 2 were represented by generalized insectivorous minnow species such as blacknose dace, whitetail shiner, creek chub. The increase in the metric of percent omnivores plus herbivores with increasing agricultural landcover was due mostly to the increase in stonerollers, which are herbivorous. The assemblage changes identified in response to an increase in agricultural land cover are consistent with the expectations for these metrics in this basin (Saylor and Scott, 1987).

The metrics significantly correlated with DCA Axis 2 were native species richness (r = –0.61, $p = 0.0162$) and percent of piscivores (r = 0.58, $p = 0.0241$) (Figure 3b). That is, the urban sites at the base of Figure 2 contained more native species than the more forested sites located at the top of Figure 2. The increase in native species richness is unexpected, since this metric is expected to decline in response to degradation (Saylor and Scott, 1987). The bottom of the figure was characterized by two native sunfish species, green sunfish and bluegill, and by omnivores such as fathead minnow, golden shiner, and blotched chub. This region of the graph is also represented by three introduced species, redbreast sunfish, flat bullhead, and fathead minnow. The sites with greater urban landcover contained a lower percentage of piscivores than the more forested sites located at the top of Figure 2, as expected (Saylor and Scott, 1987). The more forested sites at the top of Figure 2 supported two piscivore species, smallmouth bass and rock bass, and were also characterized by four specialized insectivore species (telescope shiner, Tennessee shiner, greenside darter, greenfin darter).

4. Discussion

The design of this study allowed us to examine the influence of environmental variables on fish assemblages in the upper French Broad River basin, and a significant result was the identification of agricultural and urban influences. DCA Axis 1 was represented by percentage of agricultural land cover and several water quality variables that are known to be related to agriculture (Table III, Figure 3a). Increased nitrogen concentrations often result from agricultural applications of synthetic fertilizers or manure and can be used to infer agricultural influences (Lenat and Crawford, 1994). The high loading of specific conductance values may also point to agricultural sources (Brown, 2000). Higher specific conductances in agricultural sites could reflect the use of both ammonium and nitrate based fertilizers as well as heavy applications of manure. The increased sulfate could be due to ammonium sulfate, which is used as a fertilizer in North Carolina. Many required micronutrients such as copper, zinc, and iron are also commonly applied as sulfate salts. DCA Axis 2 likely represents urban influences; variables that loaded highly on this axis were percentage urban land cover, population density, iron concentrations, and manganese concentrations (Table III, Figure 3b). Population density is strongly related to urban activity and land development. Urban streams are often characterized by higher metals concentrations (Lenat and Crawford, 1994), which may account for the relations of this axis with iron and manganese concentrations. Additional assessment is needed to further elucidate specific environmental effects of urbanization.

In addition to the human-induced factors, natural environmental characteristics also appeared to play a role in structuring fish assemblages in the upper French Broad river basin. Changes in the fish assemblage along the first DCA axis were related to rainfall; the sites at the left end of DCA Axis 1 (Figure 2) were located in the headwaters of the basin where the highest rainfall occurs. The second DCA axis, in addition to reflecting urban land use, was also related to soil erodibility, stream gradient, and elevation. Other studies have demonstrated effects of stream gradient and elevation on fish assemblages (Edds, 1993; Maret *et al.*, 1997; Brown, 2000), but unlike these studies, results from the French Broad showed that sites with lower stream gradient were located at higher elevation. Such sites contained more urban land and occurred in and around the city of Asheville, which is located in an intermontane valley of moderate relief (USGS, 1979). The sites at the top of DCA axis 2 (Figure 2) are located furthest downstream in the upper French Broad basin where the river cuts through the core of the Appalachian mountains (USGS, 1979), so it is not surprising that these streams have a higher gradient and are more forested. These findings underscore the interplay of natural and human-induced factors in this basin.

