ABSTRACT

HAIN, ERNST FREDERICK. Development of an Effective Fish Index of Biotic Integrity for the Sandhills Region of North Carolina. (Under the direction of Stacy A.C. Nelson.)

We describe a process for developing an index of biotic integrity (IBI) for resident fish assemblages in an ecoregion that exhibits low natural productivity and biodiversity. From 1990 to 2006, 55 fish community samples were collected by the North Carolina Division of Water Quality (NCDWQ) in the Cape Fear, Lumber, and Yadkin river basins within the Sandhills US EPA level IV ecoregion. Initial analyses of these data, using the 12 IBI metrics employed by the NCDWQ in other regions of the state, failed to distinguish significant differences between reference and non-reference streams. To develop a more robust method of measuring responses to anthropogenic disturbance, we delineated contributing watersheds for each of the 36 sample sites using GIS, hydrologic modeling, and 20-foot resolution Digital Elevation Models (DEM) derived from Light Detection and Ranging (LiDAR) data. The 2001 National Land Cover Database (NLCD) and *in situ* habitat data were used to determine various land use/land cover and hydrologic variables within each watershed. These variables were then used to select the sample sites with absolute minimal anthropogenic impacts. We used the Kruskal-Wallis test to identify eleven fish community metrics, two chemical metrics, and nine individual species that were significantly different in reference and non-reference sites. Of these fifteen metrics, only three exhibited higher values in reference streams. Our results demonstrate that the abundance and diversity of the Sandhills fish fauna are greater in area more highly impacted by anthropogenic activities. By automating the process by which reference sites are chosen, we

were able to produce a multi-metric IBI that reflects the varying levels of anthropogenic impacts on wadeable streams in the Sandhills ecoregion.

Development of an Effective Fish Index of Biotic Integrity for the Sandhills Region of North Carolina

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Ernst Frederick Hain

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APPROVED BY:

Halil I. Cakir

James F. Gilliam

Bryn H. Tracy

Stacy A.C. Nelson
Chair of Advisory Committee

BIOGRAPHY

Masters of Science Candidate, North Carolina State University, Fall 2008

Bachelors of Music, North Carolina School of the Arts, May 2004

North Carolina General Education Diploma, 2000

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1.1.1. Background

Land conversions from development may ultimately degrade water quality and fish habitat within areas of high change by increasing impervious surfaces, storm water runoff, excess sediment, and environmental pollutants which then drain into adjacent waterways (Paul and Meyer 2001, Wang et al. 2001, Jennings and Jarnagin 2002). Increasing developmental pressures within the Sandhills region of North Carolina may conflict with the state's ability to develop baseline reference stream sites and robust indices for the geographically unique waters of this area. An additional complication in developing water quality indices based on fish is the naturally low abundance and diversity of fish fauna in the Sandhills. Thus, it is important that agencies such as the North Carolina Division of Water Quality (NCDWQ) continue to develop more efficient monitoring tools which account for escalating levels of human population growth and development, and their associated impacts on aquatic ecosystem health (Schueler 1994, Schueler and Clayton 1997). The development of effective biological classification criteria and the use of geospatial tools, such as geographic information systems (GIS) and remote sensing, in land use and freshwater monitoring can play a vital role in reducing the cost, labor, and time required to monitor these systems over large geographic areas (Nelson et al. 2003, Nelson et al. 2006).

1.1.2. North Carolina Division of Water Quality Purpose

The Federal Clean Water Act (FCWA, sections 305b and 303d) requires all states to identify natural waters that are impaired or otherwise too degraded to support their designated and existing uses. In addition to the environmental and recreational benefits (*fishable* and *swimmable* waters as defined by the FCWA), these waters are also important to the economy of each state (Canfield et al. 2002). These resources provide a valuable source of water, critical linkages to commercial and sports fisheries, habitat support for fish and wildlife, local and regional hydroelectric power, as well as aesthetic and recreational values to the citizenry (Heiskary et al. 1994, Kerr et al. 1994, Obrecht et al. 1998, Canfield et al. 2002). In fact, the importance of these functions is so critical, that any reductions in stream function or ecosystem health could mean severe impacts, not only within the affected local region, but on a statewide level as well. Thus, routine and effective monitoring is important to measure changes in water quality and biota so appropriate management decisions can be made (Heiskary et al. 1994, Obrecht et al. 1998, Canfield et al. 2002). However, extensive monitoring and collection programs, on a statewide level still remain costly and logistically prohibitive (Nelson et al. 2003). Thus, novel approaches are required to assess water quality and stream health over large geographic areas.

