Division of Water Resources
Water Sciences Section
Biological Assessment Branch

December 16, 2014

MEMORANDUM

To: Dianne M. Reid

Through: Eric Fleek

From: Bryn H. Tracy

Subject: Fish Community Metric Calibration and Rating Development for Wadeable Streams in the Sand Hills (Cape Fear, Lumber, and Yadkin River basins)[1]

INTRODUCTION

Fish community data from wadeable Sand Hills streams have been collected by Division of Water Resources (DWR, formerly known as the Division of Water Quality) staff since 1990 following existing standard operating procedures (NCDEHNR 1995; NCDEHNR 1997; NCDENR 2006). However, metrics and biocriteria were never developed specifically for these unique communities (more aptly termed assemblages (Fauth, et al. 1996) which often have naturally low species diversity and low biological productivity (NCDENR 2004). Sand Hills fish communities were not rated accurately (i.e., bioclassifications were too low for streams with no or minimal anthropogenic impacts) after applying the existing metrics and biocriteria for streams in the Cape Fear River or in the Yadkin River basins (NCDENR 1999; Tracy 2003). Similarly, Paller et al. (1996), using NCDENR (1995) methods, were unable to discriminate between disturbed and undisturbed fish communities in Sand Hills streams even after the existing metrics and biocriteria were altered to better suit Sand Hills streams in South Carolina. Consequently, the North Carolina Sand Hills fish communities have been classified by Biological Assessment Branch (BAB, formerly known as the Biological Assessment Unit, BAU) staff as Not Rated until metrics and biocriteria could be developed (NCDENR 2004).

The purpose of this document is to describe the analytical steps followed in deriving metrics and biocriteria for rating fish communities in wadeable streams in the North Carolina Sand Hills. The final product is known as the Sand Hills North Carolina Index of Biological Integrity (Sand Hills NCIBI). The Sand Hills NCIBI would be an additional biological monitoring tool that could be used by the DWR in evaluating wadeable streams in the Sand Hills, complementing the existing benthic macroinvertebrate assessments.

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[1] This document was approved by Eric Fleek and Dianne Reid on October 15, 2015 to be released for external peer review. On October 15, 2015, it was distributed to Dr. Paul Angermeier (Virginia Tech University), Dr. Thomas Cuffney (USGS, Raleigh, NC), Dr. Johnathan Kennen (USGS, Lawrenceville, NJ), Dr. Thomas Kwak (North Carolina State University), Ms. Patti Landford (GA Department of Natural Resources), and Ms. Paula Marcinek (GA Department of Natural Resources). All comments were returned by December 10th. Revisions were made as deemed necessary.

Description of the Sand Hills in North Carolina

In North Carolina, the Sand Hills, a U.S. EPA Level IV ecoregion, encompasses 1,573 mi^2 across three river basins (Yadkin-Pee Dee, Lumber, and Cape Fear) (Rohde and Arndt 1991; Griffith et al. 2002; Figures1 and 2). The region also extends southwest into South Carolina and Georgia. Large municipal areas include the cities of Southern Pines, Rockingham, and Fayetteville; military reservations include Fort Bragg and Camp Mackall. Landuse types, based upon the 2006 National Land Cover Database (NLCD), are 61% forest, 18% grassland, 11% developed, 7% agriculture, and 3% barren and water (Figure 2). These percentages are only for the Sand Hills Level IV ecoregion and do not include any adjacent (peripheral) watersheds which were also included in this study, for example, the Cross Creek watershed draining Fayetteville which is within the Southeastern Floodplains & Low Terraces and the Atlantic Southern Loam Plains (Figures 1 and 2). [Note: for reasons why these adjacent sites were included, please refer to the text on Page 6.]

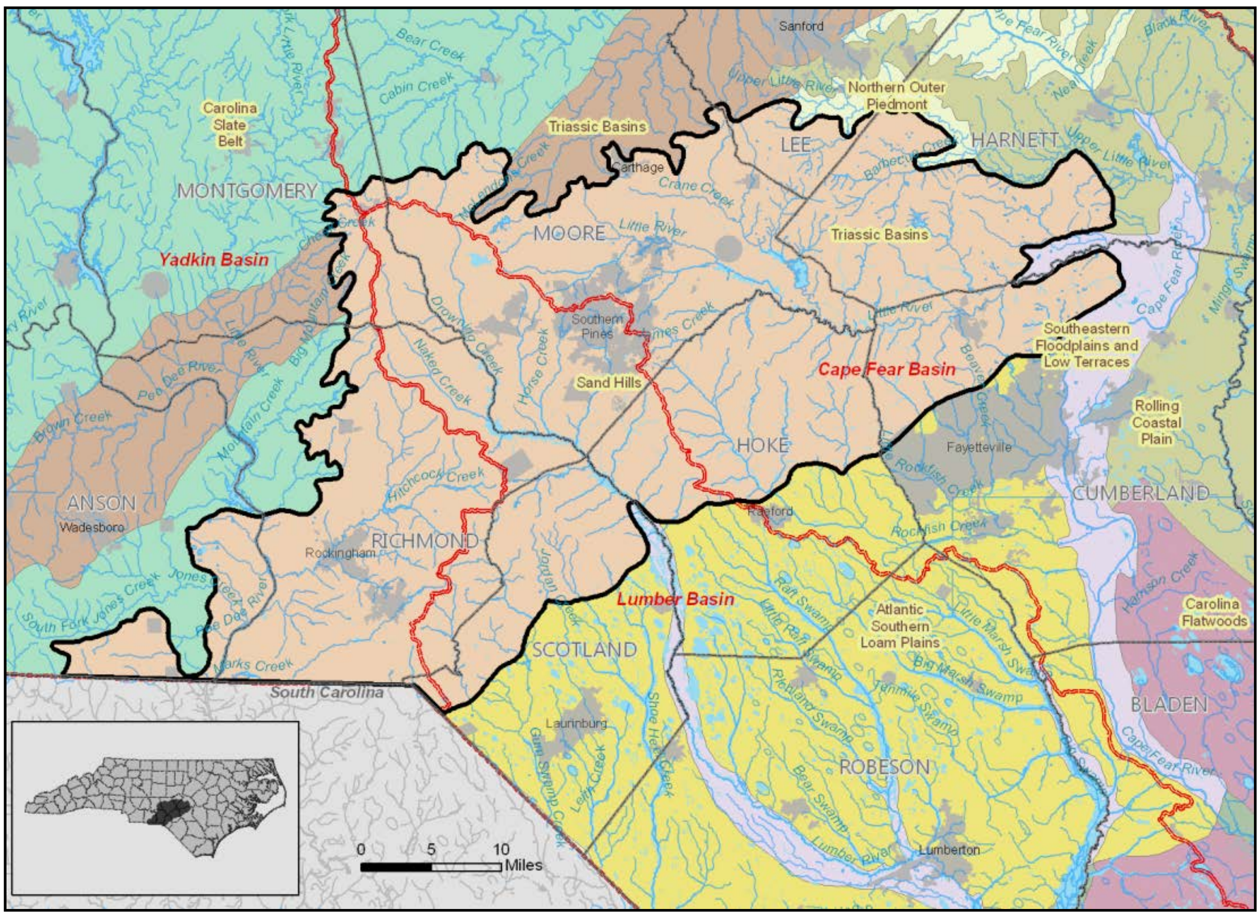


Figure 1. **The Sand Hills and adjacent Level IV ecoregions in North Carolina.**

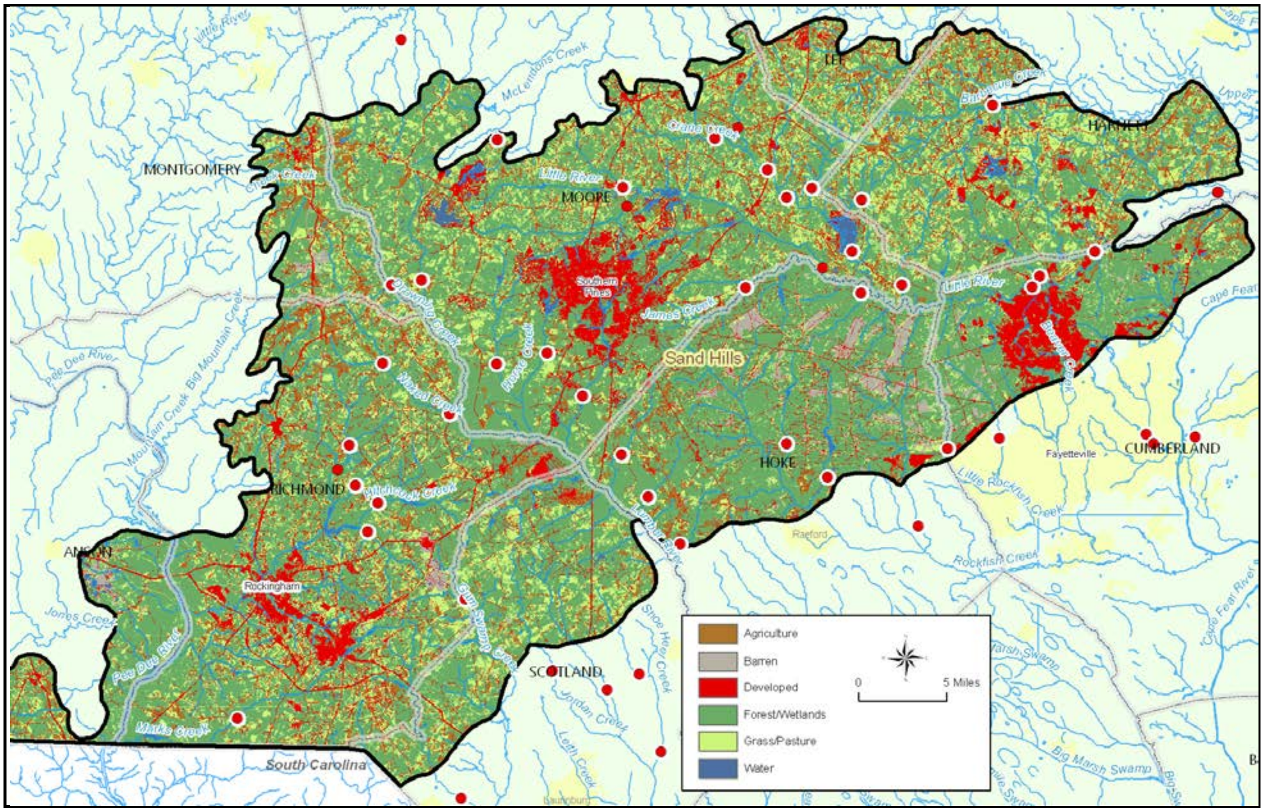


Figure 2. **Landuse types for the Sand Hills Level IV ecoregion in North Carolina. Fish community sites considered in this study are shown as red dots (n=51). Red dots shown outside the ecoregion were also included in the study; see text on Pages 4 and 5 for the explanation.**

Sand Hills streams are clear and tannin stained, with low specific conductance and pH, with moderate to swift velocities, and permanent, year-round flow in the larger watersheds. However, during extreme droughts, streams draining even the larger watersheds may cease flowing and those draining smaller watersheds may dry up entirely (e.g., Snelson and Suttkus 1978). Substrates are often white quartz sand and gravel with variable amounts of submerged, coarse, woody debris. Aquatic macrophytes and macroalgae may be abundant in sun-lit areas such as at bridge crossings and road and utility line right-of-ways. Aquatic macrophytes, including Spatterdock, Arrowhead, Golden Club, Bur-Reed, Eel-Grass, sedges, pondweed, and a red alga (*Batrachospermum* sp.), are often observed (Rohde and Ross 1987). The riparian areas are generally intact bottomland forests of American Holly, Red Bay, Red Maple, and Bald Cypress; the upland forests are composed of Loblolly Pine, Longleaf Pine, Turkey Oak, and Wire Grass (Griffith et al. 2002). Many of the first and second order headwater streams are impounded for amenity lakes, golf course ponds, and municipal drinking water supply reservoirs, fragmenting aquatic habitats and fish communities (Rohde and Arndt 1991; Figures 1 and 2). More detailed descriptions of the streams and the ecoregion may be found in summary water quality assessments for each individual river basin in North Carolina (NCDENR 2004, 2007a, 2007b).

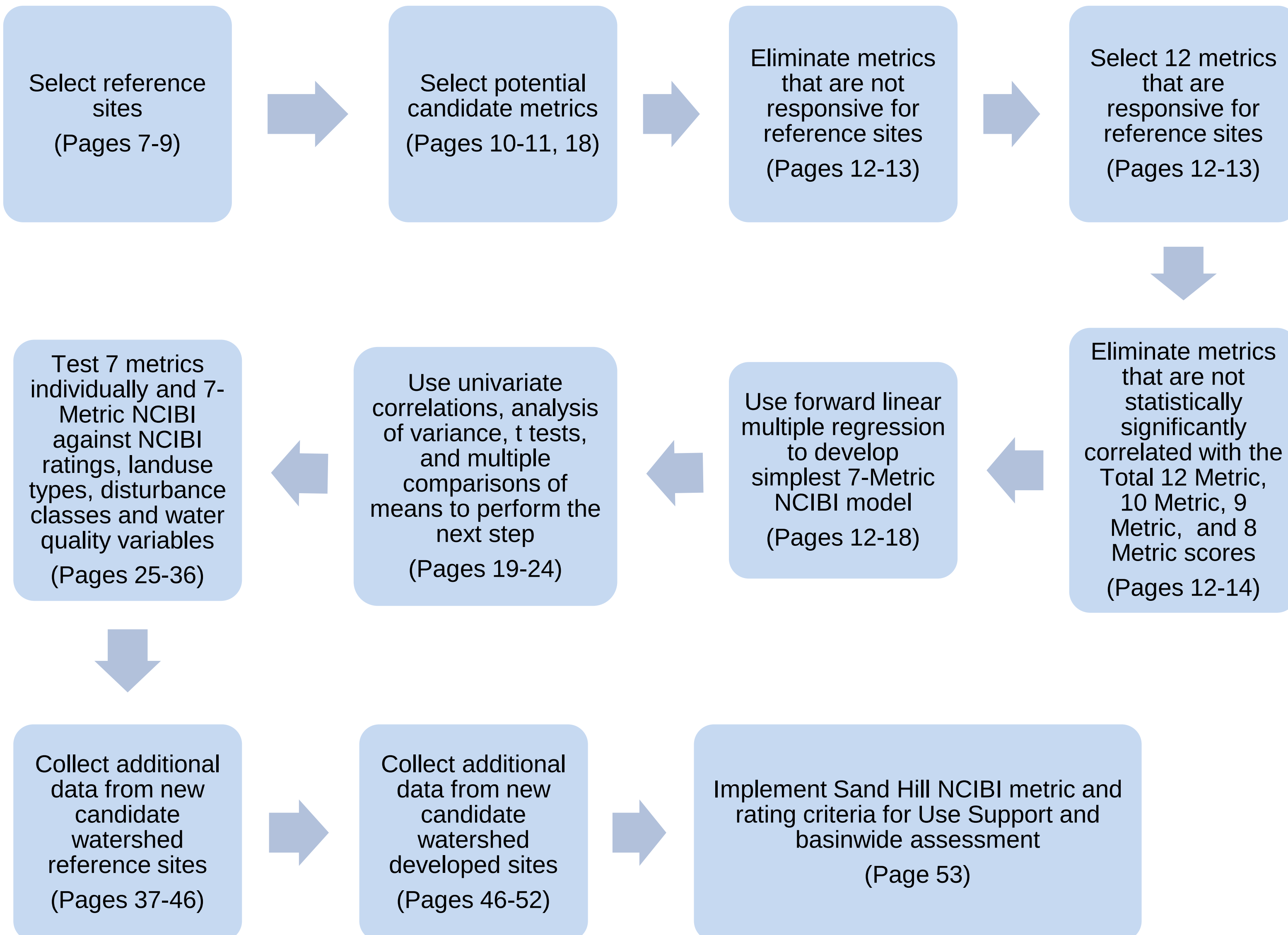
In North Carolina, the fish fauna in the Sand Hills is comprised of 67 species, including 18 species of minnows, 16 species of sunfish, and 7 species each of catfishes and darters (Appendix 1; DWR unpublished data; Menhinick 1991; North Carolina State Museum of Natural Sciences fishes database (http://collections.naturalsciences.org/searchFishes.aspx). [Note: excluded from this list are three nonindigenous species of catfish (Flathead Catfish, Blue Catfish, and Channel Catfish) that typically would not be found in wadeable Sand Hills streams, rather preferring larger waterbodies such as the mainstem reaches of the Cape Fear, Lumber, and Pee Dee rivers.] Within the Sand Hills, the fauna of the Cape Fear River Basin is the most diverse with 64 species, followed by the Lumber with 50 species, and the Yadkin with 48 species. Thirteen species are considered as Tolerant, 12 species as Intolerant, and 42 as Intermediate to the effects of "pollution" by DWR staff in consultation with other scientists familiar with North Carolina's fish fauna (Appendix 1; NCDEHNR 1995; NCDENR 2006). The Sand Hills area includes many priority aquatic species and areas for habitat conservation and protection (NCWRC 2005). Compared to other river basins and regions, the fauna of the Sand Hills has not been significantly homogenized by nonindigenous (nonnative and invasive) species. Only 5 of the 67 species, Redlip Shiner (Lumber and Cape Fear), Green Sunfish (Yadkin and Cape Fear), Redear Sunfish (Lumber, Cape Fear, and Yadkin), Spotted Bass (Cape Fear), and Yellow Perch (Yadkin), are considered nonindigenous in these basins (Appendix 1; http://portal.ncdenr.org/web/wq/ess/bau/nativefish). Endemic and state-listed species include Thinlip Chub, Sandhills Chub, and Pinewoods Darter (all state Special Concern species), and Banded Sunfish (state Significantly Rare species) (NCWRC 2005; LeGrand et al. 2014).

METHODS

> A flow chart (found on Page 5) was developed to guide the reader through the process on how an Index of Biotic Integrity was developed for the North Carolina Sand Hills. The reader is encouraged to frequently consult the flow chart as the index is being developed and tested in this document.

All data collected for these analyses were obtained prior to 2009, except for data collected in October 2013 and June 2014. Fish community data from wadeable streams have been collected sporadically since 1990, usually in the spring, as part of the Cape Fear, Lumber, and Yadkin River basinwide monitoring programs (i.e., in 1990, '91, '94, '96, '98, '01, '03, '06, '08, and '09) and for one special study (BAU 2002a; Appendices 2 and 3). The data are from lower Strahler order streams that are wadeable from one shoreline across to the other and for a stream length distance of 600 feet. Most of the stations are located at bridge crossings or other publicly accessible areas. Small headwater streams that are overgrown with riparian vegetation and difficult to sample and higher Strahler order rivers that are not wadeable were not assessed. Collection methods have not changed and have followed existing standard operating procedures (NCDEHNR 1995; NCDEHNR 1997; NCDENR 2006) with water quality (temperature, dissolved oxygen, pH, and specific conductance) and habitat data collected simultaneously at least since 1996. Habitat data, including width of riparian zones, were determined for all but three samples and specific conductance data was collected for all but one sample (Appendix 3). Landuse/landcover data for each site were determined using the 2006 NLCD coverage (Appendix 3).

Flowchart for Developing an NCIBI for the North Carolina Sand Hills



Streams draining the southeast portion of the Sand Hills in Cumberland, Scotland, and Hoke County (Figures 1-3; Appendix 2) are technically placed in the U.S. EPA Level IV ecoregions of the Atlantic Southern Loam Plains and Southeastern Floodplains and Low Terraces by Griffith et al. (2002). However, their fish communities share faunal (same species) and physico-chemical (tannin stained, low pH, and low specific conductance) similarities with those of the Sand Hills (DWR unpublished data). In fact, Griffith et al. (2002) pointed out: ". . . *some aquatic ecologists suggested our boundary be moved further southeast, as streams coming out of the Sand Hills maintain those regional characteristics downstream*". For this study, the Sand Hills dataset was expanded to include these streams (such as Jordan and Juniper creeks) in Scotland, Hoke, and Cumberland counties (Figure 3). Other sites were excluded from the Sand Hills dataset if the fauna were more similar to that of Piedmont streams than that of the Sand Hills (e.g. Gum Log Canal) or if the stream characteristics were not Sand Hills-like (Figure 3; Appendix 4). These included streams in the Carolina Slate Belt and the Triassic Basins such as Wet and McLendons creeks (Figure 1).

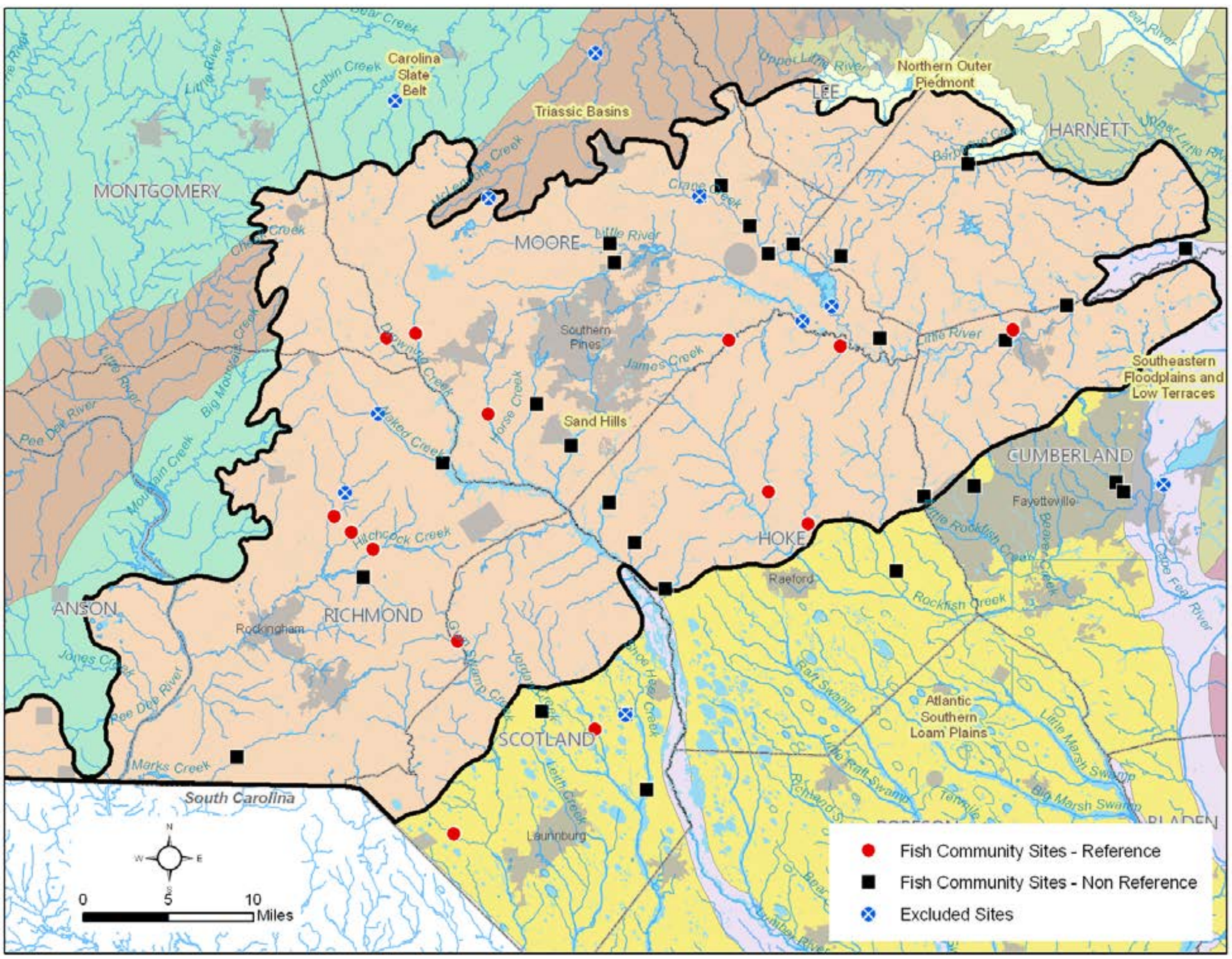


Figure 3. **Reference (n=14) and non-reference (n=29) fish community sites in the Sand Hills and adjacent ecoregions. Ten sites, shown as blue dots, were excluded from final analyses; the explanation for doing so may be found in Appendix 4.**

Reference Site Selection

As defined in the North Carolina Administrative Code (NCAC), "*biological integrity means the ability of an aquatic ecosystem to support and maintain a balanced and indigenous community of organisms having species composition, diversity, population densities and functional organization similar to that of the reference condition*" (NCAC 2007 -- 15 NCAC 02B.0202 (11)). Reference conditions are not further defined in the NCAC, but are of the utmost importance in the assessment of ecological integrity in all streams (Kwak and Freeman 2010). Although there are no clear criteria for the selection of a reference system or definition of reference conditions, their selection is crucial in the development of methods to evaluate the biological integrity of a stream. Reference sites represent the least impacted (minimally altered) streams and the best biological condition of the aquatic communities that can be attained (Hughes 1995; Hughes et al. 1986; USEPA 1996; USEPA 1999; Griffith et al. 2002; Stoddard et al. 2006). Since 2000, BAB has used watershed-derived characteristics and biocriteria (USEPA 1996; USEPA 1999) to select reference sites and to develop reference site based fish community metrics. This method was adopted in Water Sciences Section (formerly known as the Environmental Sciences Section)-approved memoranda (BAU 2002b; 2002c; 2002d), in all basinwide assessment reports since 2001 (e.g., NCDENR 2007b), and in the current standard operating procedures (NCDENR 2013). Parts of it have also been adopted in BAB's Reference Site Selection and Criteria Development (http://portal.ncdenr.org/web/wq/ess/bau/refs). Such methods, based upon systematically-gathered data, are preferable to subjective methods based upon expert opinion (Kwak and Freeman 2010). Reference sites were selected based on the six criteria presented in Table 1.