The set of environmental variables used in this study were relatively successful in explaining the species patterns identified by DCA. The r^2 values for the regression, 0.59 and 0.71, are within the range of values reported for similar studies (0.3–0.8, e.g., Matthews and Robison, 1988; Ibarra and Stewart, 1989; Goldstein *et al.*, 1996). Environmental characteristics that may explain additional variance, but were not measured in this study, include influence from impoundments, effects of past land use, land use in the riparian zone, and point-source pollution. Impoundments, which occur on the Little and Ivy Rivers, can restrict the movement of fishes and alter aquatic habitat. Past land use can have long-term consequences for fish assemblages. However, Harding *et al.* (1998), in a study of the influence of past land use on present-day fish species richness in North Carolina river basins, found that species richness in the upper French Broad was best explained by localized current land use. Localized land use effects, particularly in the riparian zone, can influence stream fish assemblages because stream ecosystems depend on inputs of woody debris and organic matter from the riparian zone (Allan, 1995). Point-source industrial and domestic pollution can also have serious effects on fish assemblages; such pollution has long been observed in the upper French Broad River basin (TVA, 1945; USGS, 1979; North Carolina Department of Environment and Natural Resources, 1998). This could explain why Mud Creek was an outlier compared to the rest of the sites in the basin. This analysis suggests that further study is needed to identify causes of impairment for this site in particular.

Multimetric and multivariate techniques used in this study were complementary in assessing the influence of environmental characteristics on fish assemblages (Norris, 1995). Metrics are useful for data interpretation because they summarize changes in multiple species and highlight biologically meaningful patterns (Fausch *et al.*, 1990; Gerritsen, 1995). Multivariate techniques provide an objective way of identifying the relative importance of various factors in structuring fish assemblages (Fausch *et al.*, 1990). A multivariate approach also allows us to go beyond the assumption used in bioassessment that anthropogenic influence occurs in a single dimension, and allows us to gain insight into multiple dimensions of fish assemblage change. Designating only two land use types, developed and undeveloped, is most likely an oversimplification for examining human impacts on fish assemblages (Hall *et al.*, 1996).

The effect of increasing agricultural land cover in the basin was reflected primarily by a shift in the trophic structure of the fish assemblage from specialized insectivores to one herbivore and generalized insectivores. The decline in specialized insectivores may be due to a reduction in their food source, because certain species of benthic invertebrates recognized as intolerant to pollution are known to decline with human impact in this geographic area (Lenat, 1993; Kerans and Karr, 1994). In particular, some benthic invertebrates have been shown to be highly sensitive to increases in sediment (Richards and Host, 1994; Wood and Armitage, 1997). The increase in generalized insectivores could be due to release from competition for food. The increase in the relative abundance of the herbivorous central stoneroller is likely due to increasing algal density, which in turn is influenced by the increased nutrient input associated with agricultural activity (Matthews *et al.*, 1987).

An increase in urban land cover along the DCA Axis 2 was related to an increase in the number of both native and introduced species. Although the native species metric is expected to decline in response to degradation, this is not always observed. For example, Lyons *et al.* (1996) found that environmental degradation caused an increase in species richness in coldwater fish assemblages, due to the addition of tolerant warmwater species, including sunfish and suckers. Land-use development and associated effluents can lead to increased stream temperature (Hynes, 1970). Temperature increases are difficult to detect through the one-time sampling methods used here, but may be biologically significant in a high-elevation system like the upper French Broad. Flow and habitat modification associated with urbanization may also promote the invasion of certain native sunfish and minnow species that are habitat generalists (Mayden, 1987). These conditions also favor invasions by non-native species.

The basin-scale design and approach used here can be applied to improve stream assessment and management. For example, this approach has shown how metrics differ from one another in their responses to environmental gradients, and as such, certain metrics may serve as more sensitive indicators than a multimetric index. Specialized insectivores were most sensitive to impairment due to an increase in agricultural land cover. Species richness may not be the best indicator of integrity in this basin, due to the increase in non-native and warmwater generalists with increasing urban impairment. The identification of useful indicators, or assessment endpoints, allows management objectives to be more explicitly defined (Serveiss, 2002). Also, this study identified two different paths of fish assemblage change associated with agricultural and urban influence; the recognition of the different changes that occur along these two pathways can be used to diagnose the possible cause of stream impairment in this basin and in similar basins (Norton *et al.*, 2000). This allows for more specific and targeted management options to be employed. For example, a stream impaired by urban influence may require restoration strategies that differ from the best management practices designed to minimize the effects of agriculture on aquatic systems. A basin-scale study such as this can support a more integrated strategy for the management of water quality and biological integrity of streams within a basin.