In recent decades numerous federal and state agencies, as well as volunteer organizations, have developed integrative approaches to efficiently monitor the health of our nation's flowing waters (Heiskary et al. 1994, Kerr et al. 1994, Obrecht et al. 1998). Traditionally, *in-situ* chemical and benthic macroinvertebrate monitoring have been the primary method of water quality and aquatic systems assessment (Hilsenhoff 1982, US EPA 1996). These methods have proven useful for reflecting local impairments to the sampled water bodies, over shorter time periods. However, both methods may be limited in providing a "*whole-systems approach*" in the assessment of environmental conditions on a larger spatial and temporal scale. Fish assemblage assessments, or fish indices of biotic integrity (IBI), have recently evolved as a prominent tool for monitoring stream ecosystem health (Zampella and Bunnell 1998, Karr and Chu 1997, Bozzetti and Schulz 2004).

1.1.3. Index of Biotic Integrity

Fish IBI's were first developed in the Midwestern United States as an innovative approach to assess the diminishing capability of freshwater systems to support stable biotic communities (Karr 1981). Significant correlation exists between fish community composition and habitat quality (Pirhalla 2004, Zampella and Bunnell 1998). The primary objective of a fish index of biotic integrity (IBI) is to first determine what fish community characteristics are typical, or can be expected in a given ecoregion (Bozzetti and Schulz 2004). In order to determine the biologic consequences of human actions, a baseline that estimates minimal human impact should be established (Karr and Chu 1997). Once a baseline is established, candidate community metrics may be chosen to describe reference conditions.

Many state monitoring and regulatory agencies have now adopted such techniques. The development of a fish IBI takes into account resident fish populations and assemblages by assessing the numbers and types of fish in comparison to populations within relatively undisturbed, or reference, waters. Fish population sizes, taxa richness, trophic structure, and species composition, as indicated by the IBI, coupled with benthic macroinvertebrate monitoring and chemical water quality monitoring, provide a much broader indication of the overall health of an ecosystem (Karr 1991). This is primarily a result of the biological communities sampled being able to provide a more accurate reflection of the long-term conditions occurring within the environment, as opposed to the "*snapshot*" conditions provided by the more traditional methods.

Fish IBI scores are usually developed using one of two general scoring techniques. The first, as proposed by Karr (et al. 1986), gives each metric a score of 1, 3, or 5 based on a given sample's performance in this metric as either approximating, deviating slightly from, or being markedly different from reference conditions. The sums of these scores represent the total index of biotic integrity score. For instance, based on Karr's twelve-metric approach, a sample will receive a score between 12 and 60. These scores will then be assigned to a given biologic integrity class from very poor to excellent (Karr 1981).

The second technique is to convert Karr's scale to a continuous 0-100 score. By converting each metric, as well as the total index of biotic integrity score, to a percent of the maximum score, a more familiar decimal system is created and may provide easier comparison to other biologic and habitat evaluations (Hughes et al. 1998, Minns et al. 1994).