Table 1. Reference site selection hierarchy -- a watershed-based approach for streams.

1. Total habitat score - ≥ 65;
2. No NPDES permitted wastewater dischargers greater than 0.01 MGD above the site or if there are small wastewater dischargers (~≤ 0.01 MGD), the dischargers are more than one mile upstream;
3. Percentage of the watershed Developed[1] (which includes open space, lawns, low-high intensity development, and golf courses and is a surrogate for imperviousness cover) - < 15%;
4. Percentage of the watershed Forested[1] (which includes deciduous, evergreen, mixed, dwarf scrub, shrub/scrub, and wetlands) - ≥ 55%;
5. Percentage of the watershed Grassland/Pasture[1] (which includes grassland/herbaceous, sedge/herbaceous, and pasture/hay) - < 25%; and
6. Width and integrity of the riparian zone (no breaks in the riparian zones or, if there are breaks, the breaks are rare) - > 18m.

[1] Landuse categories were based upon the 2006 NLCD codes for Forest (41, 42, 43, 52, 90, 91, and 95), for Developed (21, 22, 23, and 24), for Cultivation (82 and 81), for Barren Rock (31), and for Grassland/Herbaceous (71).

The process of reference site selection began with 94 samples from 51 unique sites (Appendix 3, Figures 2 and 3). From this list, 64 samples were eliminated from consideration as reference samples:

- 13 samples were eliminated for because they did not meet established DWR selection criteria as explained in Appendix 4;
- 2 samples had a total habitat score less than 65;
- 4 samples had wastewater dischargers above the site;

- 7 samples had development exceeding 15%;
- 31 samples had percent forest less than 55%;
- 5 samples had percent grass/pasture greater than 25%; and
- 2 samples had riparian zones less than 18 m wide.

After these 64 eliminations, the selection process ultimately yielded 30 samples from 14 sites that qualified as reference sites and 51 samples from 29 sites that qualified as non-reference sites (Appendices 3 and 4; Figure 3). Thirteen sites were eliminated from any further analyses in this study (Figure 3; Appendix 4).

The number of reference sites/samples from each basin were: Cape Fear (5 sites, 10 samples), Lumber (6 sites, 14 samples), and Yadkin (3 sites, 6 samples) (Table 2; Appendices 3 and 5). The ranges for drainage area, percentage of landuse types, and water quality variables are listed in Appendix 3. All of the reference and non-reference site watersheds had at least some degree of development (see the section on Landuse Types and Disturbance Classes, Pages 11-12). The non-reference sites accounted for streams ranging from impacted to minimally impacted (Appendix 3). Some of the minimally impacted sites did not qualify as reference sites, because they lacked one or more of the characteristics listed in Table 1. For example, if less than 55% of a sites' watershed was forested, it failed to qualify as a reference site (e.g., Little Rockfish Creek).

Data Analyses and Interpretation

There are many region-specific variations of accepted metrics used in the development of an Index of Biotic Integrity (IBI) (Karr 1981; Fausch et al. 1984; Karr et al. 1986; Simon and Lyons 1995; Paller et al. 1996; USEPA 1996; Hughes et al. 1998; Karr and Chu 1999; USEPA 1999; McCormick et al. 2001). DWR analyses followed previously used and approved methods for metric criteria calibration and biocriteria development (BAU 2002a; 2002b; 2002c; Tracy 2003). Criteria development of the new Sand Hills NCIBI methods considered the following objectives:

1. The criteria must be scientifically defensible.
2. The end points and criteria should be calibrated using reference data as required by NCAC 2007 -- 15 NCAC 02B.0202 (11) from the physiographic region (Mountains, Piedmont, Sand Hills, Inner Coastal Plain, and Outer Coastal Plain) or river basin under study.
3. The individual metric maximum scores should be achievable or explainable by the reference samples.
4. The criteria should be able to account for differences between reference and degraded sites.

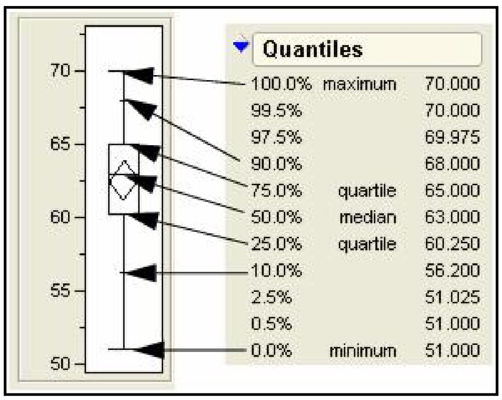
Table 2. **Reference streams including drainage area and landuse types (in percent, from the 2006 NLCD). Landuse disturbance classes are defined as: Forested (F, ≥ 65% forest cover) and Mostly Forested (MF, 55-64% forest cover).**

Basin/Waterbody	Station	County	Latitude	Longitude	Drainage Area (mi^2)	Developed	Forest	Grassland	Agr.	Barren	Impervious Cover	Land Use Class
Cape Fear												
Flat Cr	Manchester Rd	Hoke	35.182500	-79.177500	7.7	6.5	59.4	17.5	0.0	16.5	2.82	MF
James Cr	off SR 2026	Moore	35.187222	-79.293333	11.4	10.7	66.4	15.5	2.8	4.0	2.64	F
Muddy Cr	SR 1001	Cumberland	35.196667	-78.998611	15.7	8.2	73.0	16.0	1.6	0.7	5.20	F
Juniper Cr	Plank Rd	Hoke	35.058333	-79.252222	11.0	8.9	64.9	20.9	0.0	4.2	3.18	F
Nicholson Cr	SR 1301	Hoke	35.030833	-79.210556	17.0	10.8	63.1	18.3	0.1	5.8	3.41	MF
Lumber												
Deep Cr	SR 1113	Moore	35.123056	-79.542778	19.3	7.8	61.3	23.9	6.4	0.0	2.66	MF
Drowning Cr	NC 73	Moore	35.187778	-79.648611	31.0	3.3	62.2	20.7	13.1	0.0	1.92	MF
Jackson Cr	SR 1122	Moore	35.191667	-79.618611	17.8	8.7	61.7	16.2	6.1	0.0	3.28	MF
Gum Swamp Cr	SR 1344	Scotland	34.929444	-79.573056	16.2	8.5	55.3	24.6	4.4	6.3	4.04	MF
Joes Cr	NC 79	Scotland	34.765278	-79.575556	30.8	8.5	61.4	18.2	11.5	0.0	3.80	MF
Juniper Cr	SR 1405	Scotland	34.855000	-79.430278	24.0	4.9	64.2	19.0	11.5	0.0	1.68	MF
Yadkin												
Beaverdam Cr	SR 1486	Richmond	35.022222	-79.683333	4.4	10.2	57.8	23.6	8.3	0.0	1.64	MF
Hitchcock Cr	SR 1486	Richmond	35.007778	-79.660833	20.0	9.3	69.7	16.9	3.4	0.1	2.27	F
Rocky Fork Cr	SR 1487	Richmond	35.055556	-79.689722	16.3	5.8	71.4	15.0	7.4	0.0	1.14	F

As part of an initial step in multi-metric development for the Sand Hills region, it was conceptualized that a model based on instream and riparian habitats (such as total habitat score), watershed characteristics (such as landuse percentages), water quality variables (such as specific conductance and pH), and fish community metrics would individually and collectively be responsive to degradation. This model was revised after determining that landuse metrics (e.g. Percent Developed, Forested, or Grassland) were deemed inappropriate for the analyses because the NLCD is updated or obtained only once every 5-10 years. A landuse-based metric that is calculated only once every 5-10 years cannot be used when conducting assessments on a more frequent basis such as annually for several years. Water quality variables, such as specific conductance and pH, could not be used because they have existing water quality standards (NCAC 2007) and if they were included in the Sand Hills NCIBI, the index would have to go through the legislative rule making process to become a biological quality monitoring tool. A total habitat metric was also rejected from further consideration because of possible property rights and stream access conflicts. Ultimately, it was determined that the multi-metric NCIBI developed for the Sand Hills region be consistent with most published IBI's and include only fish assemblage attributes. This approach is the same as that used for other North Carolina basins (NCDENR 2006; 2013) and consistent with that implemented by other states (e.g., Georgia -- http://www.georgiawildlife.org/node/913) and other researchers (Paller et al. 1996; Smogor and Angermeier 2001; McCormick et al. 2001; and many others).

All of the fish community IBI indices used by researchers are based upon a modification of Karr's original IBI (Karr 1981; Karr et al. 1986; Karr and Chu 1999). There is no standard number of metrics that are used nor a standard suite of metrics that is used by all researchers. However, an effective ecological index should be socially-relevant, simple and easily understood, scientifically-based, quantitative, and cost effective (Kwak and Freeman 2010). Typically, a number of candidate metrics are statistically evaluated (e.g., as many as 43 in Hain et al. (2012) and 58 in McCormick et al. (2001)) and candidate metrics that are ultimately chosen strive to represent four major aspects of fish assemblage biological integrity: taxonomic richness, habitat guilds, trophic guilds, and individual health and abundance (Hughes et al. 1998). The selection of metrics, endpoints, and ratings is an iterative process (Paller et al. 1996; Hughes et al. 1998; Karr and Chu 1999; and McCormick et al. 2001). Metrics are scored a high, medium, or low value if the data approximated, deviated slightly from, or were markedly different from reference conditions (Hughes et al. 1998). Metrics and biocriteria are then tailored to the ecoregion, river basin/drainage, or watershed of interest because of faunal differences across various scales of the landscape (Miller et al. 1988; Paller et al. 1996; Angermeier et al. 2000; Schleiger 2000; McCormick et al. 2001; Smogor and Angermeier 2001; Hain et al. 2012). In fact, it is difficult to create effective biotic indices that are universal; as fauna and environmental stresses change regionally, so will suitable indicator organisms. Thus a biotic index developed for a specific region and environmental stressors may require modification for a different fauna and environmental relationships (Kwak and Freeman 2010).

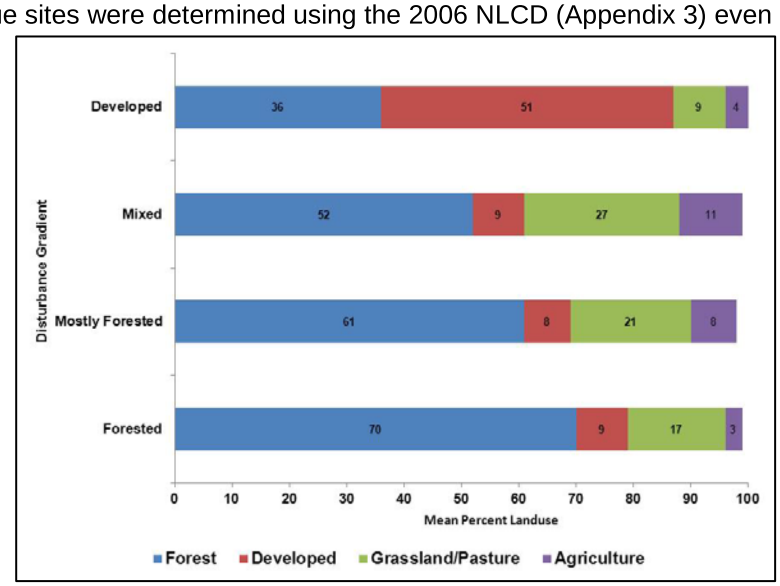
The Number of Intolerant Species and Percentage of Tolerant Fish, used in the calculation of the NCIBI in other regions of the state (NCDENR 2006), were adjusted for the Sand Hills fauna (Appendix 1). The pollution tolerances of fish species were originally determined using the Delphi Technique (NCDEHNR 1995; Zuboy 1981). Tolerances are periodically reviewed and updated as additional information becomes available from field observations, descriptions of habitat requirements in regional faunal literature (e.g., Etnier and Starnes 1993; Jenkins and Burkhead 1994; Rohde et al. 2009) and the best professional judgment of BAB scientists (NCDENR 2006; NCDENR 2013). Species that were previously classified as Intermediate in tolerance (Spotted Sucker, Mud Sunfish, Blackbanded Sunfish, Dollar Sunfish, and Spotted Sunfish) are now classified as Intolerant Species; and previously considered Intermediate Species, Eastern Mudminnow and Redear Sunfish, are now classified as an Tolerant Species.



Data were processed using Microsoft® 2007 Excel and statistically analyzed using SAS's jmp® 8.0.2 version software (SAS 2007). All reference sample data were analyzed and endpoints derived for each potential metric using box and whisker plots showing quartiles and percentiles (see insert figure to the left as an example of how to interpret a box and whisker plot). Statistical analyses performed included univariate correlations, analysis of variance, t tests, and multiple comparisons of means. The Tukey-Kramer multiple comparisons test was performed only if the analysis of variance test was significant (SAS 2007).

Landuse Types and Disturbance Classes

Landuse types for each of the 43 unique sites were determined using the 2006 NLCD (Appendix 3) even though some of the fish community data were collected well before (i.e., 1996) or well after (i.e., 2008) these data were compiled. Impervious cover (Schueler et al. 2009; http://www.mrlc.gov/nlcd06_data.php) was also determined using the 2006 NLCD (Appendix 3). Relationships between the final suite of fish community metrics and the Sand Hills NCIBI versus landuse types (categories) and disturbance classes were also determined. The disturbance classes were defined as: Forested – > 65% forest; Mostly



Forested – 55-64% forest; Mixed – 45-54% forest; and Developed - < 45% forest (see insert figure on the preceding page; Appendix 3). Metrics were plotted and correlated against landuse classes (Forested, Mostly Forested, Mixed, and Developed) and against landuse/landcover variables (Percent Developed, Percent Forested, Percent Grassland + Herbaceous + Pasture, and Percent Agriculture (cultivated row crops and orchards).

RESULTS

A list of 17 candidate metrics was created (Table 3) and metric scores and values were generated for reference site responsiveness. Several potential metrics (e.g., number and percent of Nonindigenous Species and the number and percent Redbreast Sunfish) were eliminated from further consideration (Table 3). Remaining metrics were correlated (Pearson's correlation coefficient, r) with each other metric and the overall Sand Hills NCIBI total score. The statistical significance of each of the correlations was tested to determine if the correlation coefficient was significantly different from zero. Initially, 12 metrics in keeping with a 1, 3, or 5 metric score and the 12-60 total point spread (NCDENR 2006; NCDENR 2013) were developed and tested for significant correlation with the 12 metric-Sand Hills NCIBI score. Metrics not significantly correlated ($p > 0.05$) with the Total Sand Hills NCIBI score (e.g., Percent Insectivore and Percent Piscivore) were dropped from further consideration (Table 3). Finally, stepwise, forward linear multiple regression (SAS 2007) was used to determine that the simplest and statistically significant NCIBI model with the fewest variables was one with seven metrics ($r^2 = 0.9118$).

Table 3. Metrics evaluated in the development of a Sand Hills NCIBI.

Metric	Accepted/Rejected	Reason for Rejection or Reason for Acceptance
Nonindigenous (Exotic) Species	Rejected	Few nonindigenous fish are encountered in Sand Hills streams (Appendix 1) as compared to other regions of the state (NCDWR unpublished data; http://portal.ncdenr.org/web/wq/ess/bau/nativefish). In the Sand Hills dataset, 26 of the 30 (87%) reference samples had 0 nonindigenous species and 39 of the 51 (76%) non-reference sites had 0 nonindigenous species.
Redbreast Sunfish (%)	Rejected	The percentage of all the fish that are Redbreast Sunfish. The Percentage of Tolerant Fish is the percentage of all the fish which are classified as tolerant, including the Redbreast Sunfish (Appendix 1). The Percentage of Redbreast Sunfish was significantly correlated with the Percentage of Tolerant Fish ($r^2 = 0.880$, $p < 0.01$) and rather than having two metrics so significantly correlated with one another and to reduce redundancy and over-parameterization of the Sand Hills NCIBI, this metric was not used.
No. of Species of Sunfish	Rejected	An evaluation of the number of species of sunfish (family Centrarchidae) in the sample. There are 16 species known from the Sand Hills with 13-16 species known from each river basin (Appendix 1). This metric was not used because there was no substantial difference between the reference and non-reference samples (reference site mean = 3.0 ± 0.314 S.E. vs. non-reference site mean = 4.0 ± 0.273).
Dusky Shiner (%)	Rejected	Highly correlated with the trophic metric, the Percentage of Invertivore Cyprinids which is the percentage of all fish that are minnows (i.e., cyprinids) and which are classified as invertivores (including insectivores) (Appendix 1). The Dusky Shiner is one of those species and is often the most abundant species in Sand Hills streams. The Percentage of Cyprinid Insectivores was significantly correlated with the Percent Abundance of the Dusky Shiner ($r^2 = 0.925$, $p < 0.01$) and rather than having two metrics so significantly correlated with one another and to reduce redundancy and over parameterization of the Sand Hills NCIBI, this metric was not used.
Omnivores+Herbivores (%)	Rejected	The percentage of all fish classified as omnivores and/or herbivores (Appendix 1). Unlike fish communities in the Piedmont and Mountains, Sand Hills communities, because of the limited light penetration and periphyton production, are dominated by insectivores and piscivores. This metric was not used because the percentages of omnivores+herbivores was often less than 5%-10% at reference and non-reference sites.
DELT (Disease, fin Erosion, Lesions, and Tumors) (%)	Rejected	Too rare of an occurrence with the percentages usually zero. In the Sand Hills dataset, 29 of the 30 (97%) reference samples had 0% disease and 42 of the 51 (82%) non-reference sites had a 0% disease. Paller et al. (1996) also did not observe much disease and deformities in Sand Hills fish in South Carolina.
Species with Multiple Age Classes (%)	Rejected	An indicator of the suitability of the habitat for reproduction. It is strongly influenced by rarely collected species (species that are represented by 1 or 2 fish in a sample) that are not reproducing in the stream (NCDENR 2006). This metric is naturally low for oligotrophic Sand Hills fish communities which are composed of few fish, few species, and few fish per species (NCDWR unpublished data). This metric was not used because there was no substantial difference between the reference (38%) and non-reference (40%) samples.
Insectivores (%)	Rejected	Not significantly correlated with the Sand Hills NCIBI ($p = 0.365$).
Piscivores (%)	Rejected	Not significantly correlated with the Sand Hills NCIBI ($p = 0.943$).
No. of Species	Rejected	Not significantly correlated with the Sand Hills NCIBI ($p = 0.065$).
No. of Darter And Madtom Species	Accepted	See text on Pages 14 and 16
Key Sand Hills Species (%)	Accepted	See text on Pages 14 and 16
No. of Fish	Accepted	See text on Pages 14 and 16
Key Sand Hills Fish (%)	Accepted	See text on Pages 14 and 17
No. of Intolerant Species	Accepted	See text on Pages 14 and 17
Tolerant Fish (%)	Accepted	See text on Pages 14 and 17
Invertivore Cyprinids (%)	Accepted	See text on Pages 14 and 18

Sand Hills NCIBI Metrics

The seven fish community metrics were based upon data collected from 14 reference sites encompassing 30 samples. Criteria values (Table 4) were scored a 2, 1, or 0 focusing upon an examination of the 10th, 25th, 75th, and 90th percentiles displayed in the box and whisker plots (Figure 4) for each of the seven metrics (USEPA 1996). A score of 2 represents values associated with Sand Hills reference sites, whereas a score of 0 indicates that values deviated greatly from those typically observed at the reference sites; a score of 1 indicates slight deviation. The maximum total score a site could receive would be 14 (each of the seven metrics being scored a 2), whereas the minimum score would be 0 (each of the seven metrics being scored a 0).

Table 4. Scoring criteria for the seven metric Sand Hills NCIBI for wadeable streams in the Sand Hills of the Cape Fear, Lumber, and Yadkin River basins.

Biological Integrity Component/Metric	Metric Criteria Distributions and/or Comments	Score
Species Richness		
Number of Darter and Madtom Species		
≥ 3	75th percentile; 25% of all reference samples had 3 or more species	2
1 or 2	10-25th percentile	1
0	an extreme deviation, only 6.7% of all reference samples had 0 sp.	0
Percentage of Key Sand Hills Species		
≥ 67%	25th percentile, 75% of all reference samples were ≥ 67%	2
50-66%	~10th – 25th percentile	1
< 50%	an extreme deviation, < 10% of all reference samples were < 50%	0
Fish Abundance		
Number of Fish		
≤ 150	~ 90th percentile, 90% of all reference samples had < 150 fish	2
151-250		1
> 250	an extreme deviation, no reference sample had > 155 fish	0
Percentage of Key Sand Hills Fish		
≥ 75%	25th percentile, 75% of all reference samples were ≥ 75%	2
50-74%	~10th – 25th percentile	1
< 50%	an extreme deviation, < 10% of all reference samples were < 50%	0
Pollution Indicator Species		
Number of Intolerant Species		
≥ 2	≥ median, ~ 75% of all reference samples were ≥ 2 sp.	2
1	10th percentile	1
0	an extreme deviation, only 3% of all reference samples were = 0 sp.	0
Percentage of Tolerant Fish		
≤ 20%	~ 90th percentile; 90% of all reference samples were ≤ 20%	2
21-40%		1
> 40%	>> 90th percentile; an extreme deviation from the reference condition	0
Trophic Composition		
Percentage of Invertivore Cyprinids		
≥ 25%	25th percentile, 75% of all reference samples were ≥ 25%	2
10-24%	~10th – 25th percentile	1
< 10%	~ 10th percentile, an extreme deviation, < 10% of all reference samples were < 10%	0

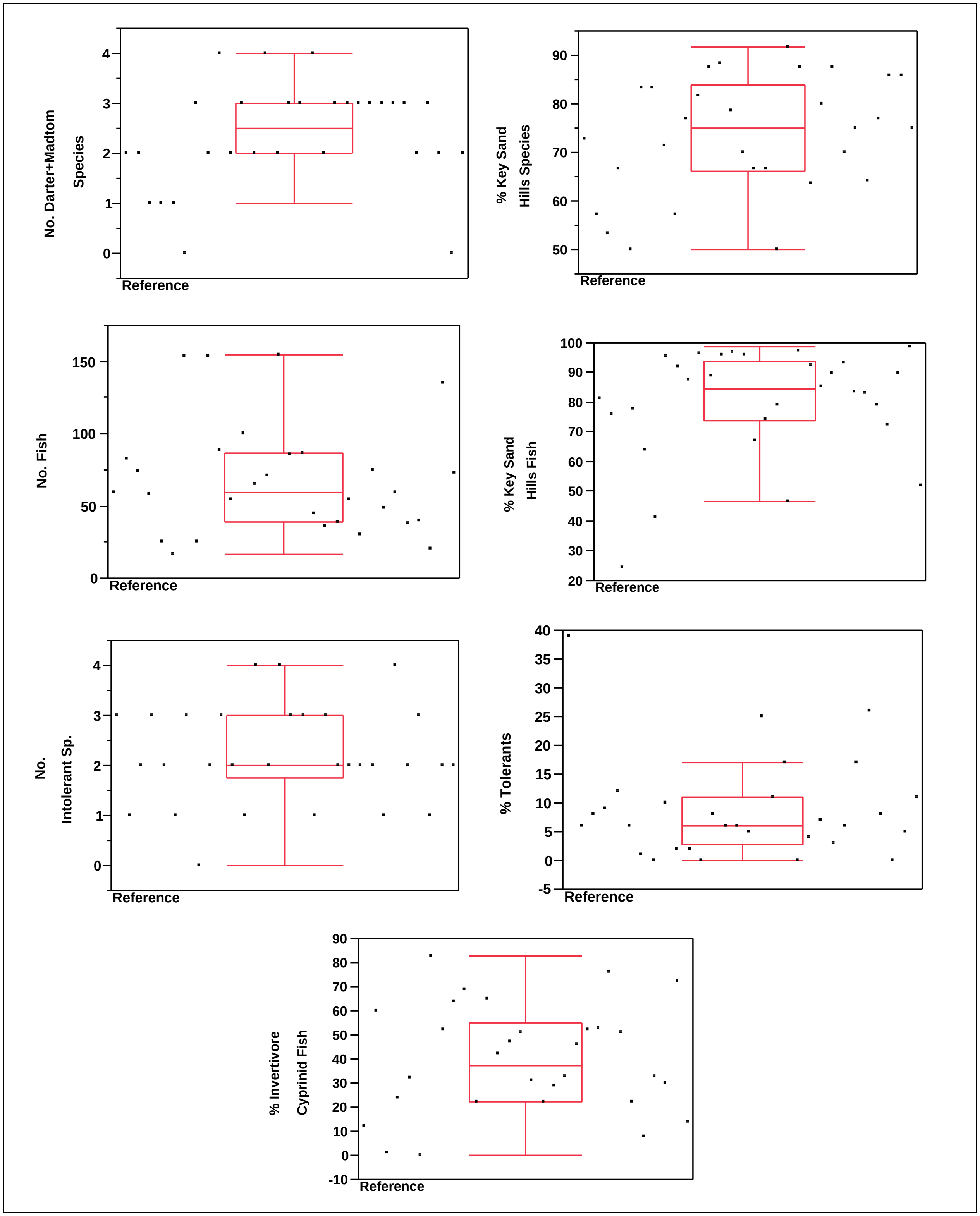


Figure 4. **Quantile box plots of fish community metrics from Sand Hills reference sites. Refer to figure insert on Page 11 for interpretation. Note: the horizontal positioning of the individual points is called "jittering" and adds random noise to the points so that coincident points do not plot atop one another (SAS 2007).**

Metric 1 – Number of Darter and Madtom Species (Table 4)
This metric; also used by Paller et al. (1996) for South Carolina Sand Hills streams, is the number of species of darters and madtoms (two groups of benthic insectivores) in a sample. Two species of madtoms and five species of darters are known from the North Carolina Sand Hills with a total of 6 or 7 species known from each river basin (Appendix 1). The Number of Darter and Madtom Species in the reference samples ranged from 0 to 4 species per sample with the median = 2.5 (Appendix 1; Figure 4). Fifty percent of the reference samples were scored a "2"; 43% of the reference samples were scored a "1"; and 7% of the reference samples were scored a "0".