Acknowledgements

We thank Jeff Powell, Steve Ahlstedt, Charlie Saylor, Chris Underwood, Rick Treece, Greg Johnson, Joe Connell, and Andy Hickey for field assistance, and Bob Baca for review. We are particularly grateful to Jonathan Kennen for assistance in statistical analysis and for his review. We thank Paul Hampson for his assistance with this paper. This paper has been reviewed in accordance with the U.S. Environmental Protection Agency's peer and administrative review policies and approved for publication.

References

Allan, J. D.: 1995, *Stream Ecology: Structure and Function of Running Waters*, Chapman & Hall, London, 388 pp.

Angermeier, P. L. and Schlosser, I. J.: 1987, 'Assessing biotic integrity of the fish community in a small Illinois stream', *N. Amer. J. Fish. Manage.* **7**, 331–338.

Angermeier, P. L. and Winston, M. R.: 1999, 'Characterizing fish community diversity across Virginia landscapes: Prerequisite for conservation', *Ecol. Appl.* **9**, 335–349.

Brown, L. R.: 2000, 'Fish communities and their associations with environmental variables, lower San Joaquin River drainage, California', *Env. Biol. Fish.* **57**, 251–269.

Bryce, S. A., Larsen, D. P., Hughes, R. M. and Kaufmann, P. R.: 1999, 'Assessing relative risks to aquatic ecosystems: A mid-Appalachian case study', *J. Amer. Water Res. Assoc.* **35**, 23–36.

Changeux, T. and Pont, D.: 1995, 'Ichthyogeographic regions and watershed size in the French river Rhone network', *Hydrobiologia* **300–301**, 355–363.

Edds, D. R.: 1993, 'Fish assemblage structure and environmental correlates in Nepal's Gandaki River', *Copeia* **1993**, 48–60.

Etnier, D. A. and Starnes, W. C.: 1993, *Fishes of Tennessee*, University of Tennessee Press, Knoxville, Tennessee, 681 pp.

Fausch, K. D., Lyons, J., Karr, J. R. and Angermeier, P. L.: 1990. 'Fish communities as indicators of environmental degradation', *Amer. Fish. Soc. Symp.* **8**, 123–144.

Gauch Jr., H. G.: 1982, *Multivariate Analysis in Community Ecology*, Cambridge University Press, Cambridge, 298 pp.

Gerritsen, J.: 1995, 'Additive biological indices for resource management', *J. N. Amer. Benthol. Soc.* **13**, 451–457.

Glantz, S. A. and Slinker, B. K.: 1990, *Primer of Applied Regression and Analysis of Variance*, McGraw-Hill, Inc., New York, 777 pp.

Goldstein, R. M., Stauffer, J. C., Larsen, P. R. and Lorenz, D. L.: 1996, *Relation of Physical and Chemical Characteristics of Streams to Fish Communities in the Red River of the North Basin, Minnesota and North Dakota, 1993–1995*, U.S. Geological Survey Water Resources Investigations Report 96-4227, Mounds View, Minnesota.

Hall Jr., L. W., Scott, M. C., Killen Jr., W. D. and Anderson, R. D.: 1996, 'The effects of land- use characteristics and acid sensitivity on the ecological status of Maryland coastal plain streams', *Environ. Toxicol. Chem.* **15**, 384–394.

Harding, J. S., Benfield, E. F., Bolstad, P. V., Helfman, G. S. and Jones III, E. B. D.: 1998, 'Stream biodiversity: The ghost of land use past', *Proc. Nat. Acad. Sci*. **95**, 14843–14847.