Indices based on fish assemblages may show the effects of land conversions on water quality conditions more quickly than conventional methods of chemical and benthic macroinvertebrate sampling (Karr 1981, Karr 1991). Ambient water quality and benthic macroinvertebrate sampling by the NCDWQ within the Sandhills region of North Carolina (Figure 1.1) suggest excellent water quality conditions, despite the fact that the fish assemblage quality has not been rated (NCDWQ 2002, NCDWQ 2003, NCDWQ 2004). Many intolerant fish species have been collected, including the endemic *Etheostoma mariae* (Pinewoods Darter) and *Semotilus lumbee* (Sandhills Chub) (NCDWQ 2002 and 2004). Any change in this level of species presence could suggest a potentially critical and ecologically important shift in the fish assemblages, and ultimately the region's water quality. Currently the NCDWQ has collected over 15 years of fish population and habitat data within the Sandhills regions, which coupled with land use and other geospatial analysis methods, may provide an exceptionally valuable dataset for assessing the future stream health and water quality changes associated with ever increasing anthropomorphic impacts. These impacts include urbanization as well as preemptive *Piocoides borealis* (Red-Cockaded Woodpecker) habitat destruction, and additional land use conversions associated with agriculture and pine plantations.

1.1.4. Study Area

The North Carolina Sandhills, designated as an U.S. EPA Level IV ecoregion (Figure 1.2) (Griffith et al. 2002), contains portions of seven state counties, as well as portions of three major river basins: the Cape Fear, Lumber and Yadkin River Basins (Figure 1.1). The Sandhills ecoregion is considered a transition zone between the coastal plain and piedmont, with a landscape characterized by rolling, sandy hills and dense hydrologic drainage networks. Soils in this region typically consist of thick, droughty, and low-nutrient, Cretaceous-aged marine sands (Griffith et al. 2002). This ecoregion is home to urban areas such as Fayetteville and Southern Pines, as well as the Fort Bragg Military Reservation and Pope Air Force Base. This region's forested areas have been drastically reduced as a result of logging and clearing, and fire suppression has altered the community properties (Landers et al. 1995). Additionally, within this area, numerous birds, mammals, mussels, insects, plants, and fish species, such as the endemic Pinewoods Darter and Sandhills Chub, are now considered rare or endangered (LeGrand et al. 2006, Rohde and Arndt 1991, Rohde and Ross 1987, Snelson and Suttkus 1978).

The characteristic tree species of the Sandhills ecosystem is the Longleaf Pine (*Pinus palustris)*, which is a long-lived tree capable of growing well in a multitude of soil types and site conditions. Longleaf Pine ecosystems once covered much of the southeastern United States coastal plain (Ware et al. 1993). Longleaf Pine savannahs reached as far north as southeastern Virginia, south into central Florida and as far west as eastern Texas (Stout and Marion 1993). Currently, the largest contiguous block of Longleaf Pine ecosystem remaining in North Carolina is found within the Sandhills ecoregion, primarily at the Fort Bragg Military Reservation and Weymouth Woods Sandhills Nature Preserve (Sorrie et al. 2006). This tree is typically considered a keystone species in a habitat usually devoid of hardwood or brush understory (Landers et al. 1995). Historically, frequent fires have prevented the establishment of less fire-resistant species. The result is a savannah-like habitat dominated by even-aged or multi-aged Longleaf Pine stands, with a groundcover of exceptionally diverse grass species (Landers et al. 1995, Outcalt and Sheffield 1996). Additionally, Longleaf Pine were drastically overexploited for lumber and turpentine, and many acres were cleared for agricultural use and yellow pine (*Pinu taeda* and others) plantations (Frost 1993). Nationwide, Longleaf Pine ecosystems have been reduced from over 92 million acres in pre-colonial times to under 4 million acres today (Kelly and Bechtold 1990).

The North Carolina Sandhills are home to the second largest population of Red-Cockaded Woodpecker (Ligon 1970, Ware et al. 1993). Red-Cockaded Woodpecker (RCW) are a nonmigratory species who are dependent upon Longleaf Pine ecosystems for survival. The birds live in cooperative-breeding colonies that build nesting cavities in living mature Longleaf Pines (Walters et al. 1992). The RCW have been listed as an endangered species since 1970 (Ligon 1970, Ware et al. 1993). Large portions of RCW habitat in North Carolina are on private lands, and the presence of RCW's has been shown to affect land owner management (Lueck and Michael 2003). Property owners whose land is in close proximity to RCW populations have been observed to be more likely to harvest trees so as to prevent the establishment of mature Longleaf Pines, thus preventing RCW habitat (Lueck and Michael 2003). This preemptive habitat destruction protects the landowner from losing certain property rights associated with the Endangered Species Act.