Metric 2 – Percentage of Key Sand Hills Species (Table 4)
A Key Sand Hills Species is defined as: "1 of 25 species which is typically associated with or that is endemic to the Sand Hills (e.g., Dusky Shiner, Dollar Sunfish, Sandhills Chub and Pinewoods Darter)" (Appendix 1). This metric takes into consideration the uniqueness of the Sand Hills fauna (Cooper et al. 1977; Rohde and Ross 1987; Rohde and Arndt 1991; NCDWR unpublished data; personal observations and professional judgment) and is the percentage of fish species in a sample that are Key Sand Hills Species. The hypothesis behind this metric is: "*Does the assemblage lose its faunal uniqueness as degradation increases, thereby shifting to an assemblage that is more ubiquitous, less unique, and more tolerant with species such as Golden Shiner, Eastern Mosquitofish, bullheads, and Green Sunfish*?" The Percentage of Key Sand Hills Species in the reference samples ranged from 50% to 92% of all the species collected with the median = 75% (Figure 4). Seventy seven percent of the reference samples were scored a "2" and 23% of the reference samples were scored a "1"; no reference samples were scored a "0".

Metric 3 – Number of Fish (Table 4)
The abundance of fish in Sand Hills streams is usually naturally low because of the streams' low biological productivity, low pH, and tannin-stained waters (Louder 1962; Louder 1963; Tatum et al. 1963; Rohde and Ross 1987; Rohde and Arndt 1991; NCDENR 1999; NCDENR 2007a; NCDWR unpublished data). High abundance of fish in these streams may indicate nutrient enrichment and habitat or watershed alterations (NCDWR unpublished data; personal observation). The Number of Fish per sample in the reference samples ranged from 17 to 155 with the median = 59 (Figure 4). Ninety percent of the reference samples were scored a "2" and 10% of the reference samples were scored a "1"; no reference samples were scored a "0".

It is conceivable that an extremely degraded stream might have as few fish as a least impaired stream and thus be scored a "2" for this metric. However, in such an instance, other metrics (e.g., Number of Intolerant Species, Percentage of Key Sand Hills Fish, and Percent Key Sand Hills Species) would more than likely all score low and the fish assemblage would be rated as Fair or Poor.

Metric 4 – Percentage of Key Sand Hills Fish (Table 4)

As stated in Metric 2, a Key Sand Hills Species is 1 of 25 species which is typically associated with or that is endemic to the Sand Hills (e.g., Dusky Shiner, Dollar Sunfish, Sandhills Chub and Pinewoods Darter) (Appendix 1). This metric takes into consideration the uniqueness of the Sand Hills fauna (Cooper et al. 1977; Rohde and Ross 1987; Rohde and Arndt 1991; NCDWR unpublished data; personal observations, and professional judgment) and is the percentage of the fish in the sample that are Key Sand Hills Fish. As with Metric No. 2, the hypothesis behind this metric is: "*Does the assemblage lose its faunal uniqueness as degradation increases, thereby shifting to an assemblage that is more ubiquitous, less unique, and more tolerant with an abundance of species such as Golden Shiner, Eastern Mosquitofish, bullheads, and Green Sunfish*?" The Percentage of Key Sand Hills Fish per sample in the reference samples ranged from 24.3% to 98.5% of all the fish collected with the median = 84.5% (Figure 4). Seventy three percent of the reference samples were scored a "2"; 20% of the reference samples were scored a "1"; and 7% of the reference samples were scored a "0".

Metric 5 – Number of Intolerant Species (Table 4)

This metric is the number of Intolerant Species in a sample which, in the Sand Hills, includes the Sandhills Chub, Thinlip Chub, Ironcolor Shiner, Taillight Shiner, Spotted Sucker, Mud Sunfish, Blackbanded Sunfish, Dollar Sunfish, Spotted Sunfish, Pinewoods Darter, Sawcheek Darter, and Piedmont Darter (NCDENR 2006; Appendix 1). The more commonly collected Intolerant Species are Spotted Sucker, Mud Sunfish, Dollar Sunfish, Pinewoods Darter (Lumber River basin only), Sawcheek Darter, and Piedmont Darter. The Number of Intolerant Species in the reference samples ranged from 0 to 4 species per sample with the median = 2 (Figure 4). Seventy seven percent of the reference samples were scored a "2"; 20% of the reference samples were scored a "1"; and 3% of the reference samples were scored a "0".

Metric 6 - Percentage of Tolerant Fish (Table 4)

This metric is the percent abundance of all the tolerant fish in a sample which in the Sand Hills includes the Bowfin, Satinfin Shiner, Golden Shiner, Creek Chub, White Catfish, Yellow Bullhead, Brown Bullhead, Flat Bullhead, Eastern Mudminnow, Eastern Mosquitofish, Redbreast Sunfish, Green Sunfish, and Redear Sunfish (NCDENR 2006; Appendix 1). Most of these Tolerant Species are likely to be encountered in wadeable streams, except for Bowfin and White Catfish. The Percentage of Tolerant Fish per sample in the reference samples from 0.0% to 39% per sample with a median = 6% (Figure 4). Ninety percent of the reference samples were scored a "2" and 10% of the reference samples were scored a "1"; no reference samples were scored a "0".

Metric 7 -- Percentage of Invertivore Cyprinids (Table 4)

This metric is the percentage of all the invertivore cyprinids in a sample. Invertivore Cyprinids are minnows that are classified as invertivores (including insectivores) (Appendix 1); there are 14 such species in the Sand Hills. The Dusky Shiner is one of those species and is often one of the most abundant species in the Sand Hills streams. The Percentage of Cyprinid Invertivores in the reference samples ranged from 0% to 83% per sample with a median = 38% (Figure 4). Seventy percent of the reference samples were scored a "2", 23% of the reference samples were scored a "1", and 10% of the reference samples were scored a "0".

In other regions of the state, a 10- or 12-metric NCIBI has been used to assess fish communities in wadeable streams (NCDENR 2013; Table 5). Although only seven metrics were used to derive a Sand Hill NCIBI, what is more important than the total number of metrics used, is that the metrics that are used and the biocriteria that are developed should be tailored to the ecoregion, river basin/drainage, or the watershed of interest based on the the different faunal assemblages across various scales of the landscape (Miller et al. 1988; Paller 1996; Angermeier et al. 2000; Schleiger 2000; McCormick et al. 2001; Smogor and Angermeier 2001; Kwak and Freeman 2010; Hain et al. 2012). This has been accomplished with the Sand Hills reference sites data set.

Table 5. Metrics used to assess fish communities in wadeable streams in North Carolina.

	River Basin/Region[1]			
Biological Integrity Component/ Metric	FRB, HIW, LTN, NEW, WAT	BRD, CTB, SAV, YAD	CPF, NEU, TAR, ROA	Sand Hills (CPF, LBR, YAD)
Species Richness				
Number of Species	X	X	X	---
Percentage of Key Sand Hills Species	---	---	---	X
Fish Abundance				
Number of Fish	X	X	X	X
Percentage of Key Sand Hills Fish	---	---	---	X
Species Composition				
Number of Species of Darters	X	X	X	---
Number of Species of Darters + Madtoms	---	---	---	X
Number of Species of Rock Bass, Smallmouth Bass, & Trout	X	---	---	---
Number of Species of Sunfish, Bass, & Trout	---	X	---	---
Number of Species of Sunfish	---	---	X	---
Number of Species of Cyprinids	X	---	---	---
Number of Species of Suckers	---	X	X	---
Pollution Indicator Species				
Number of Intolerant Species	X	X	X	X
Percentage Tolerant Individuals	X	X	X	X
Trophic Composition				
Percentage of Omnivorous + Herbivorous Individuals	X	X	X	---
Percentage of Insectivorous Individuals	X	X	X	
Percentage of Invertivore Cyprinids	---	---	---	X
Percentage of Piscivorous Individuals	---	X	X	---
Fish Condition				
Percentage of Diseased Fish	---	X	X	---
Percentage of Species With Multiple Age Groups	X	X	X	---

[1]Abbreviations are: FRB = French Broad, HIW = Hiwassee, LTN = Little Tennessee, NEW = New, WAT = Watauga, BRD = Broad, CTB = Catawba, SAV = Savannah, YAD = Yadkin, CPF = Cape Fear, NEU = Neuse, TAR = Tar, ROA = Roanoke, and LBR = Lumber.

Univariate (Pearson's) Correlations of a 7-Metric Sand Hills NCIBI

Each of the seven metrics was significantly correlated with at least two other metrics (Table 6). The number of significant correlations ranged from 5 of 6 (Percentage of Key Sand Hills Fish and Number of Intolerant Species) to 2 of 6 (Number of Fish). All seven metrics were significantly correlated with the overall 7-Metric Sand Hills NCIBI total score (Table 3 and Figure 5). Metrics positively correlated with the 7-Metric Sand Hills NCIBI were Number of Darter and Madtom Species, Percentage of Key Sand Hills Species, Percentage of Key Sand Hills Fish, Number of Intolerant Species, and Percentage of Invertivore Cyprinids. Metrics negatively correlated with the 7-Metric Sand Hills NCIBI were Number of Fish and Percentage of Tolerant Fish.

Table 6. Matrix of Pearson's correlation coefficients (r) for each metric and the 7-Metric Sand Hills NCIBI. Bolded correlation coefficients are significant at p < 0.05 level; not bolded correlation coefficients are not significant (p> 0.05); n = 81 observations.

Metric	NCIBI Score	No. D & M Sp.	% Key SH Sp.	No. Fish	% Key SH Fish	No. Intol. Sp.	% Tol.
No. D & M Sp.	**0.6463**						
% Key SH Sp.	**0.5644**	**0.2327**					
No. Fish	**-0.3177**	0.0195	**-0.3224**				
% Key SH Fish	**0.6134**	0.1815	**0.4777**	**-0.3427**			
No. Intol. Sp.	**0.6817**	**0.5337**	**0.2860**	-0.0670	**0.2435**		
% Tol. Fish	**-0.5034**	**-0.2813**	**-0.3752**	0.1252	0.0225	**-0.2921**	
% Invertivore Cyprinids	**0.5604**	**0.2963**	0.2038	0.2091	**0.4539**	**0.2645**	**-0.2943**

[1]Abbreviations are: Sp. = species, D&M = darter and madtom species, SH = Sand Hills, Intol. = intolerant, Tol. = tolerant.

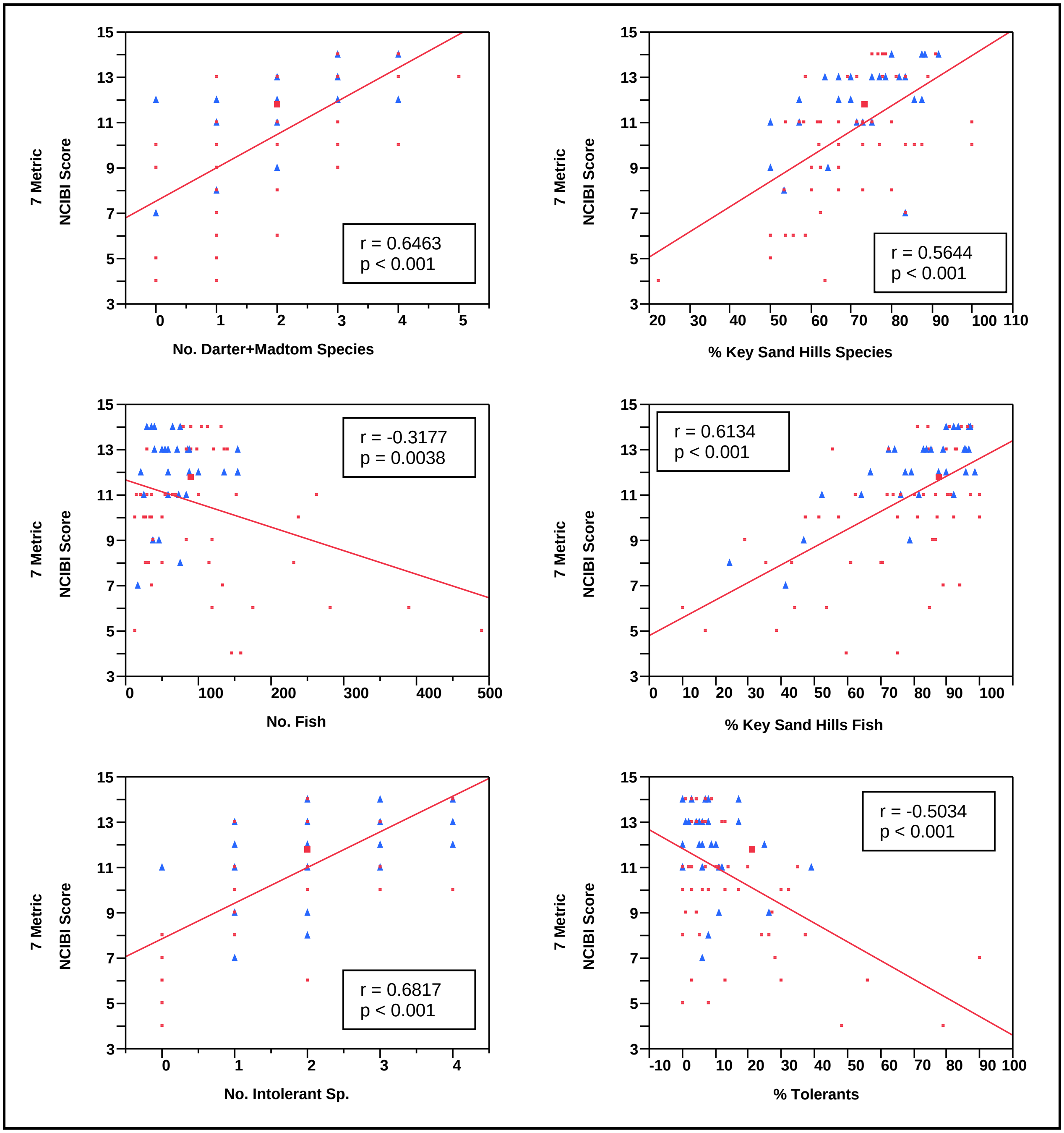


Figure 5. **Scatter plots for seven Sand Hills fish community metrics and the final 7-Metric Sand Hills NCIBI total score. Reference sites are shown as blue triangles and non-reference sites are shown as red dots. Pearson's correlation coefficients and their level of significance are found in the text boxes within each plot and also in Table 6. Each of the regression lines is significant (p < 0.01).**

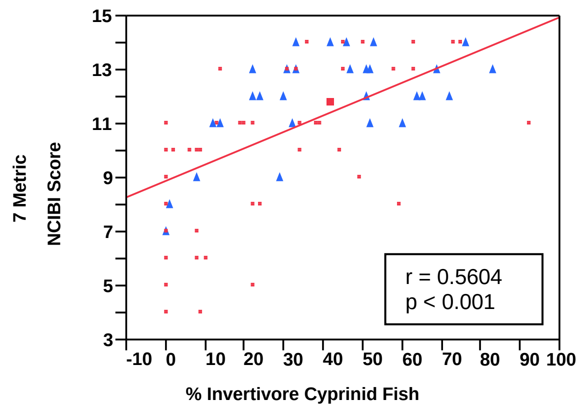


Figure 5 (continued).

The 7-Metric Sand Hills NCIBI and each of the seven metrics was significantly correlated with specific conductance, pH, and the total habitat score of each sample (Table 7). Specific conductance was negatively correlated with the 7-Metric Sand Hills NCIBI total score and four of the six metrics. Specific conductance of Sand Hills streams is naturally low and an increase in the conductivity is considered to be an indicator of watershed or water quality alterations (NCDWR unpublished data; NCDENR 1999; NCDENR 2007a; Page 272 in http://www.esb.enr.state.nc.us/documents/CapeFearAmbientMonitoring04-08.pdf); personal observations). pH was negatively correlated with the 7-Metric Sand Hills NCIBI total score and significantly correlated with 3 of the 6 metrics. As mentioned in the Description of the Sand Hills section, the pH of Sand Hills streams is naturally low because of the tannic acids and an increase in the pH is similarly considered to be an indicator of watershed or water quality alterations (Page 271 in http://www.esb.enr.state.nc.us/documents/CapeFearAmbientMonitoring04-08.pdf; NCDWR unpublished data; NCDENR 1999; NCDENR 2007a; personal observations). Total habitat score was significantly correlated with the 7-Metric Sand Hills NCIBI and 4 of the 6 metrics.

Table 7. Matrix of Pearson's correlation coefficients (r) for each metric, the 7-Metric Sand Hills NCIBI, specific conductance, pH, and total habitat score. Bolded correlation coefficients are significant at the $p < 0.05$ level; not bolded correlation coefficients are not significant ($p > 0.05$), n = 81.

Metric	Specific Conductance	pH	Total Habitat Score
No. Darter+Madtom Species	**-0.2473**	0.0257	0.1625
% Key Sand Hills Species	**-0.4547**	**-0.2705**	**0.2983**
No. Fish	**0.4430**	**0.2609**	-0.1138
% Key Sand Hills Fish	**-0.2453**	-0.1993	0.1156
No. Intolerant Species	**-0.3173**	-0.2030	**0.4007**
% Tolerants	**0.5686**	**0.3192**	**-0.5676**
% Invertivore Cyprinid Fish	-0.1602	-0.0948	**0.3400**
7-Metric Sand Hills NCIBI	**-0.5509**	**-0.2889**	**0.4784**

The 7-Metric Sand Hills NCIBI and 5 of the 7 metrics were significantly correlated with percent forest, percent developed, percent grassland/pasture, and total imperviousness (Table 8). The Number of Fish and Percentage of Key Sand Hills Fish were not significantly correlated with landuse types. None of the metrics or the 7-Metric Sand Hills NCIBI were significantly correlated with percent agriculture and only two metrics were significantly correlated with percent grassland/pasture. A similar trend was observed during development and testing of NCDWR's small stream biocriteria using benthic macroinvertebrates (NCDENR 2009). The insignificant correlations with these two landuse types may be related to their low percentages in this dataset (Appendix 3). For example, the mean percent grasslands/pasture was only 22% (range = 6%-33%) and the mean percent agriculture was only 8% (range = 0%-30%) (Appendix 3). These low percentages may be attributed to the Sand Hills soils which, in general, do not support row crops (agriculture) because of its poor water holding capacity (droughtiness) and rapid leaching of plant nutrients (Griffith et al. 2002).

Table 8. Matrix of Pearson's correlation coefficients (r) for each metric, the 7-Metric Sand Hills NCIBI and landuse types. Bolded correlation coefficients are significant at p < 0.05 level; not bolded correlation coefficients are not significant (p> 0.05), n = 81.

Metric	% Forest	% Developed	% Grass Pasture	% Agr.	Total Imperviousness
No. Darter+Madtom Species	**0.2945**	-0.1726	-0.0434	-0.0528	**-0.2282**
% Key Sand Hills Species	**0.2679**	**-0.3575**	**0.2664**	-0.1143	**-0.3784**
No. Fish	-0.0720	0.1017	-0.0923	0.0936	0.1629
% Key Sand Hills Fish	0.0006	0.0379	-0.0631	-0.1555	0.0142
No. Intolerant Species	**0.3956**	**-0.2580**	-0.0147	-0.0904	**-0.3399**
% Tolerants	**-0.5204**	**0.7533**	**-0.5256**	-0.1026	**0.8133**
% Invertivore Cyprinid Fish	**0.2951**	**-0.2442**	-0.0893	0.0546	**-0.2857**
7-Metric Sand Hills NCIBI	**0.4531**	**-0.4172**	0.1252	-0.1363	**-0.4987**

Sand Hills NCIBI Ratings Development

Sand Hills NCIBI ratings (Excellent, Good, Good-Fair, Fair, and Poor) were derived based upon a quantile (box) plot of the reference dataset (Table 9; Figure 6; Appendix 6), a method that is commonly used for setting ratings thresholds (McCormick et al. 2001). Figure 6 also shows the distribution of the non-reference samples; however, non-reference sample data were not used for setting the rating thresholds. Approximately 87% of the reference samples were scored ≥ 12 (range 12-14). These samples were assigned a Sand Hills NCIBI rating of either Good or Excellent. Based upon these new ratings, 60% of the reference samples were rated Excellent, 27% were rated Good, 10% were rated Good-Fair, and 3% were rated Fair (Appendix 6).

Table 9. Scores and ratings, based upon reference site data, for evaluating fish communities in wadeable streams in the Sand Hills in North Carolina using the North Carolina Index of Biotic Integrity (NCIBI).

NCIBI Score	Sand Hills NCIBI Rating	Comments
13 or 14	Excellent	75^{th} percentile to maximum score; 47% of all reference samples were ≥ 13; these scores represent the "best" communities
11 or 12	Good	25^{th} – 50^{th} percentile
9 or 10	Good-Fair	between the 10^{th} and 25^{th} percentiles
7 or 8	Fair	minimum score of the reference samples
≤ 6	Poor	< 10^{th} percentile of non-reference sites; represents a substantial deviation from reference samples

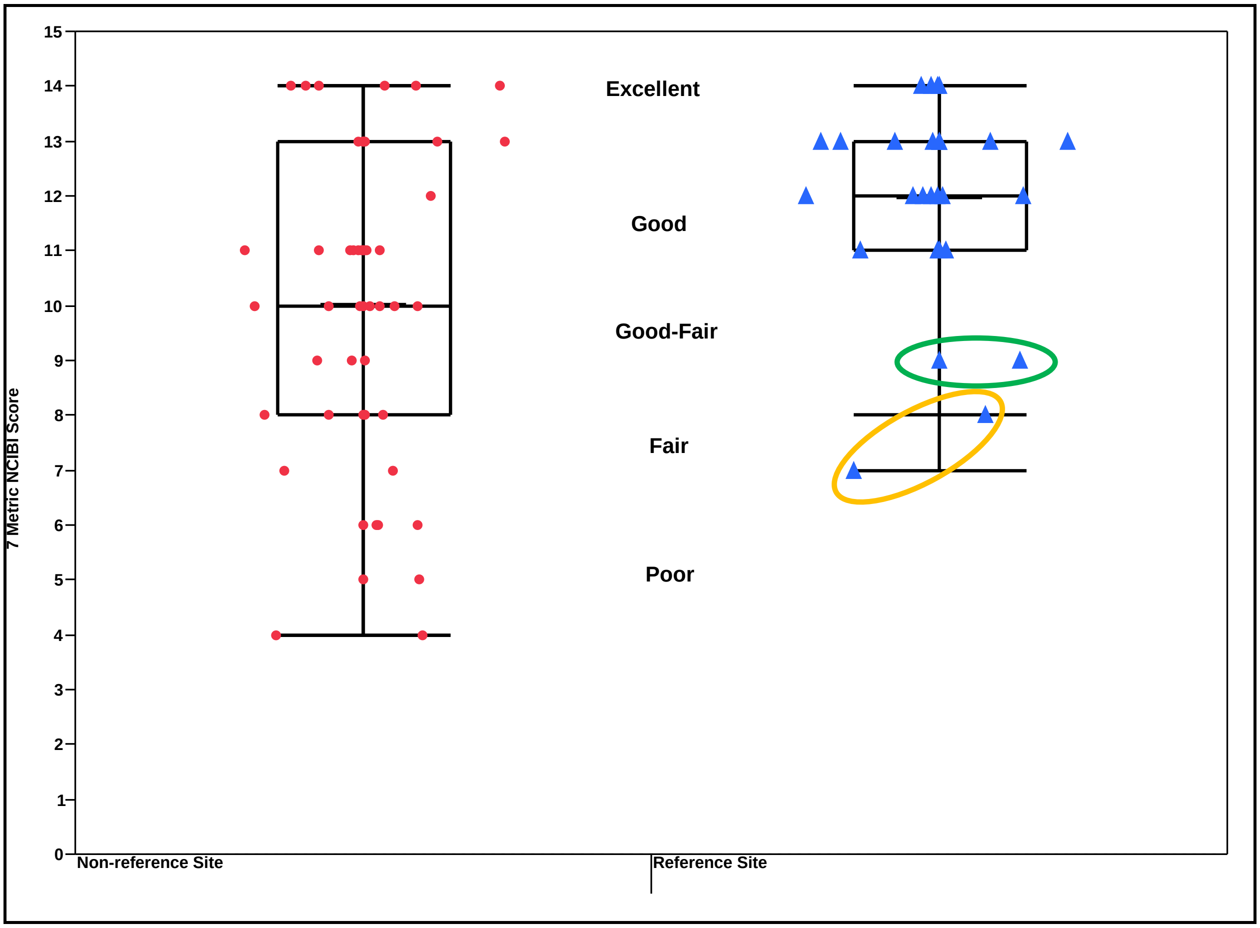


Figure 6. **Quantile box plots of the 7-Metric Sand Hills NCIBI scores for Sand Hills fish community reference (blue triangles) and non-reference (red dots) sites. See text for explanation of green and orange ellipses. Note: the horizontal positioning of the individual points for the Non-Reference and Reference sites is called "jittering" and adds some random noise to the plotted points so that coincident points do not plot atop one another (SAS 2007).**

Two reference samples rated Fair – Beaverdam Creek (Sample No. 96-13), and Hitchcock Creek (Sample No. 2006-2) (Figure 6 (orange ellipse)); Appendix 6). Beaverdam Creek had the smallest drainage area of any of the sites (4.4 mi^2) and may have dried up during the summer of 1995. The subsequent rating for Beaverdam Creek was Good in April 2006 (Appendix 6). High abundance of Yellow Perch (a nonindigenous species in the Yadkin River basin), a probable escapee from upstream McKinney Lake or the fish hatchery, likely contributed to the low rating in Hitchcock Creek in April 2006. A previous April 2001 rating had been Excellent (Appendix 6).