Harned, W. D.: 1979, *A Quantitative Survey of Fish and Macroinvertebrates of French Broad River and Selected Tributaries: June–August 1977*, Tennessee Valley Authority Technical Note B35, Division of Water Resources, Office of Natural Resources, Norris, Tennessee.

Holling, C. S.: 1992, 'Cross-scale morphology, geometry, and dynamics of ecosystems', *Ecol. Mon*. **62**, 447–502.

Hughes, R. M., Kaufmann, P. R., Herlihy, A. T., Kincaid, T. M., Reynolds, L. and Larsen, D. P.: 1998, 'A process for developing and evaluating indices of fish assemblage integrity', *Can. J. Fish. Aquat. Sci*. **55**, 1618–1631.

Hynes, H. B. N.: 1970, *The Ecology of Running Waters*, University of Toronto Press, Toronto, Canada, 555 pp.

Ibarra, M. and Stewart, D. J.: 1989, 'Longitudinal zonation of sandy beach fishes in the Napo River basin, eastern Ecuador', *Copeia* **1989**, 364–381.

Karr, J. R.: 1981, 'Assessment of biotic integrity using fish communities', *Fisheries* **6**, 21–27.

Karr, J. R. and Chu, E. W.: 1999, *Restoring Life in Running Waters: Better Biological Monitoring*, Island Press, Washington D.C., 206 pp.

Kerans, B. L. and Karr, J. R.: 1994, 'A benthic index of biotic integrity (B-IBI) for rivers of the Tennessee Valley', *Ecol. Appl.* **4**, 768–785.

Lenat, D. R.: 1993, 'A biotic index for the southeastern United States: Derivation and list of tolerance values, with criteria for assigning water-quality ratings', *J. N. Amer. Benthol. Soc*. **12**, 279–290.

Lenat, D. R. and Crawford, J. K.: 1994, 'Effects of land use on water quality and aquatic biotic of three North Carolina Piedmont streams', *Hydrobiologia* **294**, 185–199.

Leonard, P. M. and Orth, D. J.: 1986, 'Application and testing of an index of biotic integrity in small, coolwater streams', *Trans. Amer. Fish. Soc*. **115**, 401–414.

Lyons, J.: 1996, 'Patterns in the species composition of fish assemblages among Wisconsin streams', *Env. Biol. Fish*. **45**, 329–341.

Lyons, J., Wang, L. and Simonson, T. D.: 1996, 'Development and validation of an index of biotic integrity for coldwater streams in Wisconsin', *N. Amer. J. Fish. Manage*. **16**, 241–256.

Maret, T. R., Robinson, C. T. and Minshall, G. W.: 1997, 'Fish assemblages and environmental correlates in least-disturbed streams of the Upper Snake River basin', *Trans. Amer. Fish. Soc*. **126**, 200–216.

Matthews, W. J.: 1998, *Patterns in Freshwater Fish Ecology*, Chapman & Hall, New York, 757 pp.

Matthews, W. J. and Robison, H. W.: 1988, 'The distribution of the fishes of Arkansas: A multivariate analysis', *Copeia* **1988**, 358–374.

Matthews, W. J., Stewart, A. J. and Power, M. E.: 1987, 'Grazing Fishes as Components of North American Stream Ecosystems: Effects of *Campostoma anomalum*', in W. J. Matthews and D. C. Heins (eds), *Community and Evolutionary Ecology of North American Stream Fishes*, University of Oklahoma Press, Norman, Oklahoma, pp. 128–135.

Mayden, R. L.: 1987, 'Historical Ecology and North American Highland Fishes: A Research Program in Community Ecology', in W. J. Matthews and D. C. Heins (eds), *Community and Evolutionary Ecology of North American Stream Fishes*, University of Oklahoma Press, Norman, Oklahoma, pp. 210–222.

McCune, B. and Mefford, M. J.: 1999, *PC-ORD Multivariate Analysis of Ecological Data*, Version 4, MjM Software Design, Glenden Beach, Oregon, 237 pp.