1.1.5. Geography/Ecoregion Characteristics of the Study Area

Geologically, the Sandhills ecoregion is composed of dry, sandy ridgetops (Jacqmain et al. 1999). The ridgetops are commonly explained as being formed as dunes by the Cretaceous Atlantic shoreline (Griffith et al. 2002, Collins et al. 2006). This area is known as the Fall Line, and is the region that separates the Coastal Plain from the Piedmont Plateau. Rivers within this region flow across the Fall Line through a series of rapids and falls before entering the Coastal Plain. The Fall Line parallels the Atlantic Coastline from New Jersey to Georgia, and represents the range boundaries for many tree and fish species (Shankman and Hart 2007). While the area was once dominated by Longleaf Pine, *Pinus taeda* (Loblolly Pine) and *P. echinata* (Shortleaf

Pine) are now also found alongside in a mixed pine matrix. Hardwood *Quercus* (oak) forests are also found in the Sandhills, especially in more fertile and moist soils (Skeen et al. 1993, Ware et al. 1993).

The soils of the Sandhills represent a transition zone from dense clay soils to the north and west and coarse sandy soils to the south and east. This transition zone experiences greater precipitation during summer months than does surrounding areas (Koch and Ray 1997). Local variation in heat flux gradients due to soil type transitions have been shown to cause temperature and pressure gradients along with circulation and increases in precipitation and lightening (Boyles et al. 2007).

Frequent fires are thought to be responsible for the dominance of the Longleaf Pine ecosystem in the southeastern United States (Gilliam and Platt 1999, Jacqmain 1999). As fire has now long been suppressed in eastern forests by human actions, fire dependent ecosystems have vanished (Gilliam and Platt 1999, Frost 1993). Fire suppression has additionally contributed to the diminishing presence of Longleaf Pine savannahs. Prescribed fires have become a major tool for restoring and maintaining Longleaf Pine savannahs throughout there range. Prescribed fires on a 2 to 4 year interval, depending on soil type and land use, are normally required to maintain a landscape that resembles a Longleaf Pine savannah (Collins et al. 2006). However, mixed-use land matrices can often limit or prevent fire management practices (Allen et al. 2006, Schnurr and Collins 2007).

1.1.6. Biodiversity

The diversity and abundance of aquatic fauna in the North Carolina Sandhills is naturally low (NCDWQ 2003). Small scale experiments and theory of species invasion suggest that communities of low diversity may be less susceptible to successful invasion of exotic species unless resource additions occur (Levine 2000, Tilman 2004). On the other hand, other experiments have shown that increased diversity inhibits invasion more so than resource addition may facilitate it (Maron and Marler 2008). As the Sandhills possesses both low diversity and low resource availability, the region may be relatively safe from invasive species (NCDWQ 2003). However, freshwater fauna assemblages throughout the country are becoming more homogenized due to widespread introductions of sport fish (Rahel 2000). Areas such as the Sandhills, where fewer fish results in fewer gamefish may be particularly at risk of loosing the integrity of their natural communities. The stocking of gamefish then may pose a more serious threat than invasive species in altering fish assemblages from pre-anthropogenic conditions.

1.1.7. Limitations

As the objective of a fish IBI is to quantify the tolerance level of fish assemblages to anthropogenic disturbance, it must then be necessary to isolate said disturbance in order to assert correlation (Whittier et al 2007). Complications occur because anthropogenic disturbances are normally multivariate. Also, tolerance values of fish species may not remain constant throughout an area of interest. It would be useful then, if individual species' tolerance values were continuously quantified specifically for a given study area, such as the Sandhills region, as opposed to categorized as tolerant, moderately tolerant, and intolerant (Whittier et al 2007).

However, site-specific quantified tolerance values are not common data, and such studies have only recently appeared in the literature (Whittier 2007, Pirhalla 2004).