Two reference samples rated Good-Fair -- Drowning Creek (Sample No. 96-02) and Muddy Creek (Sample No. 2003-59) (Figure 6 (green ellipse); Appendix 6). Drowning Creek was sampled in very early spring (March 25, 1996) when the water temperature was only 11°C and the fish may have still been inactive and inaccessible as they are during colder months of the year (personal observation). Three subsequent ratings between May 1996 and May 2006 for Drowning Creek have been Good or Excellent (Appendix 6). Muddy Creek rated lower than expected because of the low abundance of Dusky Shiner, a Key Sand Hills Species and an Invertivore Cyprinid. A subsequent rating of Muddy Creek in 2008 was Excellent.

The revised ratings of the entire dataset (n = 81) ranged from Poor to Excellent (Appendix 6). Sites that are classified as High Quality Waters (HQW) (e.g., Deep, Drowning, and Jackson creeks) were also rated Excellent with the Sand Hills NCIBI (Appendix 6). The Little River, another HQW classified stream, was most recently rated Poor in 2008; it had rated Excellent in 2003. The fish community at this site seemed to be strongly influenced by the large number of Bluegill which were collected (n = 280) whom most likely originated in the adjacent golf course's ponds and escaped into the stream during high flow events.

Ratings for five sites in the Crane Creek watershed (three sites on Crane Creek and one site each on Herds and Beaver creeks) seemed to be rated too low (Fair or Poor) using the Sand Hills biocriteria (Appendix 6) and, except for Beaver Creek, should be rated with Cape Fear Basin Piedmont biocriteria (NCDENR 2006). Technically in the Sand Hills, the headwaters of the Crane Creek watershed border the Triassic Basin in Lee and Moore counties (Figure 3; Griffith et al. 2002). Other streams bordering the Triassic Basin, such as McLendons and Richland creeks, were not included in the Sand Hills dataset and were instead rated with Piedmont biocriteria (refer to the Methods sections and Appendix 4). The Crane Creek watershed was investigated in April 2002 for NCDWR's Wetlands Restoration Program's Watershed Planning Initiative (BAU 2002d). There were indications (e.g., a noticeable absence of long-lived stoneflies in the Crane Creek watershed) that stream flow in the smaller watersheds may become intermittent during low flow periods each year and especially during prolonged droughts as were experienced in 2001 and 2002. [Note: Crane Creek at SR 1810, Sample No. 2008-05, was deleted from the dataset (refer to the Methods section) because of drought impacts still observed in April 2008 following the 2007 drought.] In April 2002, no subwatersheds in the Crane Creek watershed were identified as impaired by either the benthic macroinvertebrate or the fish communities. If rated with the Cape Fear Basin Piedmont biocriteria rather than the Sand Hills fish community biocriteria, the four sites within the Crane Creek watershed would rate Fair, Good-Fair, or Good (Appendix 6). Two sites on Crane Creek (at US 1 and at SR 2005) would rate Fair. The fish community at the US 1 site seemed to be strongly influenced by the large number of Age 1 Bluegill which were collected in April 2002 (n = 353; 72% of all the fish collected). Visible using Google Earth® software, there is a large impoundment on an unnamed tributary just upstream of the site. Escapees from the pond during high flow events (i.e., the late September 2001 rain event) may have been washed over the spillway and temporarily taken up residence in the stream. Besides the effects of low flows and droughts, additional causes for these two sites to be rated as Fair are unknown. Beaver Creek, rated Fair in 2008, was rated Good-Fair in 2002. The lower than expected rating in 2008 may be due to lingering effects from the 2007 drought; seven species collected in 2002 were missing in 2008.

Responsiveness of the Sand Hills NCIBI Ratings to Individual Metrics

The responsiveness of the overall 7-Metric Sand Hills NCIBI ratings to each of the seven individual metrics is shown in Table 10. Samples (sites) rated Excellent had individual metric scores that on average ranged from 1.9 to 2.0. By contrast, samples (sites) rated Poor had individual metric scores that on average ranged from 0.3 to 1.1. Changes in individual metrics made greater contributions in certain rating classes than at others. For example, a Good rating had greater decreases in the scores for Number of Darter+Madtom (from 2 to 1.2) and Percentage of Invertivore Cyprinids (from 2 to 1.4) than the other five metrics which only decreased on average by 0.2 to 0.4. The Number of Intolerant Species score (from 2 to 0.3) was the lowest of any metric score for the Poor rating.

Table 10. Average metric scores for the five Sand Hills NCIBI rating classes. The possible score for any metric is 0, 1, and 2 (Table 4).

	Sand Hills NCIBI Rating				
Metric	**Excellent**	**Good**	**Good-Fair**	**Fair**	**Poor**
No. Darter+Madtom Species	1.9	1.2	1.1	0.9	0.8
% Key Sand Hills Species	1.9	1.6	1.7	1.6	0.9
No. Fish	2.0	1.8	2.0	1.9	1.0
% Key Sand Hills Fish	1.9	1.8	1.3	0.8	0.8
No. Intolerant Species	1.9	1.7	1.3	0.8	0.3
% Tolerant Fish	2.0	1.8	1.8	1.3	1.1
% Invertivore Cyprinid Fish	1.9	1.4	0.5	0.4	0.5

Sand Hills NCIBI Ratings and Intolerant Species Occurrences

As explained previously, Metric No. 5 is the Number of Intolerant Species collected in a sample (Table 4; Appendices 1 and 5). In the Sand Hills, there are 12 Intolerant Species of which 9 were encountered in this dataset (Table 11); Thinlip Chub, Ironcolor Shiner, and Taillight Shiner were not encountered in this dataset. When analyzed across the five rating classes, 90% (135 of 150) of the occurrences of Intolerant Species were at sites that were rated Excellent or Good (Table 11). When analyzed by species across the five rating classes, at least 75% of all the intolerant occurrences were at sites that rated Excellent or Good (range 75 to 100%). Two occurrences were at Little River (Sample No. 2008-04), a site rated Poor due to the high abundance of Bluegill that escaped from nearby golf course ponds. Only five occurrences (3%) were at sites rated Fair.

Table 11. Intolerant Species occurrences (number of sites with intolerant species) across the Sand Hills NCIBI ratings.

Species	Sand Hills NCIBI Rating Excellent	Good	Good-Fair	Fair	Poor	Total Sites Encountered
Sandhills Chub	10	7	0	0	0	17
Spotted Sucker	10	9	5	2	0	26
Mud Sunfish	8	5	0	0	0	13
Blackbanded Sunfish	0	1	1	0	0	2
Dollar Sunfish	16	11	3	2	1[1]	33
Spotted Sunfish	2	2	0	0	0	4
Pinewoods Darter	14	7	1	0	0	22
Sawcheek Darter	7	5	6	1	1[1]	20
Piedmont Darter	7	5	1	0	0	13
No. of sites with Intolerant Species	74	52	17	5	2	150

[1]Occurences at Little River; refer to Sand Hills NCIBI Rating Development section for explanation of the lower than expected ratings at this site.

The seven Sand Hills metrics and the 7-Metric Sand Hills NCIBI were then tested for significance with the Sand Hills NCIBI ratings, landuse types, disturbance gradients (classes), and water quality variables. These results are presented below.

Metric Differences between Sand Hills NCIBI Ratings

Responsiveness of the seven Sand Hills metrics, specific conductance, pH, and total habitat score among the five Sand Hills NCIBI rating classes were determined using analysis of variance (ANOVA) and the Tukey-Kramer Honest Significant Difference test. Each ANOVA test was significant at the $p < 0.001$ level, except for pH which was significant at the $p < 0.05$ level. Sample sizes (n) for the ratings were: 8 (Poor), 9 (Fair), 12 (Good-Fair), 25 (Good), and 27 (Excellent). Results from the multiple comparison tests (Figure 7) showed that there was at least one significant mean difference in all seven metrics, specific conductance, pH, and the total habitat score between the five rating classes (means with the same superscript letter were not significantly different):

- Number of Darter and Madtom Species – Poor $(1.1)^{b}$, Fair $(1.1)^{b}$, Good-Fair $(1.8)^{b}$, Good $(2.0)^{b}$, Excellent $(3.1)^{a}$
- Percentage of Key Sand Hills Species (%) – Poor $(50.5)^{c}$, Fair $(68.3)^{ab}$, Good-Fair $(73.0)^{ab}$, Good $(69.6)^{b}$, Excellent $(78.4)^{a}$
- Number of Fish – Poor $(221.3)^{a}$, Fair $(79.4)^{b}$, Good-Fair $(43.9)^{b}$, Good $(84.7)^{b}$, Excellent $(81.1)^{b}$
- Percentage of Key Sand Hills Fish (%) – Poor $(47.8)^{d}$, Fair $(58.7)^{cd}$, Good-Fair $(69.7)^{bc}$, Good $(81.7)^{ab}$, Excellent $(87.5)^{a}$
- Number of Intolerant Species -- Poor $(0.3)^{d}$, Fair $(0.8)^{cd}$, Good-Fair $(1.4)^{bc}$, Good $(2.1)^{ab}$, Excellent $(2.6)^{a}$

- Percentage of Tolerant Fish (%) – Poor (29.6)^{a}, Fair (24.9)^{ab}, Good-Fair (12.2)^{abc}, Good (12.0)^{bc}, Excellent (6.0)^{c}
- Percentage of Invertivore Cyprinids (%) – Poor (14.0)^{bc}, Fair (12.7)^{bc}, Good-Fair (12.9)^{c}, Good (34.0)^{ab}, Excellent (47.3)^{a}
- Specific Conductance (µS/cm) – Poor (47.5)^{a}, Fair (41.3)^{a}, Good (27.3)^{b}, Good-Fair (26.3)^{b}, Excellent (22.8)^{b}
- pH (s.u.) – Poor (6.3)^{a}, Fair (5.8)^{ab}, Good-Fair (5.5)^{ab}, Good (5.3)^{ab}, Excellent (5.3)^{b}
- Total Habitat Score – Excellent (92.8)^{a}, Good (91.2)^{a}, Good-Fair (91.0)^{a}, Fair (86.4)^{a}, Poor (74.0)^{b}

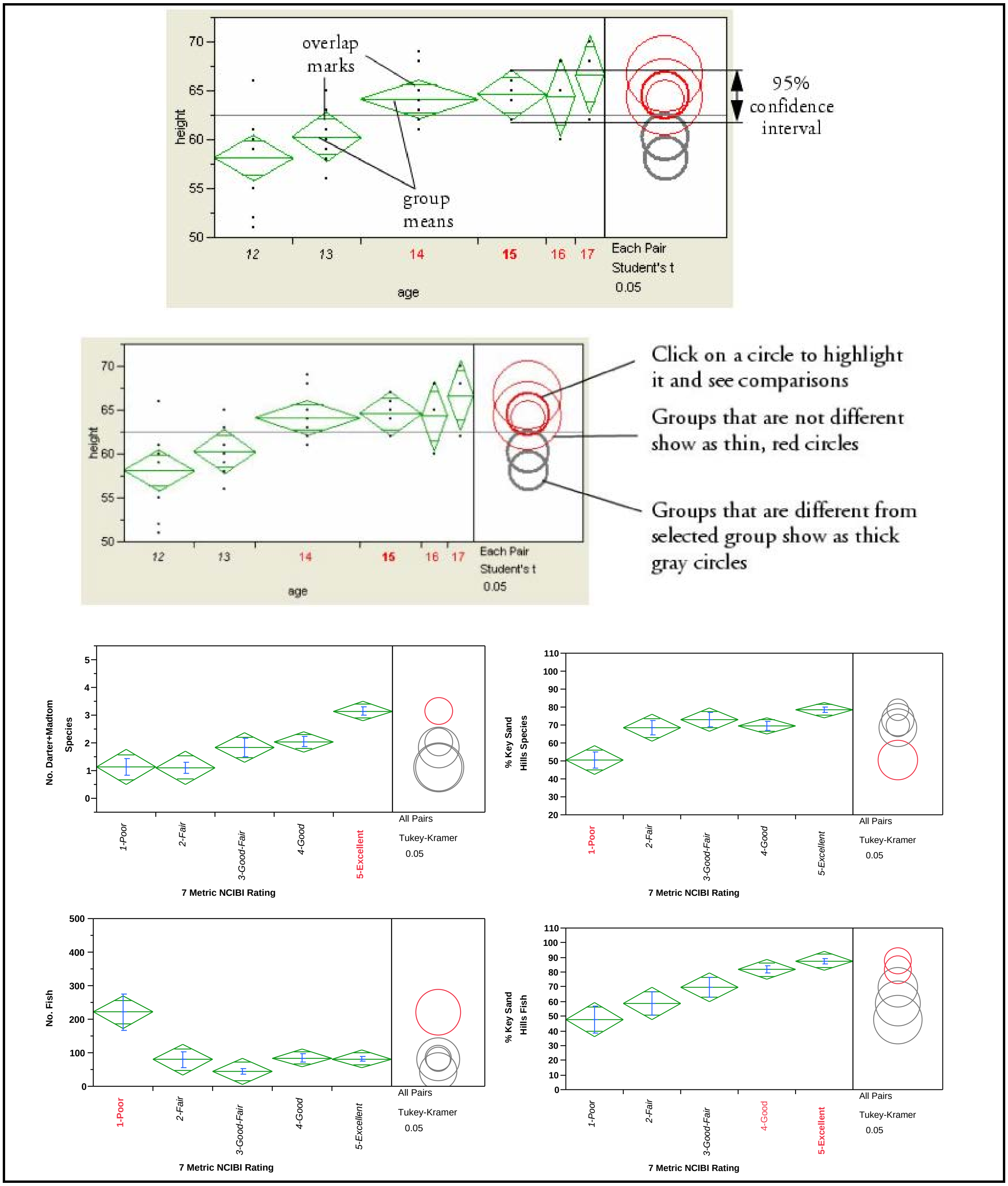


Figure 7. **Multiple comparisons of means, including mean diamonds and Tukey-Kramer tests, for seven metrics, specific conductance, pH, and total habitat scores for the five classes of the Sand Hills NCIBI ratings. The top two plots (from SAS 2007) show how to interpret the results and are not meant to imply an interactive feature. Ratings high-lighted in red are significantly different from the ratings high-lighted in gray.**

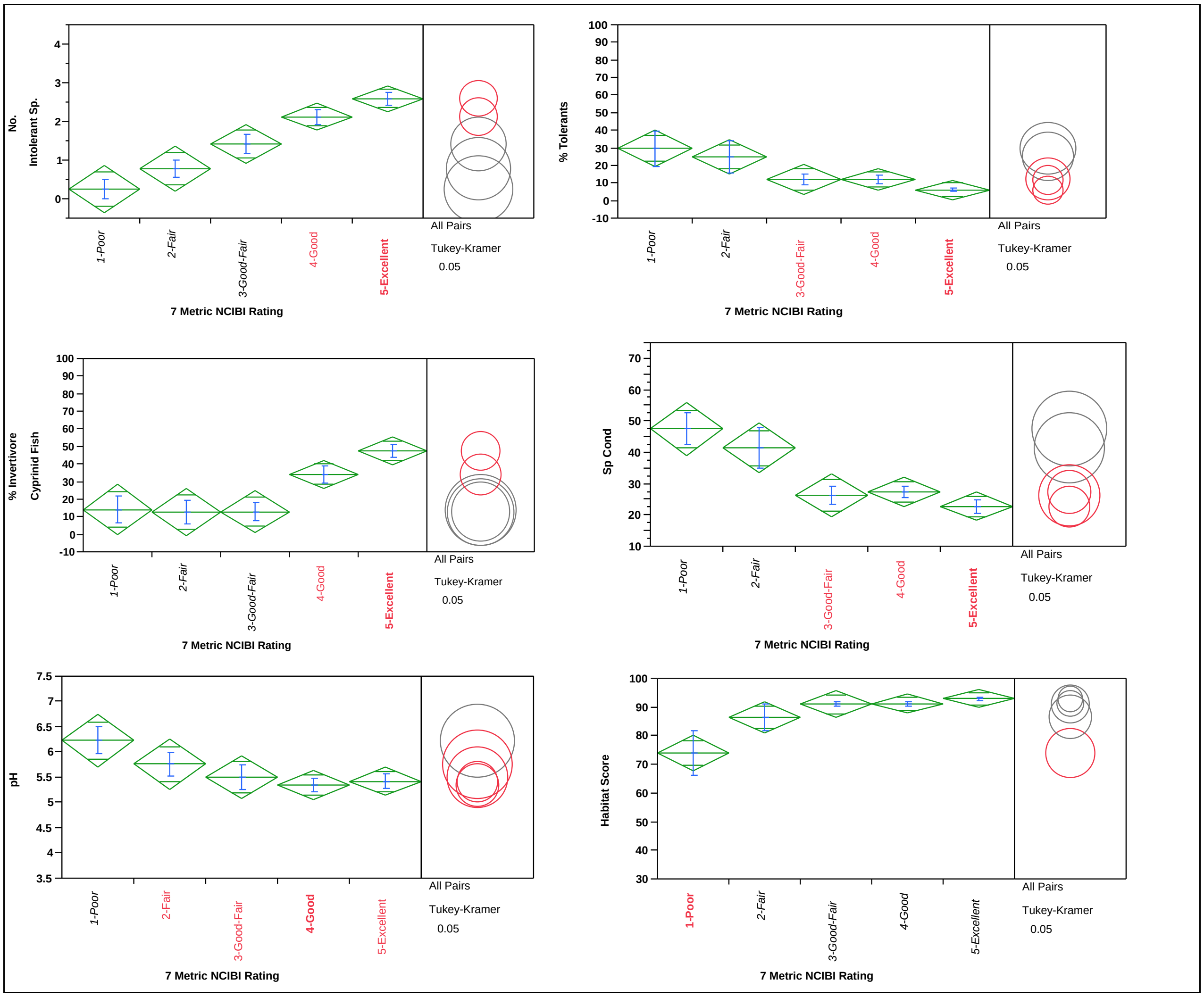


Figure 7 (continued).

Metric Differences between Sand Hills NCIBI Ratings and Most and Least Disturbed Samples

Responsiveness of the seven metrics, specific conductance, pH, and total habitat score between the non-reference samples where the Sand Hills NCIBI was ≤ 8 (Poor and Fair sites and which ultimately may be placed on the impaired streams list) and the reference and least disturbed near-reference samples where the Sand Hills NCIBI was ≥ 11 (Good and Excellent) were determined using analysis of variance (ANOVA) and t tests (Table 12; Figure 8). Each of the ANOVA tests was significant at least at the $p < 0.001$ level, except for pH which was significant at the $p < 0.01$ level. Sample sizes (n) for the ratings were 17 for Poor (8) and Fair (9) and 53 for Good (25) and Excellent (27).

Table 12. Differences in the means for seven fish community metrics, specific conductance, pH, and total habitat scores between samples rated Poor and Fair (Sand Hills NCIBI ≤ 8) and samples rated Good and Excellent (Sand Hills NCIBI ≥ 11). Mean diamonds are shown in Figure 8.

Metrics and Variables	Poor and Fair	Good and Excellent	p value (p < 0.05 were significant)	Response to stressors (compared to Sand Hills NCIBI ≥ 11)	% Difference
No. of Darter and Madtom Species	1.1	2.6	$p < 0.0001$	Decrease	-58
Percent Key Sand Hills Species	59.9	74.6	$p < 0.0001$	Decrease	-20
No. of Fish	146.2	81.8	$p < 0.01$	Increase	+79
Percent Key Sand Hills Fish	53.6	85.0	$p < 0.0001$	Decrease	-37
Number of Intolerant Species	0.5	2.3	$p < 0.0001$	Decrease	-78
Percentage of Tolerant Fish	27.1	8.7	$p < 0.0001$	Increase	+211
Percentage Invertivore Cyprinids	13.3	40.2	$p < 0.0001$	Decrease	-67
Specific Conductance (µS/cm)	44.2	24.9	$p < 0.0001$	Increase	+78
pH (s.u.)	6.0	5.4	$p < 0.01$	Increase	+75
Total Habitat Score	81.0	91.9	$p < 0.0001$	Decrease	-12

Results from the multiple comparison tests showed that there were significant mean differences in all metrics, specific conductance, pH, and the Total Habitat Score between sites rated Poor and Fair and sites rated Good and Excellent (Table 12; Figure 8):

- Number of Darter and Madtom Species – Excellent and Good > Fair and Poor
- Percentage of Key Sand Hills Species – Excellent and Good > Poor and Fair
- Number of Fish – Excellent and Good < Poor and Fair
- Percentage of Key Sand Hills Fish – Excellent and Good > Poor and Fair
- Number of Intolerant Species – Excellent and Good > Poor and Fair
- Percentage of Tolerant Fish – Excellent and Good < Poor and Fair
- Percentage of Invertivore Cyprinids – Excellent and Good > Poor and Fair
- Specific Conductance – Excellent and Good < Poor and Fair
- pH – Excellent and Good < Poor and Fair
- Total Habitat Score – Excellent and Good > Poor and Fair

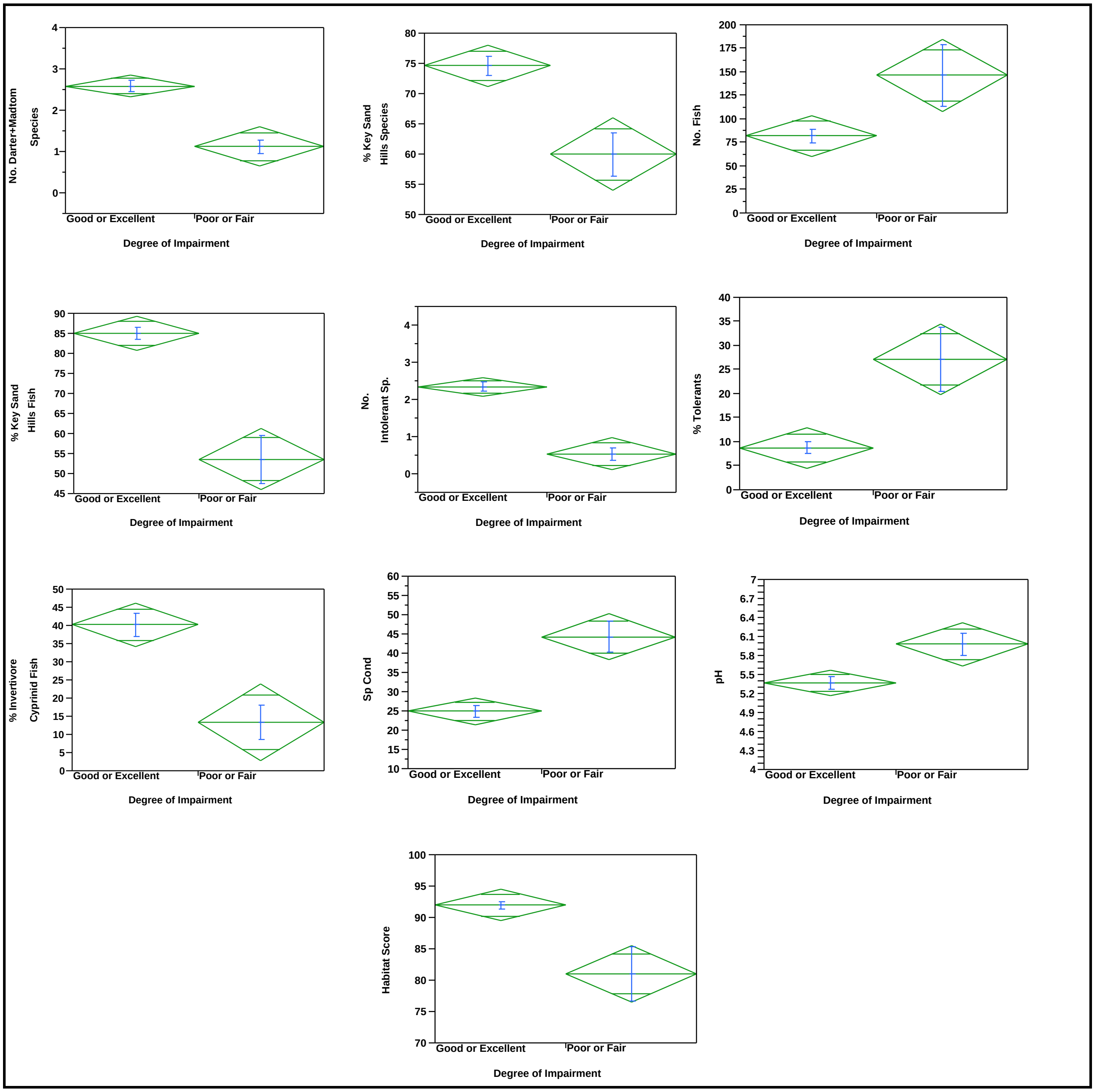


Figure 8. **Comparisons of means for seven metrics, specific conductance, pH, and total habitat scores for Good or Excellent vs. Poor or Fair sites in the Sand Hills. Significance of tests are listed in Table 12.**

All metrics, as well as habitat, pH, and specific conductance, were able to discriminate between Poor and Fair and Excellent and Good sites (Table 13). Contrasting the sites, the Excellent and Good sites had higher quality habitats, lower specific conductance, pH, and percentage of tolerant fish, greater percentages of Key Sand Hills Species and Key Sand Hills Fish, fewer fish, and more Intolerant Species than the Poor and Fair sites. The percent differences in the seven metrics between Poor and Fair sites (samples) and Good and Excellent sites (samples) ranged from -20% (Percent Key Sand Hills Species) to + 211% (Percentage of Tolerant Fish) (Table 12).