Meador, M. R., Cuffney, T. F. and Gurtz, M. E.: 1993, *Methods for Characterizing Fish Communities as Part of the National Water Quality Assessment Program*, U.S. Geological Survey Open File Report 93-104, Raleigh, North Carolina, 40 pp.

Miller, D. L., Leonard, P. M., Hughes, R. M., Karr, R., Moyle, P. B., Schrader, L. H., Thompson, B. A., Daniles, R. A., Fausch, K. D., Ftizhugh, G. A., Gammon, J. R., Halliwell, D. B., Angermieier, P. L. and Orth, D. J.: 1988, 'Regional application of an index of biotic integrity for use in water resource management', *Fisheries* **13**, 12–20.

Norris, R. H.: 1995, 'Biological monitoring: The dilemma of data analysis', *J. N. Amer. Benthol. Soc.* **14**, 440–450.

North Carolina Department of Environment and Natural Resources: 1998, *Basinwide Assessment Report: French Broad River Basin*, Division of Water Quality, Raleigh, North Carolina, 203 pp.

Norton, S. B., Cormier, S. M., Smith, M. and Jones, R. C.: 2000, 'Can biological assessment discriminate among types of stress? A case study from the Eastern Corn Belt Plains Ecoregion', *Environ. Toxicol. Chem.* **19**, 1113–1119.

Omernick, J. M.: 1987, 'Ecoregions of the conterminous United States', *Ann. Assoc. Amer. Geograph.* **77**, 118–125.

Richards, C. and Host, G.: 1994, 'Examining land use influences on stream habitat and macroinvertebrates: A GIS approach', *Water Resour. Bull.* **30**, 729–38.

SAS Institute, Inc.: 1988, *SAS/STAT user's guide, Release 6.03 Edition*, SAS Institute Inc., Cary, North Carolina, 1028 pp.

Saylor, C. and Scott Jr., E. M.: 1987, *Application of the Index of Biotic Integrity to Existing TVA Data*, Tennessee Valley Authority, Division of Air and Water Resources, TVA/ONRED/ AWR 87/32, Norris, Tennessee, 25 pp.

Serveiss, V. B.: 2002, 'Applying ecological risk principles to watershed assessment and management', *Env. Manag.* **29**, 145–154.

Shelton, L. R.: 1994, *Field Guide for Collecting and Processing Stream-water Samples for the National Water-Quality Program*, Open File Report 94-455, U.S. Geological Survey, Reston, Virginia, 42 pp.

Tennessee Valley Authority: 1945, *Studies of the Pollution of the Tennessee River System III. French Broad System*, Tennessee Valley Authority, Health and Safety Department, Knoxville, Tennessee, 62 pp.

Ter Braak, C. J. F. and Smilauer, P.: 1998, *CANOCO Reference Manual and User's Guide to Canoco for Windows: Software for Canonical Community Ordination (Version 4)*, Microcomputer Power, Ithaca, New York, 351 pp.

U.S. Environmental Protection Agency: 2001, *Protecting and Restoring America's Watersheds*, EPA 840-R-00-001, 56 pp.

U.S. Geological Survey: 1979, *Water Quality of the French Broad River, North Carolina*, Water Resource Investigations 79-87, Raleigh, North Carolina, 53 pp.

Waite, I. R. and Carpenter, K. D.: 2000, 'Fish communities in the Willamette Valley, Oregon, and their relationships to stream habitat and water quality: a multivariate statistical approach', *Trans. Amer. Fish. Soc.* **129**, 754–770.

Whittier, T. R., Hughes, R. M. and Larsen, D. P.: 1988, 'Correspondence between ecoregions and spatial patterns in stream ecosystems in Oregon', *Can. J. Fish. Aquat. Sci.* **45**, 1264–1278.

Wood, P. J. and Armitage, P. D.: 1997, 'Biological effects of fine sediment in the lotic environment', *Environ. Manage.* **21**, 203–217.

Zampella, R. A. and Bunnell, J. F.: 1998, 'Use of reference-site fish assemblages to assess aquatic degradation in pinelands streams', *Ecol. Appl.* **8**, 645–658.