Although biologic indices based on a single taxonomic group have been used for some time, multiple criteria indices are a relatively younger tool (Johnson et al. 2006, Paavola 2003). Different taxonomic groups have been shown to exhibit unique responses to various ecological stressors (O'Connor 2000). Thus, it should be understood that not all stressors will be expressed through fish assemblages. Fish IBI's are most appropriately used in conjunction with other taxonomic or chemical monitoring (Paller 2001).

1.1.8. Current Status of Sandhills Fish IBI

In North Carolina, the Biological Assessment Unit (BAU) of the Division of Water Quality (NCDWQ) is responsible for collecting fish community data on the biological integrity of the state's *wadeable* streams, classifying each stream on a scale from poor to excellent, and reporting this assessment to the public (NCDWQ 2006). Each of North Carolina's 17 river basins are sampled and assessed by the NCDWQ on a five-year rotation. While exhaustive sampling in the Sandhills region of the state has been conducted, time constraints and resources have prevented BAU from developing statistically defensible criteria for categorizing the health of the fish communities in these streams (*NCDWQ personal communication 2006*). Previous indices used for this region were designed primarily for the Piedmont region streams of North Carolina and did not take into consideration the unique faunal assemblages, low abundance and diversity of fish communities, low pH levels, and the low primary productivity of the large number of tanninstained and blackwater streams found within the Sandhills region (*NCDWQ personal communication 2006*).

1.1.9. Geographic Information Systems Approach

New geospatial tools, such as Light Detection and Ranging (LiDAR) technology, provide increased potential for providing improved landscape information useful in stream-based watershed applications (Lefsky et al. 2002, Reutebuch et al. 2005). Data derived from LiDAR imagery are capable of mapping both topography and vegetation. These data differ from imagery-based remote sensing technology by incorporating a *z*-value for surface elevation. The *z*-value is estimated by measuring the time between laser pulse and reflections returning to the sensor from the earth's surface (Bachman 1979). Because LiDAR emits and records the reflection of the sensor's laser pulse, it is considered an "active" remote sensing platform (Wehr and Lohr 1999). The resulting output of a LiDAR platform is a series of points representing *x,y, and z* coordinates, known as a "point cloud". The sensors employed by this platform emit pulses at a rate between 10,000 and 100,000 per second (Reutebuch et al. 2005). The result is very high resolution (1-5m) and accurate elevation data (Reutebuch et al. 2005).

Topographic surfaces are derived from LiDAR data by extracting the bare earth values through a series of automated and manual procedures (Killian et al. 1996, Kraus and Pfeifer 1998). A continuous surface in raster format may then be created from the point data using one of several geostatistic interpolatation techniques, including inverse distance weighting, kriging, and regularized spline with tension (Lloyd and Atkinson 2006, Mitasova et al. 2005).

Elevation data derived from LiDAR has been used in hydrologic modeling and to produce highly accurate hydrologic maps (Wehr and Lohr 1999, Colson 2007). The high resolution digital elevation models (DEM) produced from LiDAR data are ideal for topography-based watershed delineations (Thompson 2001). In an effort to produce better floodplain maps, the state of North Carolina has flown statewide LiDAR surveys. The resulting data have been made available to the public by the N.C. Floodplain Mapping Program and can be found at www.ncfloodmaps.com.

1.2. Research Objectives and Justifications

1.2.1. Statement of Problem

This study worked in conjunction with the North Carolina Division of Water Quality (NCDWQ). My objective was to develop more efficient assessment techniques for the biological monitoring of aquatic systems within the North Carolina Sandhills region of the Cape Fear, Lumber, and Yadkin-Pee Dee River Basins. This region, due to its unique geography, has proven to be challenging in the assessment of long-term stream health based on a biotic index of resident fish assemblages. Similar indices of biotic integrity (IBI) have been successfully employed by the NCDWQ in other parts of the state to assess habitat and water quality for compliance with the Federal Clean Water Act. Using data collected by the NCDWQ from 1990 to 2006, multiple fish, stream, and landscape criteria were used to evaluate streams’ ability to support fish communities comparable to reference streams.