Differences in Metrics Among and Between Landuse Disturbance Classes

Responsiveness of the seven metrics, 7-Metric Sand Hills NCIBI total score, specific conductance, pH, and total habitat score among the landuse disturbance classes was determined using analysis of variance (ANOVA) and the Tukey-Kramer Honest Significant Difference tests (Figure 9). The disturbance classes were defined in the Methods section (Page 11) and are listed by sample in Appendix 3. Because landuse is updated only every 10 years or so, the 2006 NLCD landuse data were used for samples collected for all years prior to and after 2006. Sample sizes (n) for each of the landuse disturbance classes were: Forested (10), Mostly Forested (31), Mixed (34) and Developed (6).

Results from the multiple comparison tests showed that there were significant mean differences in the Number of Intolerant Species, Percentage of Tolerant Fish, the 7-Metric Sand Hills NCIBI Total Score, specific conductance, pH, and the Total Habitat Score among the four landuse disturbance classes (Figure 9). There were no significant mean differences (i.e., they were not sensitive to different landuse classes for the other five metrics among the four landuse disturbance classes (Figure 9). Specifically (means with the same superscript letter were not significantly different):

- Number of Darter and Madtom Species – no significant difference, $p > 0.05$
- Percentage of Key Sand Hills Species – no significant difference, $p > 0.05$
- Number of Fish – no significant difference, $p > 0.05$
- Percentage of Key Sand Hills Fish – no significant difference, $p > 0.05$
- Number of Intolerant Species – Forested $(2.2)^{a}$, Mostly Forested $(2.2)^{a}$, Mixed $(1.7)^{ab}$, Developed $(0.5)^{b}$; $p < 0.01$
- Percentage of Tolerant Fish – Forested $(12.2)^{b}$, Mostly Forested $(6.3)^{b}$, Mixed $(12.7)^{b}$, Developed $(53.3)^{a}$; $p < 0.0001$
- Percentage of Invertivore Cyprinids – no significant difference, $p = 0.05$
- 7-Metric Sand Hills NCIBI Total Score – Forested $(11.6)^{a}$, Mostly Forested $(11.6)^{a}$, Mixed $(10.4)^{a}$, Developed $(6.8)^{b}$; $p < 0.001$
- Specific Conductance (µS/cm) – Forested $(22.0)^{bc}$, Mostly Forested $(23.6)^{c}$, Mixed $(32.9)^{b}$, Developed $(48.8)^{a}$; $p < 0.001$
- pH (s.u.) – Forested $(4.9)^{c}$, Mostly Forested $(5.4)^{bc}$, Mixed $(5.6)^{ab}$, Developed $(6.4)^{a}$; $p < 0.001$
- Total Habitat Score – Forested $(92.5)^{a}$, Mostly Forested $(92.3)^{a}$, Mixed $(89.7)^{a}$, Developed $(67.6)^{b}$; $p < 0.001$

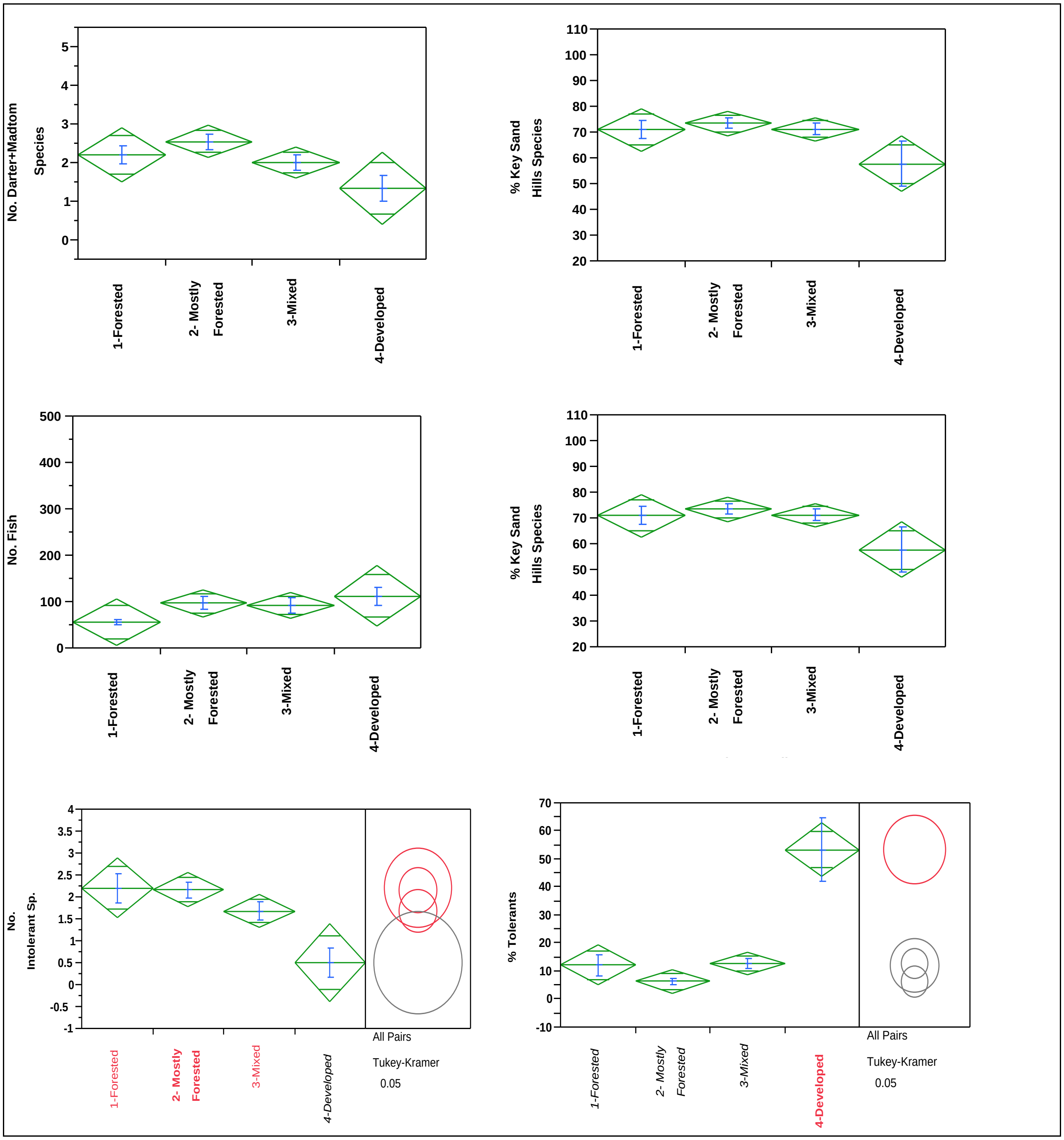


Figure 9. Multiple comparisons of means for seven metrics, 7-Metric Sand Hills NCIBI total score, specific conductance, pH, and total habitat score versus landuse disturbance classes. If the ANOVA was not significant, only a quantile plot is displayed. Tukey-Kramer tests and mean diamonds are shown for significant ANOVAs. Classes high-lighted in red are significantly different from the classes high-lighted in gray.

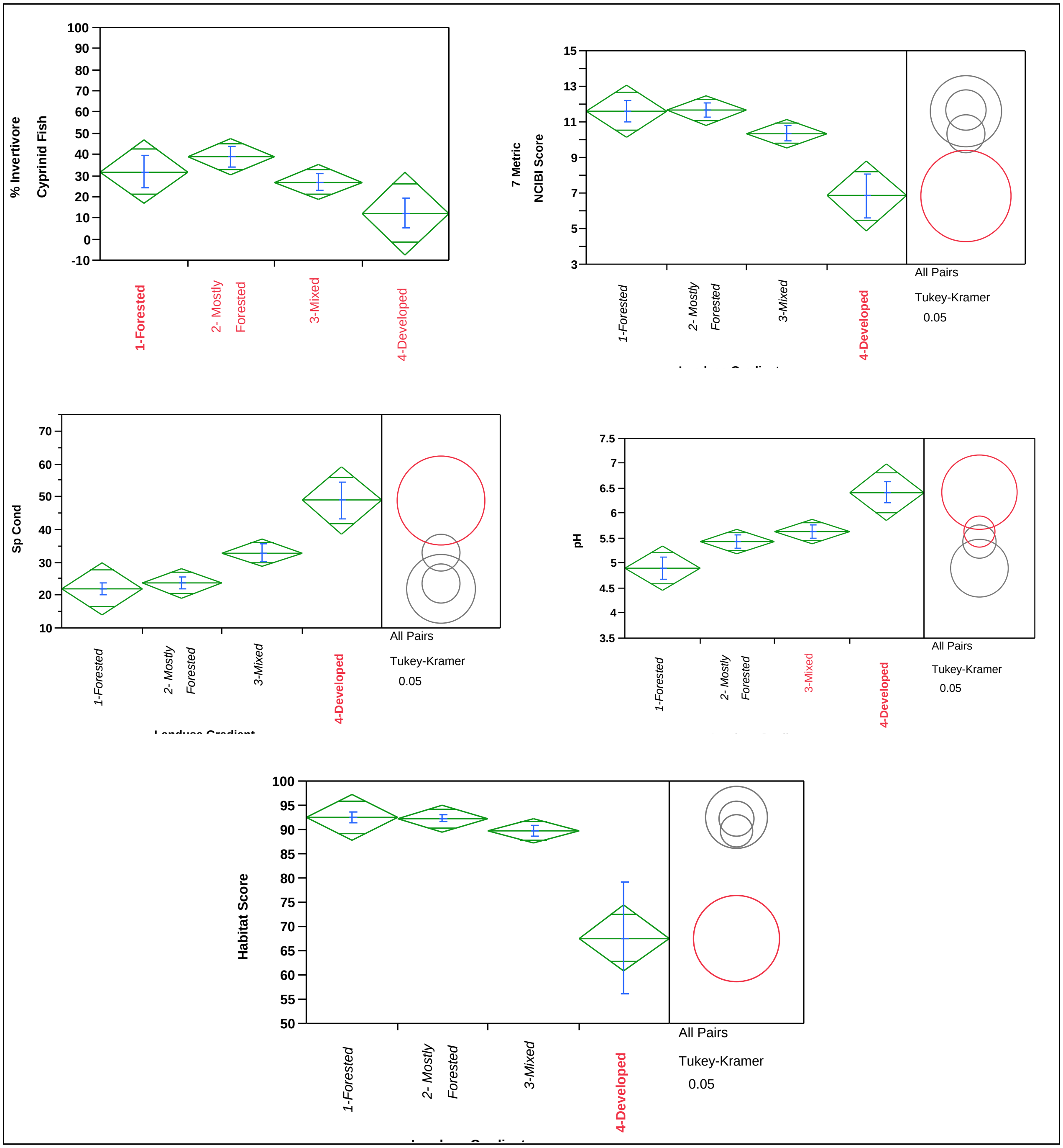


Figure 9 (continued).

Responsiveness of the seven metrics, specific conductance, pH, and total habitat score between Developed and Forested samples were further determined using analysis of variance (ANOVA) and t tests (Table 13; Figure 10). Sample sizes (n) for the ratings were 6 for Developed and 10 for Forested. Four of the seven metrics were not significantly different (i.e., were not sensitive) between Forested and Developed sites; however, 3 of the 7metrics, the 7-Metric Sand Hills NCIBI total score, specific conductance, pH, and total habitat score were able to discriminate between Developed and Forested sites (Table 13).

Table 13. Differences in the means for seven fish community metrics, total 7-metric Sand Hills NCIBI, specific conductance, pH, and total habitat scores between Forested (n=10) and Developed (n=6) sites/samples.

Metrics and Variables	Forested	Developed	p value (p < 0.05 were significant)	Response to increased development
No. of Darter and Madtom Species	2.2	1.3	p = 0.0542	No response
Percent Key Sand Hills Species	70.9	57.6	p = 0.1198	No response
No. of Fish	55.5	112.0	p = 0.0048	Increase
Percent Key Sand Hills Fish	76.1	78.6	p = 0.7794	No response
Number of Intolerant Species	2.2	0.5	p = 0.0043	Decrease
Percentage of Tolerant Fish	12.2	53.3	p = 0.0010	Increase
Percentage Invertivore Cyprinids	31.7	12.2	p = 0.1040	No response
7-Metric Sand Hills NCIBI Score	11.6	6.8	p = 0.0015	Decrease
Specific Conductance (µS/cm)	22.0	48.8	p < 0.0001	Increase
pH (s.u.)	4.9	6.4	p < 0.0005	Increase
Total Habitat Score	92.5	67.6	p < 0.01	Decrease

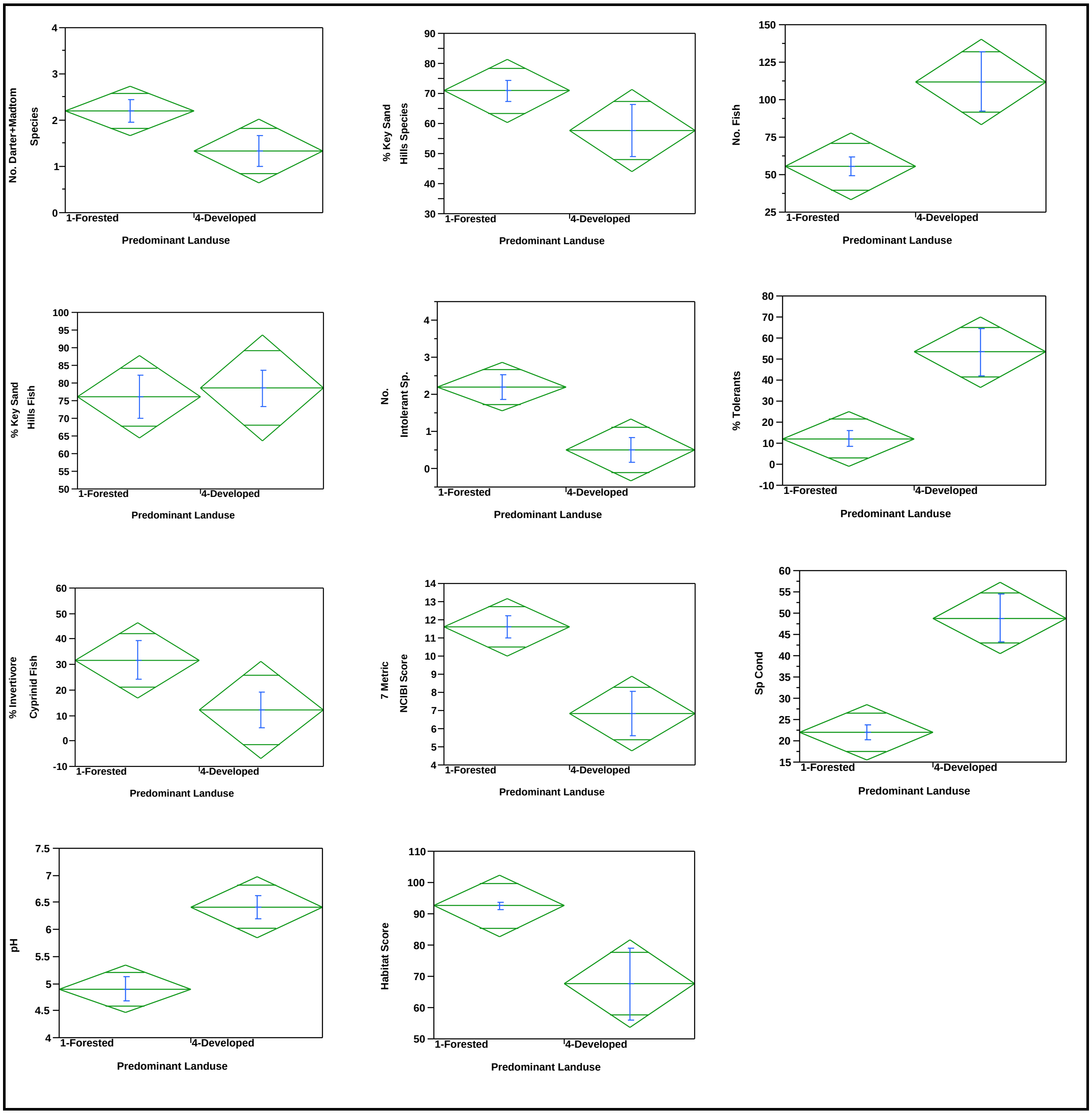


Figure 10. **Comparisons of means for seven metrics, the 7-Metric Sand Hills NCIBI total score, specific conductance, pH, and total habitat score versus two predominant landuse classes.**

SAND HILLS NCIBI VALIDATION

Because no new data had been collected from the Sand Hills since 2009, it was desired to identify, if possible, sites that encapsulated both ends of the disturbance gradient including least impaired (reference or near-reference) sites as well as substantially impaired sites to increase sample size and to help validate the metrics and biocriteria that have been developed (Tables 4 and 9). Unfortunately, a portion of the least-impaired sites (those with a high percentage of forested lands) are located within inaccessible or difficult to reach areas on the Fort Bragg property (e.g., upper Rockfish Creek watershed). Also, many of the smaller streams have been impounded or the free-flowing sections are fragmented by small reservoirs. Similarly, potentially degraded streams in the vicinity of the cities of Rockingham (e.g., Falling Creek) and Fayetteville (e.g., Beaver and Little Cross creeks) are also impounded or are swamp-like.

Additional Reference Watershed Sites

In the Summer of 2012, using Terrain Navigator® and USGS's StreamStats software (http://water.usgs.gov/osw/streamstats/north_carolina.html), 48 candidate watersheds were examined to determine their 2006 landuse characteristics (Tracy 2012). From this desk-top exercise, 26 sites qualified as potential reference sites which also were at least 65% forested. On September 12-13, 2012, each site was visited to determine: 1) if the site could be sampled, 2) if the habitat score was indeed greater than 65, and 3) if the width of the riparian zones were ≥ 18 m. After the site visit, 21 sites qualified as reference sites (Table 14; Tracy 2012). Some summary statistics for these 21 sites were:

- Drainage areas ranged from 3.3 mi^2 to 44.8 mi^2;
- Percent Forest/Wetland/Shrub ranged from 65% to 83%;
- Landuse class – all qualified as "Forested" (≥ 65%); and
- At least two sites were from each of the three river basins in the Sand Hills.

Table 14. Drainage area and 2006 NLCD database landuse types (in percentages) for potential reference sites in the Sand Hills. The Forested landuse class (LU) is defined as having ≥ 65% Forest/Wetland/Shrubland cover).

Basin/Waterbody	Location	County	d.a. (mi^2)	Barren	Cultiv. (Agric.)	Dev.	For./Wet./Shrb.	Grass./Herb.	Water	LU Class
CPF										
Deep Cr	Fire Break No. 10	Hoke	5.0	12.5	0.0	7.0	73.3	6.8	0.4	F
Hector Cr	SR 1001	Moore	4.7	0.1	4.4	3.0	69.4	22.0	1.1	F
Horse Cr	Manchester Rd	Hoke	3.3	15.2	0.0	4.5	72.5	7.8	0.0	F
Jumping Run	Manchester Rd	Hoke	5.0	22.4	0.0	4.1	64.6	8.7	0.4	F
Little Cr	Manchester Rd	Cumberland	2.2	7.0	0.0	1.7	80.0	10.2	1.2	F
Gum Br	Chicken Rd	Hoke	5.2	4.7	0.8	7.8	77.0	9.6	0.1	F
Piney Bottom Cr	Fire Break No. 25	Hoke	8.1	12.3	0.0	10.8	66.0	10.8	0.0	F
Rockfish Cr	King Road	Hoke	4.7	1.2	0.0	9.9	82.5	5.7	0.7	F
Rockfish Cr	Quewhiffle Rd	Hoke	22.1	7.0	0.0	9.8	75.4	7.7	0.2	F
Rockfish Cr	Chicken Rd	Hoke	27.2	7.0	0.0	8.9	75.9	8.0	0.2	F
Rockfish Cr	Plank Rd	Hoke	39.0	5.6	0.1	8.3	77.9	7.9	0.2	F
Rockfish Cr	All American Trail	Hoke	44.8	5.0	0.5	8.0	78.5	7.7	0.2	F
McDuffie Cr	Plank Road cut-off	Hoke	4.2	2.4	0.0	9.9	82.5	5.2	0.0	F
Puppy Cr	Plank Rd	Hoke	19.5	16.2	0.0	9.0	66.0	8.6	0.1	F
L Rockfish Cr	Plank Rd	Hoke	11.2	10.9	0.0	5.1	72.1	11.3	0.5	F
LBR										
Hills Cr	SR 1400	Scotland	6.0	0.0	6.8	6.7	71.9	11.7	2.8	F
UT Drowning Cr	SR 1141	Moore	7.8	0.0	10.5	3.0	69.6	15.1	1.7	F
Juniper Cr	SR 1324	Scotland	15.5	0.0	5.5	4.4	68.3	21.4	0.4	F
Juniper Cr	US 15/501	Scotland	19.5	0.0	6.9	4.8	67.3	20.4	0.5	F
YAD										
Baggetts Cr	US 1	Richmond	4.4	0.4	10.2	6.8	69.0	13.6	0.1	F
Bones Fk	SR 1487	Richmond	7.6	0.0	3.8	10.1	70.4	15.4	0.3	F

1Abbreviations are: Cultiv. (Agric.) = Cultivated (Agriculture), Dev. = Developed, For./Wet./Shrb. = Forest/Wetland/Shrubland, Grass,/Herb = Grassland/Herbaceous.

However, rather than sampling 21 sites, it was suggested that 10 would be adequate to validate the proposed metrics and biocriteria (Tables 4 and 9). On October 15-18, 2013, 11 sites were visited and/or sampled where fish community, habitat, and water quality data were collected according to standard operating procedures (NCDENR 2006) (Figure 11). Two sites could not be sampled due to unfavorable environmental conditions:

- Gum Branch (Hoke County, Cape Fear River Basin) -- even though the habitats and water quality were of high quality, the substrate was deep, soft muck and therefore, the creek was physically non-wadeable.
- Bones Creek (Richmond County, Yadkin River Basin) -- this creek was braided with no well-defined main channel either upstream or downstream of the bridge and therefore, the creek was not sampled.

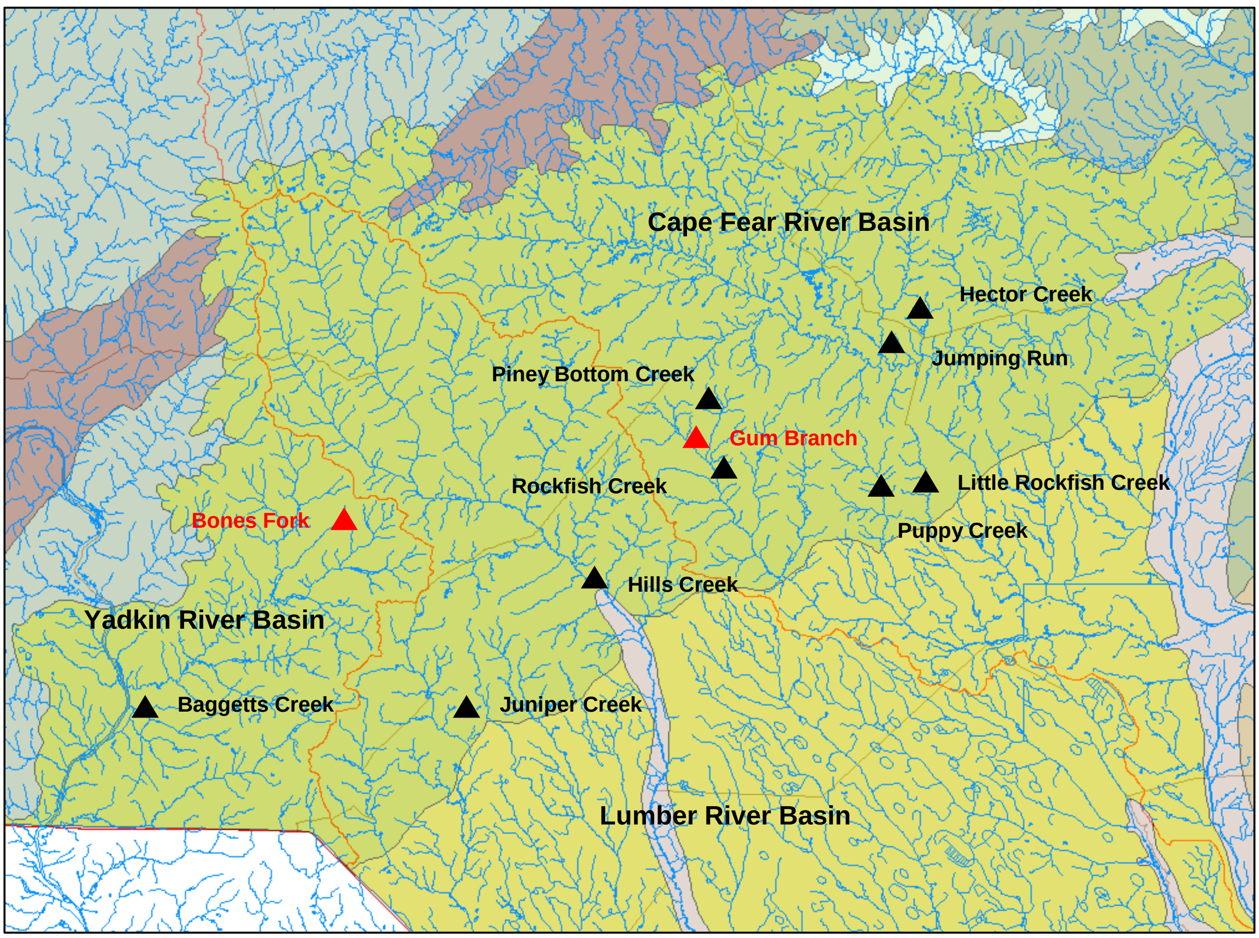


Figure 11. **Location of sample sites in the Sand Hills, October 15-18, 2013. Red triangles represent sites that were not sampled due to unfavorable environmental conditions (see text for explanations).**

Because the samples were collected in the Fall, all young-of-year were excluded from the data analyses. Flow conditions during the sampling event were slightly less than the median flow based upon the USGS gages at Rockfish Creek at Raeford and Flat Creek near Iverness in the Cape Fear River Basin and at Big Shoe Heel Creek near Laurinburg in the Lumber River Basin. Further up in the Lumber River Basin, the USGS gage on Drowning Creek near Hoffman was slightly greater than median flow.

Watershed and Landuse Characteristics

Summary statistics for these nine reference sites (Table 15):

- drainage areas ranged from 4.4 to 39.0 mi^2;
- percent agriculture ranged from 0.0 to 10.2%;
- percent developed (including unpaved military aircraft runways) ranged from 3.0 to 10.8%;
- percent grassland/herbaceous ranged from 7.9 to 22.0%; and
- percent forest/wetland/shrub ranged from 65 to 78%.

Contrasted to the original 14 reference sites (Table 2 and Appendix 3), these nine sites were slightly more forested (69.5 vs. 63.6%), less developed (6.5 vs. 7.7%), less cultivated (3.0 vs. 6.0%), and have less grassland (12.9 vs. 19.1%).

Water Quality and Habitat Assessments

Typical of other Sand Hills streams, the specific conductance and pH were very low (range 11-26 µS/cm and range 3.5-4.8 s.u., respectively; Table 15). All of the streams were clear, but darkly tannin-stained and are commonly known as “blackwater” streams (Figure 12). As expected, the habitat characteristics were of exceptionally high quality (total habitat scores range 85-98; Table 15, Figure 12) with stable vegetated banks, wide forested riparian zones, densely shaded canopies, and the channels were sinuous. However, Puppy and Baggetts creeks appeared to have been channelized historically because the channels were straight, the banks were entrenched, and the alternating pools and riffles were not as frequent as that found at other reference sites. Additionally, the habitat characteristics at Baggetts Creek were more typical of a Carolina Slate Belt-type stream than that of a Sand Hills stream. Unlike Sand Hills streams, the substrate was comprised of a moderately embedded cobble and gravel mix, there were frequent riffles as wide as the stream and extending twice the width of the stream, and macrophytes such as *Valisneria* and *Batrachospermum* were absent. Using the Piedmont/Mountain habitat criteria (NCDENR 2006), the total habitat score for Baggetts Creek was 84 as compared with the Sand Hills habitat score of 87, both of high quality.

Table 15. Site locations, habitat and landuse characteristics, and physical-chemical measurements for nine fish community sites in the Sand Hills, October 15-18, 2013. The Forested landuse class is defined as having ≥ 65% forest/wetland/shrub cover.

Waterbody	Hector Cr	Jumping Run	Rockfish Cr	Piney Bottom Cr	Puppy Cr	L Rockfish Cr	Hills Cr	Juniper Cr	Baggetts Cr
Location	SR 1001	Manchester Rd	Plank Rd	Fire Break No. 25	Plank Rd	Plank Rd	SR 1400	US 15/501	US 1
County	Moore	Hoke	Hoke	Hoke	Hoke	Hoke	Scotland	Scotland	Richmond
Latitude	35.18310	35.16378	35.05906	35.10267	35.04980	35.05444	34.98289	34.88394	34.86672
Longitude	79.09846	-79.11695	-79.27803	-79.30029	-79.12949	-79.09083	-79.40504	-79.45198	-79.83545
Drainage Area (mi^2)	4.7	5.0	39.0	8.1	19.5	11.2	6.0	19.5	4.4
Elevation (ft.)	180	200	215	240	200	180	250	225	160
Level IV Ecoregion	65c	65c	65c	65c	65c	65c	65c	65l	65c
Date Sampled	10/15/13	10/15/13	10/16/13	10/18/13	10/16/13	10/16/13	10/17/13	10/17/13	10/17/13
Fish Sample No.	2013-72	2013-73	2013-74	2013-80	2013-75	2013-76	2013-77	2013-78	2013-79
Habitat Characteristics									
Channel Modification (15)	15	15	15	15	10	15	15	15	10
Instream Habitat (20)	20	20	20	20	20	20	20	20	20
Bottom Substrate (15)	13	13	13	13	13	13	10	13	15
Pool Variety (10)	10	10	10	10	8	10	10	10	8
Erosion (10)	10	10	10	10	8	10	10	10	8
Bank Vegetation (10)	10	8	10	10	8	10	10	10	7
Light Penetration (10)	10	10	10	10	8	10	10	10	10
Riparian Zone-L (5)	5	5	5	5	5	5	5	5	5
Riparian Zone-R (5)	5	5	5	5	5	5	4	4	4
Total Score (0-100)	98	96	98	98	85	98	94	97	87
Visible Landuse (%)									
Forest	100	100	100	100	100	100	90	100	100
Rural Residential	0	0	0	0	0	0	10	0	0
2006 NLCD[2]									
Barren	0.1	22.4	5.6	12.3	16.2	10.9	0.0	< 0.1	0.4
Cultivated (Agriculture)	4.4	0.0	0.1	0.0	0.0	< 0.1	6.8	6.9	10.2
Developed	3.0	4.1	8.3	10.8	9.0	5.1	6.7	4.8	6.8
Forest/Wetlands/Shrubland	69.4	64.6	77.9	66.0	66	72.1	71.9	67.3	69
Grassland/Herbaceous	22.0	8.7	7.9	10.8	8.6	11.3	11.7	20.4	13.6
Water	1.1	0.4	0.2	0.0	0.1	0.5	2.8	0.5	0.1
Landuse Class	F	F	F	F	F	F	F	F	F
Physical-Chemical									
Ave. Width (m)	2	3	8	4	6	5	3	6	4
Ave. Depth (m)	0.4	0.4	0.5	0.4	0.4	0.5	0.4	0.4	0.4
Water Clarity	Blackwater	Blackwater	Blackwater	Blackwater	Blackwater	Blackwater	Blackwater	Blackwater	Blackwater
Temperature (°C)	18.3	18.0	18.2	18.3	18.6	18.6	17.8	17.7	19.0
Dissolved Oxygen (mg/L)	8.5	8.6	8.4	7.5	6.5	7.9	8.0	8.0	8.7
Dissolved Oxygen (%)	90	91	89	80	70	85	84	84	94
Specific Conductance (µS/cm)	18	11	13	13	12	12	15	25	26
pH (s.u.)	4.8	4.5	3.5	3.6	3.8	3.9	3.8	4.4	4.6

[1]65c=Sand Hills; 65l= Atlantic Southern Loam Plains.
[2]http://water.usgs.gov/osw/streamstats/north_carolina.html.

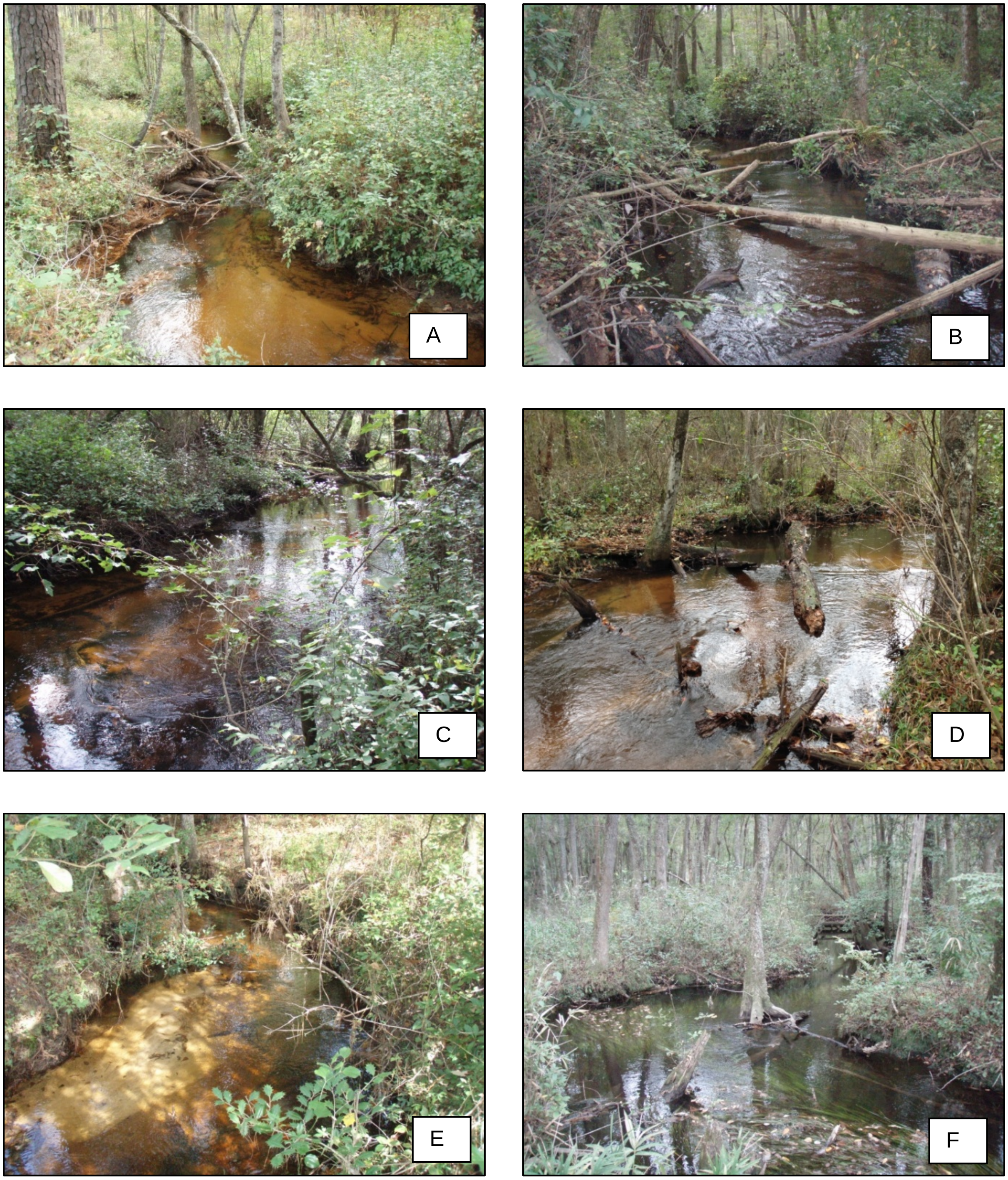


Figure 12. **Habitat characteristics of Sand Hills streams, October 15-18, 2013. Streams are: A) Jumping Run, B) Hills Creek, C) Juniper Creek, D) Piney Bottom Creek, E) Hector Creek, and F) Gum Branch.**

Fish Community Assessments

Twenty-two species were collected from the nine sites of which 17 were classified as Key Sand Hills Species (Table 16). The most wide spread species, collected at a majority of the sites, were Pirate

Perch, Redfin Pickerel, and Dusky Shiner, - all are classified as Key Sand Hills Species (Table 17). Metric values, metric scores, Total Sand Hills NCIBI scores, and Sand Hills NCIBI ratings are listed in Tables 18 and 19.

Table 16. Species collected from nine fish community sites in the Sand Hills, October 15-18, 2013.

Scientific Name	Common Name	Tolerance Rating[1]	Trophic Guild of Adults	Key Sand Hills Species
Anguillidae	**Freshwater Eels**			
Anguilla rostrata	American Eel	Intermediate	Piscivore	X
Cyprinidae	**Carps and Minnows**			
Notemigonus crysoleucas	Golden Shiner	Tolerant	Omnivore	
Notropis cummingsae	Dusky Shiner	Intermediate	Insectivore	X
Semotilus lumbee	Sandhills Chub	Intolerant	Insectivore	X
Catostomidae	**Suckers**			
Erimyzon oblongus	Creek Chubsucker	Intermediate	Omnivore	X
Ictaluridae	**North American Catfishes**			
Ameiurus brunneus	Snail Bullhead	Intermediate	Insectivore	
A. natalis	Yellow Bullhead	Tolerant	Omnivore	
Noturus insignis	Margined Madtom	Intermediate	Insectivore	
Esocidae	**Pikes**			
Esox americanus	Redfin Pickerel	Intermediate	Piscivore	X
E. niger	Chain Pickerel	Intermediate	Piscivore	X
Aphredoderidae	**Pirate Perches**			
Aphredoderus sayanus	Pirate Perch	Intermediate	Insectivore	X
Fundulidae	**Topminnows**			
Fundulus lineolatus	Lined Topminnow	Intermediate	Insectivore	X
Centrarchidae	**Sunfishes**			
Acantharchus pomotis	Mud Sunfish	Intolerant	Insectivore	X
Centrarchus macropterus	Flier	Intermediate	Insectivore	X
Enneacanthus chaetodon	Blackbanded Sunfish	Intolerant	Insectivore	X
E. gloriosus	Bluespotted Sunfish	Intermediate	Insectivore	X
Lepomis auritus	Redbreast Sunfish	Tolerant	Insectivore	X
L. macrochirus	Bluegill	Intermediate	Insectivore	
L. marginatus	Dollar Sunfish	Intolerant	Insectivore	X
Percidae	**Perches**			
Etheostoma mariae	Pinewoods Darter	Intolerant	Insectivore	X
E. olmstedi	Tessellated Darter	Intermediate	Insectivore	X
E. serrifer	Sawcheek Darter	Intolerant	Insectivore	X

[1]Based upon NCDENR (2006) and Appendix 1.

Table 17. Abundance of species collected from nine fish community sites in the Sand Hills, October 15-18, 2013.

		Waterbody								
Scientific Name	**Common Name**	**Hector Cr**	**Jumping Run**	**Rockfish Cr**	**Piney Bottom Cr**	**Puppy Cr**	**L Rockfish Cr**	**Hills Cr**	**Juniper Cr**	**Baggetts Cr**
Anguillidae	**Freshwater Eels**									
Anguilla rostrata	American Eel	---	---	1	---	---	---	1	3	---
Cyprinidae	**Carps and Minnows**									
Notemigonus crysoleucas	Golden Shiner	---	---	---	---	---	---	---	---	1
Notropis cummingsae	Dusky Shiner	25	29	10	25	37	23	38	12	---
Semotilus lumbee	Sandhills Chub	3	---	---	---	1	---	---	---	---
Catostomidae	**Suckers**									
Erimyzon oblongus	Creek Chubsucker	---	---	---	---	---	3	1	---	3
Ictaluridae	**North American Catfishes**									
Ameiurus brunneus	Snail Bullhead	---	---	---	---	---	---	1	1	---
A. natalis	Yellow Bullhead	2	3	1	2	2	---	2	YOY	8
Noturus insignis	Margined Madtom	16	7	2	1	---	---	---	3	---
Esocidae	**Pikes**									
Esox americanus	Redfin Pickerel	1	1	1	4	1	2	3	3	6
E. niger	Chain Pickerel	---	YOY	---	1	2	2	2	2	---
Aphredoderidae	**Pirate Perches**									
Aphredoderus sayanus	Pirate Perch	3	2	4	5	7	3	9	2	7
Fundulidae	**Topminnows**									
Fundulus lineolatus	Lined Topminnow	---	---	---	---	6	2	---	---	---
Centrarchidae	**Sunfishes**									
Acantharchus pomotis	Mud Sunfish	---	1	---	1	2	---	3	---	1
Centrarchus macropterus	Flier	---	---	---	---	---	---	---	2	---
Enneacanthus chaetodon	Blackbanded Sunfish	---	---	---	---	---	1	---	---	---
E. gloriosus	Bluespotted Sunfish	---	---	2	6	1	5	---	---	---
Lepomis auritus	Redbreast Sunfish	27	5	2	4	---	---	---	---	9
L. macrochirus	Bluegill	---	---	1	---	---	---	---	---	7
L. marginatus	Dollar Sunfish	---	---	---	1	---	3	1	1	19
Percidae	**Perches**									
Etheostoma mariae	Pinewoods Darter	---	---	---	---	---	---	4	3	---
E. olmstedi	Tessellated Darter	---	---	5	3	---	1	---	3	---
E. serrifer	Sawcheek Darter	---	---	---	---	5	1	---	---	---

Table 18. Values of seven Sand Hills NCIBI metrics from nine fish community sites in the Sand Hills, October 15-18, 2013.

Basin/Waterbody	No. Darter & Madtom Sp.	% Key SH Sp.	No. Fish	% Key SH Fish	No. Intol. Sp.	% Tol. Fish	% Invert. Cyprinids
Cape Fear							
Hector Cr	1	71	77	77	1	38	36
Jumping Run	1	71	48	79	1	17	60
Rockfish Cr	2	70	29	86	0	10	34
Piney Bottom Cr	2	82	53	94	2	11	47
Puppy Cr	1	90	64	97	3	3	59
L Rockfish Cr	2	100	46	100	3	0	50
Lumber							
Hills Cr	1	82	65	95	3	3	58
Juniper Cr	3	82	35	89	2	0	34
Yadkin							
Baggetts Cr	0	67	61	74	2	30	0

Table 19. Scores of seven Sand Hills NCIBI metrics and ratings from nine fish community sites in the Sand Hills, October 15-18, 2013.

Basin/Waterbody	No. Darter & Madtom Sp.	% Key SH Sp.	No. Fish	% Key SH Fish	No. Intol. Sp.	% Tol. Fish	% Invert. Cyprinids	Total NCIBI Score	Sand Hills NCIBI Rating
Cape Fear									
Hector Cr	1	2	2	2	1	1	2	11	Good
Jumping Run	1	2	2	2	1	2	2	12	Good
Rockfish Cr	1	2	2	2	0	2	2	11	Good
Piney Bottom Cr	1	2	2	2	2	2	2	13	Excellent
Puppy Cr	1	2	2	2	2	2	2	13	Excellent
L Rockfish Cr	1	2	2	2	2	2	2	13	Excellent[1]
Lumber									
Hills Cr	1	2	2	2	2	2	2	13	Excellent
Juniper Cr	2	2	2	2	2	2	2	14	Excellent
Yadkin									
Baggetts Cr	0	2	2	1	2	1	0	8	Fair[2]

[1]Little Rockfish Creek at Plank Road in Hoke County has been sampled three times -- in 2003, 2008, and most recently in October 2013. The landuse data reported in Appendix 3 were incorrectly calculated; data reported in Table 15 are correct. Little Rockfish Creek should have been considered a reference site for all the statistical analyses previously reported in this document. This misclassification was not detected until recently. Regardless of its classification, the fish community has consistently been rated Excellent (Appendix 6).
[2]See text for explanation.

7-Metric Sand Hills NCIBI Validation for Sand Hills Streams

Eight of the nine new reference sites (except for Baggetts Creek) rated Good (n=3, 37.5% of all sites) or Excellent (n=5, 62.5% of all sites) (Table 19). The percentage of sites rated as Excellent was greater than that for the original 26 reference sites (54% rated Excellent and 46% rated Good; Appendix 6). The Number of Darter and Madtom Species metric was the only metric that consistently scored lower (< 2) than what might be expected for reference streams (Tables 2 and 19). However, 15 of the 30 samples from the reference sites achieved the maximum score by having 3 or more species (Appendix 5), so the maximum score is achievable.

The fish community at Baggetts Creek was rated only as Fair using the 7-Metric Sand Hills NCIBI (Table 19). This was unexpected. However, the reasons why can be explained. Although the creek and its watershed are shown as being in the Sand Hills (Griffith et al. 2002), characteristics of this creek are more closely related to a Carolina Slate Belt-type stream than of a Sand Hills stream. Baggetts Creek was the only site from which the Dusky Shiner, a Key Sand Hills Species, was not collected. If this site was instead rated with the Yadkin River Basin Piedmont biocriteria (NCDENR 2006), the community would rate Good-Fair with a Piedmont NCIBI = 44. This scenario is similar to that in Mills Creek which is also shown as being in the Sand Hills (Griffith et al. 2002), but when rated using the Yadkin River Basin Piedmont biocriteria, it was rated as Excellent in 2006 and 2011 (NCDWR unpublished data). Therefore, it is suggested that Baggetts Creek be rated using the Piedmont biocriteria rather than the Sand Hills biocriteria.

Except for one stream which is more appropriately classified as a Carolina Slate Belt stream within the Piedmont, all communities were rated Good or Excellent. The new data further substantiated the effectiveness of the 7-Metric NCIBI for the Sand Hills region in determining the biological condition. Reference sites and other least impacted fish community sites in the Sand Hills will typically have:

- low specific conductance, low pH, low abundances of fish, and low percentages of tolerant fish;
- high percentages of Key Sand Hills Species, Key Sand Hills fish, and Invertivore Cyprinids;
- at least two Intolerant Species; and
- high quality instream habitat characteristics.

Additional Developed Watershed Sites

In January 2014, another suggestion was put forth that additional impaired sites in Developed watersheds should be sampled. During February and March 2014, landuse characteristics were determined for 15 candidate watersheds (Tracy 2014). From that desk-top exercise, seven sites were identified as potentially impaired with a very high percentage of their watershed developed or in cultivated crops (agriculture). On March 21, 2014, these sites were visited to determine if the site could be sampled. From this list, four sites were determined to be “sampleable” (Table 20 and Figure 13).

Table 20. **Proposed sampling sites and landuse/land classifications for four potentially impaired sites in the Sand Hills.**

Basin	CPF	LBR	CPF	CPF
Waterbody	Blounts Cr	Aberdeen Cr	Cross Cr	Walkers Cr
Location	Person St	US 15/501	Langdon St	NC 27
County	Cumberland	Moore	Cumberland	Harnett
Latitude	35.04981	35.12880	35.07960	35.36020
Longitude	-78.87060	-79.43167	-78.88850	-78.89950
Drainage Area (mi^2)	12.2	19.5	14.4	9.5
Barren (%)	0.0	0.1	0.9	0.0
Cultivated (Agriculture) (%)	0.8	0.9	2.5	33.8
Developed (%)	78.9	56.9	48.0	6.6
Forest (%)	12.9	28.9	31.3	34.7
Wetland (%)	4.5	5.4	6.6	5.1
Shrubland (%)	1.9	3.6	5.3	4.4
Forest + Wetland + Shrubland (%)	19.3	37.9	43.2	44.2
Grassland/Herbaceous (%)	0.6	2.0	3.4	15.1
Water (%)	0.5	2.1	1.9	0.4
LU Class[1]	Developed	Developed	Developed	Developed

[1] The Developed landuse class was defined as having less than 45% forest.

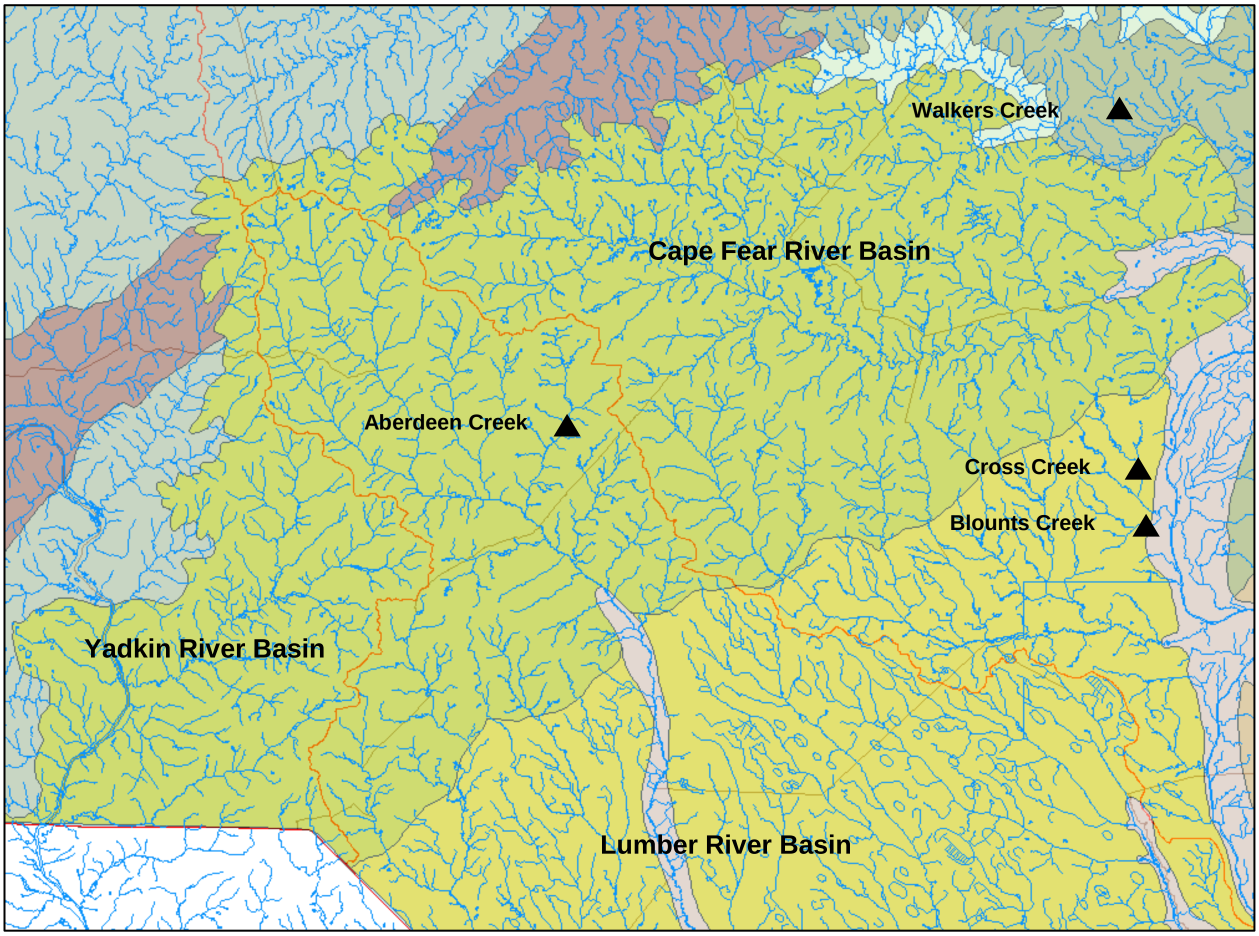


Figure 13. **Location of proposed developed sample sites in the Sand Hills.**

The four sites were visited and/or sampled on June 24, 2104 where fish community, habitat, and water quality data were collected according to NCDENR (2006). Two sites could not be sampled due to unfavorable hydrologic conditions:

- Aberdeen Creek (Moore County, Lumber River Basin) -- at this site, the creek is a dark, slow moving, deep pool (waist to chest deep), with an entrenched channel and is downstream from Pages Lake. Even though its watershed is more than 55% developed, it is not an ideal site to sample because of its close proximity to the lake (less than 0.5 mile downstream). Flows are also regulated by upstream Watson Lake Dam No. 1 and Watson Lake Dam No. 2 (Lake Dornach). And even though the flow conditions on June 24th were 50% of the median flow (57 cfs vs. 115 cfs) based upon the USGS gages at Drowning Creek near Hoffman, the creek was still too deep to effectively sample.
- Walkers Creek (Harnett County, Cape Fear River Basin) -- was not flowing; it is a small tributary to the Little River which was also well below median flow on June 24th.

Flow conditions on June 24th were slightly less than the median flow (5.7 cfs vs. 7.0 cfs) based upon the USGS gage at Flat Creek near Iverness in the Cape Fear River Basin. Early in the spring (approximately mid-May), both streams were well out of their banks due to exceptionally heavy rainfall in a short period of time.

Of the six sites/samples categorized as having predominantly developed watersheds (Tank, Blounts, Cross, and Aberdeen creeks; Appendix 3 and Table 20), Blounts Creek had the greatest percentage of its watershed developed (79%) and the least in forest (13%). The specific conductance and pH were elevated at Blounts and Cross creeks (81 µS/cm and 67 µS/cm, respectively, and 6.4 s.u. and 6.7 s.u., respectively) (Table 21) which is typical of Sand Hills streams with developed watersheds (Appendix 3). Both streams were also tannin stained. As commonly found in urban streams, the habitat characteristics were also of lower quality (total habitat scores were 71 and 65; Table 21) with eroding banks, substrates of urban debris; invasive plants within the riparian zones; rip/rap and other bank stabilization devices; urban debris stranded in the bank vegetation from high water events; and portions of the stream were channelized and the banks were often incised (Figure 14).

Figure 14. **Habitat characteristics of Blounts Creek at Person Street (A-C), June 24, 2014 and Cross Creek at Langdon Street (D-F), March 21, 2014, Cumberland County.**

Table 21. Site locations, habitat and landuse characteristics and physical-chemical measurements for two fish community sites in the Sand Hills, June 24, 2014. The Developed landuse disturbance class is defined as having < 45% forest/wetland/shrub cover.

Waterbody	Blounts Cr	Cross Cr
Location	Person Street	Langdon Street
County	Cumberland	Cumberland
Latitude	35.04981	35.07960
Longitude	-78.87060	-78.88850
Drainage Area (mi^2)	12.2	14.4
Elevation (ft.)	85	105
Level IV Ecoregion	Atlantic Southern Loam Plains	Atlantic Southern Loam Plains
Date Sampled	06/24/2014	06/24/2014
Fish Sample No.	2014-57	2014-58
Habitat Characteristics		
Channel Modification (15)	12	5
Instream Habitat (20)	12	15
Bottom Substrate (15)	13	9
Pool Variety (10)	10	10
Erosion (10)	2	2
Bank Vegetation (10)	8	8
Light Penetration (10)	9	9
Riparian Zone-L (5)	3	3
Riparian Zone-R (5)	2	4
Total Score (0-100)	71	65
Visible Landuse (%)		
Commercial	50	---
Industrial	50	---
Forest	---	25
Residential	---	75
2006 NLCD[1]		
Barren	0.0	0.9
Cultivated (Agriculture)	0.8	2.5
Developed	78.9	48.0
Forest + Wetlands + Shrubland	12.9	31.3
Grassland/Herbaceous	0.6	3.4
Water	0.5	1.9
Landuse Gradient	Developed	Developed
Physical-Chemical		
Ave. Width (m)	7	7
Ave. Depth (m)	0.4	0.4
Water Clarity	Clear, slightly tannic	Slightly turbid, tannic
Temperature (°C)	24.3	24.6
Dissolved Oxygen (mg/L)	7.2	6.8
Dissolved Oxygen (%)	86	82
Specific Conductance (µS/cm)	81	67
pH (s.u.)	6.4	6.7

[1]http://water.usgs.gov/osw/streamstats/north_carolina.html.

Fish Community Assessments

Twenty-two species were collected from the two sites of which nine were classified as Key Sand Hills Species (Table 22). The most abundant species was Bluegill at Blounts Creek; the Redbreast Sunfish, a Key Sand Hills Species, but also a Tolerant Species, was also abundant at Blounts and Cross creeks. Non-native species collected included the highly tolerant Green Sunfish and Redear Sunfish. Only one specimen of an Intolerant Species, Dollar Sunfish, was collected at each site; one species of darter, Tessellated Darter, was collected only at Cross Creek. Metric values and metric scores are listed in Table 23.

Table 22. Abundance of species collected from Blounts and Cross creeks, Cumberland County, June 24, 2014.

Scientific Name	Common Name	Tolerance Rating[1]	Trophic Guild of Adults[2]	Key Sand Hills Species	Blounts Creek	Cross Creek
Anguillidae	**Freshwater Eels**					
Anguilla rostrata	American Eel	Inter.	Pisc.	X	6	1
Cyprinidae	**Carps and Minnows**					
Cyprinella nivea	Whitefin Shiner	Inter.	Insect.		1	---
Nocomis leptocephalus	Bluehead Chub	Inter.	Insect.		1	---
Notemigonus crysoleucas	Golden Shiner	Tol.	Omni.		4	---
Notropis cummingsae	Dusky Shiner	Inter.	Insect.	X	---	3
N. hudsonius	Spottail Shiner	Inter.	Omni.		16	---
N. petersoni	Coastal Shiner	Inter.	Insect.	X	---	8
Ictaluridae	**North American Catfishes**					
Ameiurus brunneus	Snail Bullhead	Inter.	Insect.		1	---
A. natalis	Yellow Bullhead	Tol.	Omni.		1	3
A. platycephalus	Flat Bullhead	Tol.	Insect.		1	---
Aphredoderidae	**Pirate Perches**					
Aphredoderus sayanus	Pirate Perch	Inter.	Insect.	X	4	6
Fundulidae	**Topminnows**					
Fundulus rathbuni	Speckled Killifish	Inter.	Insect.	X	2	---
Poeciliidae	**Livebearers**					
Gambusia holbrooki	Eastern Mosquitofish	Tol.	Insect.		---	1
Centrarchidae	**Sunfishes**					
Enneacanthus gloriosus	Bluespotted Sunfish	Inter.	Insect.	X	---	1
Lepomis auritus	Redbreast Sunfish	Tol.	Insect.	X	53	53
L. cyanellus	Green Sunfish	Tol.	Insect.		2	---
L. gulosus	Warmouth	Inter.	Insect.		3	2
L. macrochirus	Bluegill	Inter.	Insect.		77	7
L. marginatus	Dollar Sunfish	Intol.	Insect.	X	1	1
L. microlophus	Redear Sunfish	Inter.	Insect.		12	---
Micropterus salmoides	Largemouth Bass	Inter.	Pisc.		1	1
Percidae	**Perches**					
Etheostoma olmstedi	Tessellated Darter	Inter.	Insect.	X	10	---

[1]Based upon NCDENR (2013) and Appendix 1. Intol. = Intolerant, Inter. = Intermediate, and Tol. = Tolerant.
[2]Based upon NCDENR (2013). Pisc. = piscivore, Omni. = omnivore, Insect. = insectivore.

Table 23. Values and scores of seven Sand Hills NCIBI metrics from two fish community sites in the Sand Hills, June 24, 2014.

Values/Scores//Waterbody	No. Darter & Madtom Sp.	% Key SH Sp.	No. Fish	% Key SH Fish	No. Intol. Sp.	% Tol. Fish	% Invert. Cyprinids
Sand Hills NCIBI Values							
Blounts Cr	1	28	196	38	1	31	< 1
Cross Cr	0	58	87	84	1	66	13
Sand Hills NCIBI Scores							
Blounts Cr	1	0	1	0	1	1	0
Cross Cr	0	1	2	2	1	0	1

7-Metric Sand Hills NCIBI Validation for Sand Hills Streams

Both sites rated Poor (Sand Hills NCIBI Total Score = 4 and 6 for Blounts and Cross creeks, respectively). Other developed watersheds (Tank Creek, Cross Creek at NC 87/210, Cross Creek at NC 87/201/24, and Aberdeen Creek) also rated Poor or Fair (Appendix 6). All three sites on Cross Creek were found to be impaired. At its confluence with the Cape Fear River, the almost 40 mi^2 Cross Creek watershed is 63% developed. Similarly, Tank Creek's watershed, which was also found to be impaired, is almost 80% developed (http://water.usgs.gov/osw/streamstats/north_carolina.html).

The new data further substantiated the effectiveness of the 7-Metric NCIBI for the Sand Hills region in determining the biological condition. Impaired fish community sites in the Sand Hills will typically have:

- elevated specific conductance and pH, high abundances of fish, and high percentages of tolerant fish;
- low percentages of Key Sand Hills Species, Key Sand Hills fish, and Invertivore Cyprinids;
- only one or an absence of Intolerant Species; and
- lower quality instream habitat characteristics.

CONCLUSIONS

Statistical analyses were used to derive a 7-Metric Sand Hills NCIBI for evaluating and rating fish communities in wadeable streams in the Sand Hills of North Carolina (Table 4). The processes for selecting and calibrating the biocriteria and derivation of ratings were based upon regional reference samples (Figures 4 and 6; Appendices 3 and 5) and methods published in the scientific literature (Karr 1981; Fausch et al. 1984; Karr et al. 1986; Miller et al. 1988; Simon and Lyons 1995; Paller et al. 1996; USEPA 1996; Hughes et al. 1998; Karr and Chu 1999; USEPA 1999; Angermeier et al. 2000; Schleiger 2000; McCormick et al. 2001; Smogor and Angermeier 2001; Hain et al. 2012). The seven metrics were able to identify impairment and to differentiate between highly impacted non-reference samples (rated Poor or Fair) and reference and near-reference samples (rated Good or Excellent) (Figures 7 and 8; Table 12). Relationships between the seven metrics versus specific conductance, pH, total habitat score, landuse types, and landuse disturbance classes were evaluated (Figures 9 and 10; Table 13). The metrics and biocriteria were further validated with additional data collected in 2013 and 2014.

Based upon a data set of more than 90 samples and the seven metrics used to derive the Sand Hills NCIBI, reference sites and other least impacted fish community sites in the Sand Hills should have low specific conductance, low pH, low abundances of fish, and low percentages of tolerant fish; high percentages of Key Sand Hills Species, Key Sand Hills fish, and Invertivore Cyprinids; at least two Intolerant Species; and high quality instream habitat characteristics (Figures 4, 7, and 8; Tables 4, 10, 12, 18, 19, and 24). Impacted sites would be expected to have the converse of these characteristics.

Table 24. Responses of the 7-Metric Sand Hills NCIBI for Sand Hills Streams

Biological Integrity Component/Metric	Reference & Least-Impacted Sites	Impaired Sites
Species Richness		
Number of Darter & Madtom Species	2 or more	0 or 1
Percentage of Key Sand Hills Species	High	Low
Fish Abundance		
Number of Fish	Low	High
Percentage of Key Sand Hills Fish	High	Low
Pollution Indicator		
Number of Intolerant Species	2 or more	0 or 1
Percentage of Tolerant Fish	Low	High
Trophic Composition		
Percentage of Invertivore Cyprinids	High	Low
Water Quality and Habitat		
Specific Conductance	Low	High
pH	Low	High
Habitat Quality	High	Low

Overall, the metrics for the Sand Hills NCIBI were able to show that as the habitat quality, water quality, and landuse practices changed, so did many integral functional and structural components of the Sand Hills communities. The 7-Metric Sand Hills NCIBI can be an effective water quality assessment tool in evaluating the biological integrity of fish communities in wadeable streams in the Sand Hills and adjacent ecoregions across the Lumber, Cape Fear, and Yadkin River basins.

RECOMMENDATIONS

Two recommendations are submitted for implementation:

1. Update NCDWR's fish community assessment database and Standard Operating Procedures (NCDENR 2013) which will enable the Sand Hills sites to be rated.
2. Implement metrics and rating criteria so that the ratings of fish communities in the Sand Hills may be used for Use Support and in future basinwide assessment and planning reports. Adoption of this monitoring tool will support the mission of the Water Sciences Section in . . . "*providing the Division with accurate information pertaining to waters of the state*"; . . ."*water quality monitoring . . .that provide scientifically defensible data*"; and . . ."*supporting the management and protection of North Carolina's water resources for the health and welfare of the citizens of North Carolina and the economic well-being of the state*".

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Appendix 1. Phylogenetic listing of the fishes in the Sand Hills of North Carolina.

Scientific Name	Common Name	River Basin Yadkin	River Basin Lumber	River Basin Cape Fear	Tolerance Rating[2]	Trophic Guild of Adults[2]	Key Sand Hills Species
Petromyzontidae	**Lampreys**						
Petromyzon marinus	Sea Lamprey			X	Intermediate	Parasitic	
Amiidae	**Bowfins**						
Amia calva	Bowfin		X	X	Tolerant	Piscivore	
Anguillidae	**Freshwater Eels**						
Anguilla rostrata	American Eel	X	X	X	Intermediate	Piscivore	X
Clupeidae	**Herrings**						
Dorosoma cepedianum	Gizzard Shad		X		Intermediate	Omnivore	
Cyprinidae	**Carps and Minnows**						
Clinostomus funduloides	Rosyside Dace			X	Intermediate	Insectivore	
Cyprinella analostana	Satinfin Shiner			X	Tolerant	Insectivore	
C. nivea	Whitefin Shiner	X		X	Intermediate	Insectivore	
C. sp. cf. *zanema*	Thinlip Chub	X	X	X	Intolerant	Insectivore	
Luxilus albeolus	White Shiner			X	Intermediate	Insectivore	
Nocomis leptocephalus	Bluehead Chub	X	X	X	Intermediate	Omnivore	
Notemigonus crysoleucas	Golden Shiner	X	X	X	Tolerant	Omnivore	
Notropis altipinnis	Highfin Shiner	X		X	Intermediate	Insectivore	
N. amoenus	Comely Shiner			X	Intermediate	Insectivore	
N. chalybaeus	Ironcolor Shiner		X	X	Intolerant	Insectivore	
N. chiliticus	Redlip Shiner	X	X[1]	X[1]	Intermediate	Insectivore	
N. cummingsae	Dusky Shiner	X	X	X	Intermediate	Insectivore	X
N. hudsonius	Spottail Shiner	X		X	Intermediate	Omnivore	
N. maculatus	Taillight Shiner	X			Intolerant	Insectivore	
N. petersoni	Coastal Shiner	X	X	X	Intermediate	Insectivore	X
N. scepticus	Sandbar Shiner			X	Intermediate	Insectivore	
Semotilus atromaculatus	Creek Chub	X		X	Tolerant	Insectivore	
S. lumbee	Sandhills Chub	X	X	X	Intolerant	Insectivore	X
Catostomidae	**Suckers**						
Erimyzon oblongus	Creek Chubsucker	X	X	X	Intermediate	Omnivore	X
E. sucetta	Lake Chubsucker	X	X	X	Intermediate	Insectivore	
Minytrema melanops	Spotted Sucker	X	X	X	Intolerant	Insectivore	X
Moxostoma collapsum	Notchlip Redhorse			X	Intermediate	Insectivore	
Ictaluridae	**North American Catfishes**						
Ameiurus brunneus	Snail Bullhead	X	X	X	Intermediate	Insectivore	
A. catus	White Catfish	X	X	X	Tolerant	Omnivore	
A. natalis	Yellow Bullhead	X	X	X	Tolerant	Omnivore	
A. nebulosus	Brown Bullhead	X		X	Tolerant	Omnivore	
A. platycephalus	Flat Bullhead	X	X	X	Tolerant	Insectivore	
Noturus gyrinus	Tadpole Madtom	X	X	X	Intermediate	Insectivore	X
N. insignis	Margined Madtom	X	X	X	Intermediate	Insectivore	
Esocidae	**Pikes**						
Esox americanus	Redfin Pickerel	X	X	X	Intermediate	Piscivore	X
E. niger	Chain Pickerel	X	X	X	Intermediate	Piscivore	X

[1]Nonindigenous, introduced into the drainage (Menhinick 1991, Rohde et al. 2009; and NCDWR and NCSM databases).
[2]Based upon NCDENR (2006).

Appendix 1 (continued).

Scientific Name	Common Name	River Basin: Yadkin	Lumber	Cape Fear	Tolerance Rating[2]	Trophic Guild of Adults[2]	Key Sand Hills Species
Umbridae	**Mudminows**						
Umbra pygmaea	Eastern Mudminnow	X	X	X	Tolerant	Insectivore	
Aphredoderidae	**Pirate Perches**						
Aphredoderus sayanus	Pirate Perch	X	X	X	Intermediate	Insectivore	X
Amblyopsidae	**Cavefishes**						
Chologaster cornuta	Swampfish		X	X	Intermediate	Insectivore	
Atherinopsidae	**New World Silversides**						
Labidesthes sicculus	Brook Silverside	X	X	X	Intermediate	Insectivore	
Fundulidae	**Topminnows**						
Fundulus lineolatus	Lined Topminnow	X	X	X	Intermediate	Insectivore	X
F. rathbuni	Speckled Killifish			X	Intermediate	Insectivore	
Poeciliidae	**Livebearers**						
Gambusia holbrooki	Eastern Mosquitofish	X	X	X	Tolerant	Insectivore	
Centrarchidae	**Sunfishes**						
Acantharchus pomotis	Mud Sunfish	X	X	X	Intolerant	Insectivore	X
Centrarchus macropterus	Flier	X	X	X	Intermediate	Insectivore	X
Enneacanthus chaetodon	Blackbanded Sunfish	X	X	X	Intolerant	Insectivore	X
E. gloriosus	Bluespotted Sunfish	X	X	X	Intermediate	Insectivore	X
E. obesus	Banded Sunfish		X	X	Intermediate	Insectivore	X
Lepomis auritus	Redbreast Sunfish	X	X	X	Tolerant	Insectivore	X
L. cyanellus	Green Sunfish	X[1]		X[1]	Tolerant	Insectivore	
L. gibbosus	Pumpkinseed	X	X	X	Intermediate	Insectivore	X
L. gulosus	Warmouth	X	X	X	Intermediate	Insectivore	
L. macrochirus	Bluegill	X	X	X	Intermediate	Insectivore	
L. marginatus	Dollar Sunfish	X	X	X	Intolerant	Insectivore	X
L. microlophus	Redear Sunfish	X[1]	X[1]	X[1]	Tolerant	Insectivore	
L. punctatus	Spotted Sunfish		X	X	Intolerant	Insectivore	X
Micropterus punctulatus	Spotted Bass			X[1]	Intermediate	Piscivore	
M. salmoides	Largemouth Bass	X	X	X	Intermediate	Piscivore	
Pomoxis nigromaculatus	Black Crappie	X	X	X	Intermediate	Piscivore	
Percidae	**Perches**						
Etheostoma flabellare	Fantail Darter			X	Intermediate	Insectivore	
E. fusiforme	Swamp Dater	X	X	X	Intermediate	Insectivore	
E. mariae	Pinewoods Darter		X		Intolerant	Insectivore	X
E. olmstedi	Tessellated Darter	X	X	X	Intermediate	Insectivore	X
E. serrifer	Sawcheek Darter	X	X	X	Intolerant	Insectivore	X
Perca flavescens	Yellow Perch	X[1]	X	X	Intermediate	Piscivore	
Percina crassa	Piedmont Darter	X	X	X	Intolerant	Insectivore	X

[1]Nonindigenous, introduced into the drainage (Menhinick 1991, Rohde et al. 2009; NCDWR and NCSM databases).
[2]Based upon NCDENR (2006).

Appendix 1 (continued).

Scientific Name	Common Name	River Basin: Yadkin	Lumber	Cape Fear	Tolerance Rating[2]	Trophic Guild of Adults[2]	Key Sand Hills Species
Elassomatidae	**Pygmy Sunfishes**						
Elassoma evergladei	Everglades Pygmy Sunfish		X	X	Intermediate	Insectivore	
E. zonatum	Banded Pygmy Sunfish		X	X	Intermediate	Insectivore	X
No. of Species	67	48	50	64			
No. of Intolerant Species	12	9	11	10			
No. Tolerant Species	13	11	9	13			
No. Key Sand Hills Species		21	25	24			25
No. Insectivorous Cyprinids	15	9	6	15			

[1]Nonindigenous, introduced into the drainage (Menhinick 1991, Rohde et al. 2009; NCDWR and NCSM databases).
[2]Based upon NCDENR (2006).

Appendix 2. Original fish community dataset (n = 94) for streams in the Sand Hills and adjacent ecoregions.

Basin/Waterbody	Station	County	Latitude	Longitude	Level IV Ecoregion	Date	Sample No.
Cape Fear							
McLendons Cr	SR 1210	Moore	35.307778	-79.543056	Sand Hills	05/05/98	98-29
						06/08/09	2009-58
Richland Cr	SR 1640	Moore	35.431944	-79.4327778	Triassic Basins	05/20/94	94-22
						04/24/98	98-24
Wet Cr	NC 24/27	Moore	35.390278	-79.640833	Carolina Slate Belt	06/27/03	2003-41
Barbeque Cr	SR 1285	Harnett	35.337965	-79.045544	Sand Hills	04/16/08	2008-17
(Lower) Little R	SR 2023	Moore	35.203611	-79.216389	Sand Hills	04/20/94	94-12
Anderson Cr	SR 2031	Harnett	35.265833	-78.819444	SE Floodplains & Low Terraces	05/06/98	98-33
						10/02/03	2003-63
						04/16/08	2008-16
Beaver Cr	SR 1825	Moore	35.269167	-79.226944	Sand Hills	04/22/02	2002-26
						04/09/08	2008-06
Buffalo Cr	SR 1001	Moore	35.189722	-79.136667	Sand Hills	05/07/98	98-34
						09/15/03	2003-56
						04/10/08	2008-08
Crane Cr	SR 1810	Moore	35.310000	-79.324444	Sand Hills	04/23/02	2002-29
						04/09/08	2008-05
Crane Cr	US 1	Moore	35.284444	-79.271944	Sand Hills	05/07/98	98-35
						04/22/02	2002-28
Crane Cr	SR 2005	Moore	35.261389	-79.252222	Sand Hills	04/22/02	2002-27
Crane Cr	SR 1001	Moore	35.216944	-79.186389	Sand Hills	04/20/94	94-11
Cypress Cr	SR 1103	Harnett	35.259444	-79.176944	Sand Hills	04/22/02	2002-25
Flat Cr	Manchester Rd	Hoke	35.182500	-79.177500	Sand Hills	09/16/03	2003-58
						04/10/08	2008-09
Herds Cr	NC 24/27	Moore	35.319167	-79.301667	Sand Hills	04/23/02	2002-30
James Cr	off SR 2026	Moore	35.187222	-79.293333	Sand Hills	09/16/03	2003-57
						04/10/08	2008-07
Jumping Run Cr	NC 210	Cumberland	35.217222	-78.943056	Sand Hills	10/02/03	2003-64
Little R	NC 22	Moore	35.269444	-79.416944	Sand Hills	09/15/03	2003-54
						04/09/08	2008-04
Muddy Cr	SR 1001	Cumberland	35.196667	-78.998611	Sand Hills	09/16/03	2003-59
						04/18/08	2008-20
Nicks Cr	NC 22	Moore	35.253333	-79.412500	Sand Hills	05/31/96	96-64
						09/15/03	2003-55
Tank Cr	Manchester Rd	Cumberland	35.187730	-79.006230	Sand Hills	06/27/08	2008-71
Bones Cr	SR 1400	Cumberland	35.063333	-79.038889	Atlantic Southern Loam Plains	10/20/03	2003-66
						04/11/08	2008-10
Cross Cr	NC 87/210	Cumberland	35.066667	-78.891667	Atlantic Southern Loam Plains	05/03/94	94-14
Cross Cr	NC 87/210/24	Cumberland	35.058333	-78.884444	Atlantic Southern Loam Plains	05/21/98	98-46
						10/20/03	2003-69
Gum Log Canal	SR 1728	Cumberland	35.064444	-78.842500	SE Floodplains & Low Terraces	10/02/03	2003-65
						04/16/08	2008-15
Juniper Cr	Plank Rd	Hoke	35.058333	-79.252222	Sand Hills	10/21/03	2003-70
						04/15/08	2008-11

Appendix 2 (continued).

Basin/Waterbody	Station	County	Latitude	Longitude	Level IV Ecoregion	Date	Sample No.
Cape Fear							
Little Rockfish Cr	Plank Rd	Hoke	35.054444	-79.090833	Sand Hills	10/20/03	2003-67
						04/15/08	2008-13
Nicholson Cr	SR 1301	Hoke	35.030833	-79.210556	Sand Hills	10/20/03	2003-68
						04/15/08	2008-12
Puppy Cr	SR 1406	Hoke	34.990833	-79.119722	Atlantic Southern Loam Plains	05/21/98	98-47
						10/21/03	2003-71
						04/15/08	2008-14
Lumber							
Aberdeen Cr	SR 1105	Moore	35.096944	-79.456111	Sand Hills	06/07/01	2001-58
						05/23/06	2006-58
Buffalo Cr	SR 1203	Hoke	34.975278	-79.358056	Sand Hills	06/05/01	2001-52
Deep Cr	SR 1113	Moore	35.123056	-79.542778	Sand Hills	06/07/01	2001-59
						05/22/06	2006-54
Drowning Cr	NC 73	Moore	35.187778	-79.648611	Sand Hills	03/25/96	96-02
						05/31/96	96-66
						06/06/01	2001-55
						05/22/06	2006-56
Horse Cr	SR 1112	Moore	35.132222	-79.492222	Sand Hills	05/23/06	2006-59
Jackson Cr	SR 1122	Moore	35.191667	-79.618611	Sand Hills	06/06/01	2001-56
						05/22/06	2006-55
Mountain Cr	SR 1215	Hoke	35.014167	-79.390556	Sand Hills	06/05/01	2001-53
						05/24/06	2006-61
Naked Cr	SR 1003	Richmond	35.081944	-79.589444	Sand Hills	03/25/96	96-01
						05/31/96	96-65
						06/06/01	2001-57
						05/22/06	2006-57
Quewhiffle Cr	SR 1225	Hoke	35.048889	-79.416944	Sand Hills	06/05/01	2001-54
						05/23/06	2006-60
Rocky Ford Br	SR 1424	Richmond	35.123333	-79.656667	Sand Hills	08/20/90	90-09
Big Shoeheel Cr	SR 1433	Scotland	34.803889	-79.376667	Atlantic Southern Loam Plains	05/23/01	2001-47
						05/24/06	2006-62
Gum Swamp Cr	SR 1344	Scotland	34.929444	-79.573056	Sand Hills	05/24/01	2001-51
						05/25/06	2006-64
Joes Cr	NC 79	Scotland	34.765278	-79.575556	Atlantic Southern Loam Plains	05/24/01	2001-50
						05/25/06	2006-65
Jordan Cr	SR 1324	Scotland	34.870556	-79.485278	Atlantic Southern Loam Plains	05/23/01	2001-48
						05/24/06	2006-63
Juniper Cr	SR 1405	Scotland	34.855000	-79.430278	Atlantic Southern Loam Plains	05/23/01	2001-49
						05/25/06	2006-66
Little Shoeheel Cr	SR 1405	Scotland	34.868056	-79.398889	Atlantic Southern Loam Plains	09/30/91	91-25
						03/25/96	96-03
Yadkin							
Beaverdam Cr	SR 1486	Richmond	35.022222	-79.683333	Sand Hills	04/15/96	96-13
						04/24/06	2006-22
Chock Cr	SR 1475	Richmond	34.984167	-79.671111	Sand Hills	04/25/06	2006-24

Appendix 2 (continued).

Basin/Waterbody	Station	County	Latitude	Longitude	Level IV Ecoregion	Date	Sample No.
Yadkin							
Hitchcock Cr	SR 1486	Richmond	35.007778	-79.660833	Sand Hills	04/05/01	2001-01
						04/24/06	2006-21
Marks Cr	SR 1104	Richmond	34.829722	-79.799722	Sand Hills	04/06/01	2001-04
						04/25/06	2006-23
Rocky Fork Cr	SR 1424	Richmond	35.035278	-79.701111	Sand Hills	04/05/01	2001-02
						04/24/06	2006-20
Rocky Fork Cr	SR 1487	Richmond	35.055556	-79.689722	Sand Hills	08/21/90	90-10

Appendix 3. **Drainage area and 2006 NLCD landuse types for reference and non-reference sites. Landuse disturbance classes are defined as: Forested (F, ≥ 65% forest cover), Mostly Forested (MF, 55-64% forest cover), Mixed (M, 45-54% forest cover), and Developed (D, < 45% forest cover).**

Basin/Waterbody	Sample No.	Total Habitat	Sp C (µS/cm)	pH (s.u.)	Drainage Area (mi^2)	Developed (%)	Forest (%)	Grassland (%)	Agr. (%)	Barren (%)	Impervious Cover (%)	Land Use Class
Reference												
Cape Fear												
Flat Cr	2003-58	90	15	4.8	7.7	6.5	59.4	17.5	0.0	16.5	2.82	MF
	2008-09	98	17	4.5								
James Cr	2003-57	88	26	5.6	11.4	10.7	66.4	15.5	2.8	4.0	2.64	F
	2008-07	92	28	4.5								
Muddy Cr	2003-59	90	22	4.4	15.7	8.2	73.0	16.0	1.6	0.7	5.20	F
	2008-20	98	31	4.3								
Juniper Cr	2003-70	94	13	4.8	11.0	8.9	64.9	20.9	0.0	4.2	3.18	F
	2008-11	95	14	3.8								
Nicholson Cr	2003-68	89	14	5.6	17.0	10.8	63.1	18.3	0.1	5.8	3.41	MF
	2008-12	95	13	5.0								
Lumber												
Deep Cr	2001-59	92	16	5.5	19.3	7.8	61.3	23.9	6.4	0.0	2.66	MF
	2006-54	96	20	4.8								
Drowning Cr	96-02	90	26	7.2	31.0	3.3	62.2	20.7	13.1	0.0	1.92	MF
	96-66	86	29	6.6								
	2001-55	95	32	6.6								
	2006-56	88	32	4.7								
Jackson Cr	2001-56	95	21	6.1	17.8	8.7	61.7	16.2	6.1	0.0	3.28	MF
	2006-55	87	24	5.3								
Gum Swamp Cr	2001-51	91	18	5.0	16.2	8.5	55.3	24.6	4.4	6.3	4.04	MF
	2006-64	95	20	5.4								
Joes Cr	2001-50	92	18	5.6	30.8	8.5	61.4	18.2	11.5	0.0	3.80	MF
	2006-65	96	21	5.4								
Juniper Cr	2001-49	91	19	4.6	24.0	4.9	64.2	19.0	11.5	0.0	1.68	MF
	2006-66	97	19	5.3								
Yadkin												
Beaverdam Cr	96-13	91	13	6.1	4.4	10.2	57.8	23.6	8.3	0.0	1.64	MF
	2006-22	87	19	5.6								
Hitchcock Cr	2001-01	96	20	5.4	23.2	9.3	69.7	16.9	3.4	0.1	2.27	F
	2006-21	87	18	5.2								
Rocky Fork Cr	2001-02	90	25	4.8	16.3	5.8	71.4	15.0	7.4	0.0	1.14	F
	2006-20	95	23	6.2								
Non-reference												
Cape Fear												
Barbeque Cr	2008-17	95	32	4.9	31.4	8.3	52.4	26.5	12.1	0.0	5.70	M
Anderson Cr	98-33	91	35	5.6	34.2	7.5	53.5	27.6	11.0	0.0	3.04	M
	2003-63	94	49	5.0								
	2008-16	94	54	5.3								
Beaver Cr	2002-26	91	27	5.4	13.6	4.9	57.8	28.1	8.9	0.0	3.71	MF
	2008-06	97	36	4.6								

Appendix 3 (continued)

Basin/Waterbody	Sample No.	Total Habitat	Sp C (µS/cm)	pH (s.u.)	Drainage Area (mi^2)	Developed (%)	Forest (%)	Grassland (%)	Agr. (%)	Barren (%)	Impervious Cover (%)	Land Use Class
Non-reference												
Cape Fear												
Buffalo Cr	98-34	88	21	4.5	18.2	5.5	52.9	32.7	8.6	0.0	3.11	M
	2003-56	89	26	4.9								
	2008-08	96	34	4.3								
Crane Cr	2002-29	88	53	6.3	16.8	8.1	51.3	27.4	12.4	0.0	5.14	M
Crane Cr	98-35	77	41	6.4	32.9	7.1	53.4	25.6	13.1	0.0	5.35	M
	2002-28	83	56	6.6								
Crane Cr	2002-27	82	63	6.3	60.3	7.9	50.3	27.4	13.7	0.0	4.62	M
Cypress Cr	2002-25	86	27	5.5	4.5	2.5	57.0	30.5	9.8	0.0	1.64	MF
Herds Cr	2002-30	94	67	6.8	9.0	7.9	51.8	24.8	14.6	0.0	5.07	M
Jumping Run Cr	2003-64	88	30	5.2	28.0	11.9	54.0	27.8	4.1	0.0	8.76	M
Little R	2003-54	88	65	6.2	27.3	9.1	59.6	23.0	7.4	0.0	5.01	MF
	2008-04	94	35	4.7								
Nicks Cr	96-64	91	18	5.7	26.4	22.6	56.8	16.9	3.1	0.0	9.94	MF
	2003-55	88	24	4.7								
Tank Cr	2008-71	76	70	5.9	5.7	71.6	15.2	6.0	6.9	0.0	60.51	D
Bones Cr	2003-66	89	14	5.8	12.5	5.9	54.3	28.4	0.2	11.1	4.81	M
	2008-10	88	18	4.8								
Cross Cr	94-14	---	47	6.6	15.2	44.7	41.3	8.5	2.9	1.0	40.63	D
Cross Cr	98-46	52	54	6.4	26.0	51.3	37.2	7.1	2.1	0.6	41.19	D
	2003-69	31	54	7.3								
Little Rockfish Cr	2003-67	89	11	5.7	11.2	4.0	51.5	31.7	0.0	12.2	2.94	M
	2008-13	92	12	4.3								
Puppy Cr	98-47	87	18	4.9	25.8	10.4	45.3	26.2	5.7	12.2	6.44	M
	2003-71	92	17	5.4								
	2008-14	94	20	4.4								
Lumber												
Aberdeen Cr	2001-58	86	31	6.4	28.0	42.8	40.7	11.2	3.8	0.0	17.93	D
	2006-58	93	37	5.9								
Buffalo Cr	2001-52	91	25	6.0	10.3	5.6	51.7	25.3	16.9	0.0	2.01	M
Horse Cr	2006-59	96	31	5.9	10.6	24.2	44.9	22.3	5.5	0.0	10.22	M
Mountain Cr	2001-53	95	29	6.2	9.7	6.9	63.4	18.1	11.3	0.0	2.64	MF
	2006-61	97	36	5.7								
Naked Cr	96-01	97	23	6.9	38.6	5.7	53.6	22.3	16.7	1.2	1.88	M
	96-65	84	14	6.3								
	2001-57	93	28	6.5								
	2006-57	89	33	6.0								
Quewhiffle Cr	2001-54	93	28	5.8	18.0	10.8	50.0	26.5	12.1	0.0	4.64	M
	2006-60	97	30	5.5								
Big Shoeheel Cr	2001-47	93	54	5.5	21.3	4.6	48.4	19.5	26.8	0.0	1.42	M
	2006-62	97	36	5.7								
Jordan Cr	2001-48	95	14	4.3	11.2	3.3	58.0	27.5	10.6	0.2	1.46	MF
	2006-63	97	16	5.8								
Little Shoeheel Cr	96-03	69	24	6.0	8.1	3.2	44.8	21.6	30.2	0.0	1.44	M

Appendix 3 (continued)

Basin/Waterbody	Sample No.	Total Habitat	Sp C (µS/cm)	pH (s.u.)	Drainage Area (mi^2)	Developed (%)	Forest (%)	Grassland (%)	Agr. (%)	Barren (%)	Impervious Cover (%)	Land Use Class
Non-reference Yadkin												
Chock Cr	2006-24	89	30	5.9	13.8	11.2	51.3	23.9	11.2	1.8	4.57	M
Marks Cr	2001-04	82	43	6.3	29.7	18.6	53.7	19.4	6.8	0.5	12.67	M
	2006-23	90	49	5.8								
Maximum	**---**	**31**	**11**	**3.8**	**4.4**	**3**	**15**	**6**	**0**	**0**	**1**	**---**
Minimum	**---**	**98**	**70**	**7.3**	**60.3**	**72**	**73**	**33**	**30**	**17**	**61**	**---**

Appendix 4. Data (n=13) deleted from the dataset and from any further consideration for deriving a Sand Hills Index of Biotic Integrity.

Basin/Waterbody	Station	County	Sample No.	Level IV Ecoregion	Reason(s) for Exclusion
Cape Fear					
McLendons Cr	SR 1210	Moore	98-29	Sand Hills	Borders the Triassic Basins, faunal similarities, rated with Piedmont criteria
McLendons Cr	SR 1210	Moore	2009-58	Sand Hills	Borders the Triassic Basins, faunal similarities, rated with Piedmont criteria
Richland Cr	SR 1640	Moore	94-22	Triassic Basins	Faunal similarities, rated with Piedmont criteria
Richland Cr	SR 1640	Moore	98-24	Triassic Basins	Faunal similarities, rated with Piedmont criteria
Wet Cr	NC 24/27	Moore	2003-41	Carolina Slate Belt	Faunal similarities, a Piedmont site; may go dry; beaver impacted since 2003
(Lower) Little R	SR 2023	Moore	94-12	Sand Hills	Should not have been sampled; too wide to sample with a crew of four; Not Rated
Crane Cr	SR 1810	Moore	2008-05	Sand Hills	Drought impacted, Not Rated
Crane Cr	SR 1001	Moore	94-11	Sand Hills	Downstream from Lake Surf, should not have been sampled because of lake effects and too wide to sample with a crew of four; Not Rated
Gum Log Canal	SR 1728	Cumberland	2003-65	SE Floodplains & Low Terraces	Atypical community, faunal similarities, rated with Piedmont criteria
Gum Log Canal	SR 1728	Cumberland	2008-15	SE Floodplains & Low Terraces	Atypical community, faunal similarities, rated with Piedmont criteria
Lumber					
Rocky Ford Br	SR 1424	Richmond	90-09	Sand Hills	No habitat data, data were collected with only two staff, Not Rated
Little Shoeheel Cr	SR 1405	Scotland	91-25	Atlantic Southern Loam Plains	No water quality or habitat data were collected, Not Rated
Yadkin					
Rocky Fork Cr	SR 1487	Richmond	90-10	Sand Hills	Below Millstone Lake; should not have been sampled because of lake effects; Not Rated

Appendix 5. Values of seven Sand Hills NCIBI metrics for reference and non-reference sites in the Sand Hills in North Carolina.

Basin/Waterbody	Sample No.	No. Darter & Madtom Sp.	% Key SH Sp.	No. Fish	% Key SH Fish	No. Intol. Sp.	% Tol. Fish	% Invert. Cyprinids
Reference sites								
Cape Fear								
Flat Cr	2003-58	2	75	73	52	2	11	14
	2008-09	0	86	135	99	2	5	72
James Cr	2003-57	2	86	20	90	1	0	30
	2008-07	3	77	40	73	3	8	33
Muddy Cr	2003-59	2	64	38	79	2	26	8
	2008-20	3	75	59	83	4	17	22
Juniper Cr	2003-70	3	70	49	84	1	6	51
	2008-11	3	88	75	93	2	3	76
Nicholson Cr	2003-68	3	80	30	90	2	7	53
	2008-12	3	64	54	85	2	4	52
Lumber								
Deep Cr	2001-59	3	88	39	92	2	0	46
	2006-54	3	92	36	97	3	17	33
Drowning Cr	96-02	2	50	45	47	1	11	29
	96-66	4	67	87	79	3	25	22
	2001-55	3	67	86	74	3	5	31
	2006-56	3	70	155	67	4	6	51
Jackson Cr	2001-56	2	79	71	96	2	6	47
	2006-55	4	88	65	97	4	8	42
Gum Swamp Cr	2001-51	2	88	100	96	1	0	65
	2006-64	3	82	54	89	2	2	22
Joes Cr	2001-50	2	77	88	97	3	2	69
	2006-65	4	57	154	88	2	10	64
Juniper Cr	2001-49	2	71	25	92	0	0	52
	2006-66	3	83	154	95	3	1	83
Yadkin								
Beaverdam Cr	96-13	0	83	17	41	1	6	0
	2006-22	1	50	25	64	2	12	32
Hitchcock Cr	2001-01	1	67	58	78	3	9	24
	2006-21	1	53	74	24	2	8	1
Rocky Fork Cr	2001-02	2	57	83	76	1	6	60
	2006-20	2	73	59	81	3	39	12
Non-reference Sites								
Cape Fear								
Barbeque Cr	2008-17	0	60	37	86	1	27	49
Anderson Cr	98-33	2	58	64	72	2	11	39
	2003-63	3	57	69	62	3	20	19
	2008-16	1	63	36	89	0	28	8
Beaver Cr	2002-26	3	67	118	29	1	4	0
	2008-06	1	80	31	35	1	0	0
Buffalo Cr	98-34	1	100	28	100	1	0	0
	2003-56	1	100	14	100	2	0	0
	2008-08	1	73	29	83	3	14	0
Crane Cr	2002-29	1	56	281	53	0	30	63

Appendix 5 (continued).

Basin/Waterbody	Sample No.	No. Darter & Madtom Sp.	% Key SH Sp.	No. Fish	% Key SH Fish	No. Intol. Sp.	% Tol. Fish	% Invert. Cyprinids
Non-reference Sites								
Cape Fear								
Crane Cr	98-35	2	59	176	44	0	13	24
	2002-28	2	50	489	17	0	8	10
Crane Cr	2002-27	1	67	114	43	0	5	22
Cypress Cr	2002-25	1	63	83	86	1	1	0
Herds Cr	2002-30	1	60	231	70	1	24	59
Jumping Run Cr	2003-64	1	73	51	61	1	37	0
Little R	2003-54	4	69	121	55	3	3	31
	2008-04	2	54	389	10	2	3	8
Nicks Cr	96-64	3	67	152	74	3	14	22
	2003-55	4	67	36	47	4	17	6
Tank Cr	2008-71	0	22	145	75	0	79	0
Bones Cr	2003-66	2	77	49	57	2	6	2
	2008-10	3	59	139	93	2	3	33
Cross Cr	94-14	1	64	159	60	0	48	9
Cross Cr	98-46	1	83	134	94	0	90	0
	2003-69	2	50	118	85	0	56	0
Little Rockfish Cr	2003-67	1	89	29	93	3	7	45
	2008-13	3	91	80	98	2	1	73
Puppy Cr	98-47	2	63	35	80	1	3	34
	2003-71	2	73	24	75	1	13	0
	2008-14	2	75	21	90	2	10	0
Lumber								
Aberdeen Cr	2001-58	2	73	89	88	2	21	42
	2006-58	2	53	27	70	1	26	22
Buffalo Cr	2001-52	4	83	136	85	3	6	14
Horse Cr	2006-59	4	78	112	95	4	3	50
Mountain Cr	2001-53	4	79	131	96	2	4	75
	2006-61	2	80	263	97	3	2	92
Naked Cr	96-01	3	88	33	52	1	3	9
	96-65	5	81	98	72	3	13	31
	2001-57	4	75	105	81	4	7	63
	2006-57	4	76	89	84	2	9	45
Quewhiffle Cr	2001-54	2	86	13	92	1	8	8
	2006-60	2	71	15	87	1	7	13
Big Shoeheel Cr	2001-47	0	83	23	87	1	30	44
	2006-62	2	71	90	90	3	12	63
Jordan Cr	2001-48	3	78	84	85	1	4	58
	2006-63	3	79	76	91	2	4	36
Little Shoeheel Cr	96-03	0	50	13	38	0	0	0
Yadkin								
Chock Cr	2006-24	1	54	54	76	3	7	20
Marks Cr	2001-04	3	62	100	91	1	35	38
	2006-23	2	62	238	81	3	32	34

Appendix 6. Sand Hills NCIBI scores and ratings for reference and non-reference sites in the Sand Hills in North Carolina.

Waterbody	Station	County	Stream Class	Date	Sample No.	Sand Hills NCIBI Score	Sand Hills NCIBI Rating
Reference sites							
Cape Fear							
Flat Cr	Manchester Rd	Hoke	WS-III	09/16/03	2003-58	11	Good
				04/10/08	2008-09	12	Good
James Cr	off SR 2026	Moore	WS-III	09/16/03	2003-57	12	Good
				04/10/08	2008-07	13	Excellent
Muddy Cr	SR 1001	Cumberland	C	09/16/03	2003-59	9	Good-Fair
				04/18/08	2008-20	13	Excellent
Juniper Cr	Plank Rd	Hoke	C	10/21/03	2003-70	13	Excellent
				04/15/08	2008-11	14	Excellent
Nicholson Cr	SR 1301	Hoke	C	10/20/03	2003-68	14	Excellent
				04/15/08	2008-12	13	Excellent
Lumber							
Deep Cr	SR 1113	Moore	WS-II;B,HQW	06/07/01	2001-59	14	Excellent
				05/22/06	2006-54	14	Excellent
Drowning Cr	NC 73	Moore	WS-II;Sw,HQW	03/25/96	96-02	9	Good-Fair
				05/31/96	96-66	12	Good
				06/06/01	2001-55	13	Excellent
				05/22/06	2006-56	12	Good
Jackson Cr	SR 1122	Moore	WS-II;HQW	06/06/01	2001-56	13	Excellent
				05/22/06	2006-55	14	Excellent
Gum Swamp Cr	SR 1344	Scotland	C	05/24/01	2001-51	12	Good
				05/25/06	2006-64	13	Excellent
Joes Cr	NC 79	Scotland	C;Sw	05/24/01	2001-50	13	Excellent
				05/25/06	2006-65	12	Good
Juniper Cr	SR 1405	Scotland	C;Sw	05/23/01	2001-49	11	Good
				05/25/06	2006-66	13	Excellent
Yadkin							
Beaverdam Cr	SR 1486	Richmond	WS-III	04/15/96	96-13	7	Fair[1]
				04/24/06	2006-22	11	Good
Hitchcock Cr	SR 1486	Richmond	WS-III	04/05/01	2001-01	12	Good
				04/24/06	2006-21	8	Fair[1]
Rocky Fork Cr	SR 1424	Richmond	WS-III	04/05/01	2001-02	11	Good
				04/24/06	2006-20	11	Good
Non-reference Sites							
Cape Fear							
Barbeque Cr	SR 1285	Harnett	C	04/16/08	2008-17	9	Good-Fair
Anderson Cr	SR 2031	Harnett	C	05/06/98	98-33	11	Good
				10/02/03	2003-63	11	Good
				04/16/08	2008-16	7	Fair
Beaver Cr	SR 1825	Moore	WS-III	04/22/02	2002-26	9	Good-Fair
				04/09/08	2008-06	8	Fair[1]
Buffalo Cr	SR 1001	Moore	WS-III	05/07/98	98-34	10	Good-Fair
				09/15/03	2003-56	11	Good
				04/10/08	2008-08	11	Good

Appendix 6 (continued).

Waterbody	Station	County	Stream Class	Date	Sample No.	Sand Hills NCIBI Score	Sand Hills NCIBI Rating
Non-Reference sites							
Cape Fear							
Crane Cr	SR 1810	Moore	WS-III	04/23/02	2002-29	6	Poor[2]
Crane Cr	US 1	Moore	WS-III	05/07/98	98-35	8	Fair[2]
				04/22/02	2002-28	6	Poor[2]
Crane Cr	SR 2005	Moore	WS-III	04/22/02	2002-27	5	Poor[3]
Cypress Cr	SR 1103	Harnett	WS-III	04/22/02	2002-25	9	Good-Fair
Herds Cr	NC 24/27	Moore	WS-III	04/23/02	2002-30	8	Fair[4]
Jumping Run Cr	NC 210	Cumberland	C	10/02/03	2003-64	8	Fair
Little R	NC 22	Moore	WS-III;HQW	09/15/03	2003-54	13	Excellent
				04/09/08	2008-04	6	Poor
Nicks Cr	NC 22	Moore	WS-III	05/31/96	96-64	11	Good
				09/15/03	2003-55	10	Good-Fair
Tank Cr	Manchester Rd	Cumberland	C	06/27/08	2008-71	4	Poor
Bones Cr	SR 1400	Cumberland	C	10/20/03	2003-66	10	Good-Fair
				04/11/08	2008-10	13	Excellent
Cross Cr	NC 87/210	Cumberland	C	05/03/94	94-14	4	Poor
Cross Cr	NC 87/210/24	Cumberland	C	05/21/98	98-46	7	Fair
				10/20/03	2003-69	6	Poor
Little Rockfish Cr	Plank Rd	Hoke	B	10/20/03	2003-67	13	Excellent
				04/15/08	2008-13	14	Excellent
Puppy Cr	SR 1406	Hoke	C	05/21/98	98-47	11	Good
				10/21/03	2003-71	10	Good-Fair
				04/15/08	2008-14	11	Good
Lumber							
Aberdeen Cr	SR 1105	Moore	C	06/07/01	2001-58	12	Good
				05/23/06	2006-58	8	Fair
Buffalo Cr	SR 1203	Hoke	C	06/05/01	2001-52	13	Excellent
Horse Cr	SR 1112	Moore	WS-II;HQW	05/23/06	2006-59	14	Excellent
Mountain Cr	SR 1215	Hoke	C	06/05/01	2001-53	14	Excellent
				05/24/06	2006-61	11	Good
Naked Cr	SR 1003	Richmond	WS-II;ORW	03/25/96	96-01	10	Good-Fair
				05/31/96	96-65	13	Excellent
				06/06/01	2001-57	14	Excellent
				05/22/06	2006-57	14	Excellent
Quewhiffle Cr	SR 1225	Hoke	C	06/05/01	2001-54	10	Good-Fair
				05/23/06	2006-60	11	Good
Big Shoeheel Cr	SR 1433	Scotland	C;Sw	05/23/01	2001-47	10	Good-Fair
				05/24/06	2006-62	13	Excellent
Jordan Cr	SR 1324	Scotland	C;Sw	05/23/01	2001-48	13	Excellent
				05/24/06	2006-63	14	Excellent
Little Shoeheel Cr	SR 1405	Scotland	C;Sw	03/25/96	96-03	5	Poor
Yadkin							
Chock Cr	SR 1475	Richmond	WS-III	04/25/06	2006-24	11	Good
Marks Cr	SR 1104	Richmond	C	04/06/01	2001-04	11	Good
				04/25/06	2006-23	10	Good-Fair

[1]See text for further explanation;[2]rated Good-Fair with Piedmont criteria; [3]rated Fair with Piedmont criteria; and [4]rated Good with Piedmont criteria.