The NCDWQ has routinely sampled fish assemblage data within the Sandhills region on 5-year increments, from 1990 to 2008. Repeated samples were collected from 36 sites comprising this dataset, with 7 sites within the Yadkin River Basin, 11 sites within the Lumber River Basin, and 19 sites within the Cape Fear River Basin. Fifty-five samples have been collected from the Sandhills region.

To produce robust criteria for designating reference sites that demonstrate minimal anthropogenic impacts, a database of detailed land use/land cover and habitat variables must be produced for each site. To accomplish this, high-resolution digital elevation models (DEM) were developed from LiDAR data, obtained from the North Carolina Flood Plains Mapping Program. Using the hydrology tools in the spatial analyst toolbox within ESRI's ArcGIS 9.2 (ESRI 2006), watersheds draining to each sample site were delineated. Land use, habitat variables, physical characteristics and hydrologic features collected with remote sensing and GIS technologies were then analyzed to determine anthropogenic impacts within each watershed. These data were then used to select the least impacted sites to be used in describing reference conditions. Quantitative statistics were then developed for the land use/land cover and habitat variables in each of these watersheds.

1.2.2. Objective 1; Develop a GIS-Based Data Management Model for Integrative Water Quality Monitoring

To develop a more robust method of measuring responses to anthropogenic disturbance, I created a model that delineates local water catchments using 20-foot resolution LiDAR data and NCDWQ sample sites as the mouth of watershed catchments. This model extracts 2001 National Land Cover Dataset (NLCD) land use data from these catchments, and along with *in situ* data in the attribute table of the NCDWQ shapefiles and additional data stored in Microsoft Access, the model determines if each sample site meets reference conditions. Reference criteria data and fish assemblage data for each site were then exported to SAS for statistical analyses.

1.2.3. Justification

An automated model for developing a fish index of biotic integrity has multiple benefits. Primarily, the automated model allows biologists to effectively manage and analyze data without necessitating the time and GIS skills normally required. Additionally, the model statistics are derived from all of the currently available data. As the NCDWQ continues to collect fish community data, the model results can be easily updated. This allows for any moving baselines to be observed and tracked. Since the IBI is derived by comparing fish community metrics of “reference streams” to those of “non-reference streams,” it is possible that even the reference conditions are changing over time. A traditional IBI may evaluate metrics based on current reference conditions, and then continue to use those metrics in rating streams for years or decades into the future. An automated model allows users to run all data or only data from a particular time, such as the first year of monitoring. By comparing model results after each sampling season, biologist can more easily observe changes in significant metrics that may occur. Also, it will be easier to observe if reference and non-reference streams are becoming more similar or more different through time.

1.2.4. Objective 2; Apply Model to NC Sand Hills Data

The fish IBI model was applied to the NC Sandhills using fish community data collected by the NCDWQ. I used this model to establish which community metrics are significantly different between reference and non-reference sites. These metrics were used to create a scoring technique whereby sampled sites can be rated as either poor, fair, good-fair, good, or excellent.

1.2.5. Justification

The mission of the Fish Community Program of the NCDWQ BAU is to assess the water quality conditions of wadeable streams of North Carolina and report their results to the Basinwide Planning Unit (NCDWQ 2006). The program has been in operation since 1990. This fish program compliments other NCDWQ programs, such as the Benthic Macroinvertebrate and Ambient Monitoring Systems.

Although fish community sampling has been conducted in the Sandhills region since 1990, an IBI has yet to be successfully employed (*NCDWQ personal communication 2006*). In regions of the state where the NCDWQ does have an effective IBI, the program has proven useful as a tool for monitoring water quality and anthropogenic disturbance, aiding in state, federal and university research, and assisting in other water projects such as TMDL's, Use Attainability Studies, and the Environmental Enhancement Program (EEP). All of these uses are seriously hindered by the absence of an effective IBI rating system for Sandhills streams. IBI's are also very useful in communicating conditions to non-biologists and non-scientists. Thus, an effective IBI for the NC Sandhills will be of exceptional value in the protection and conservation of this very unique ecosystem.

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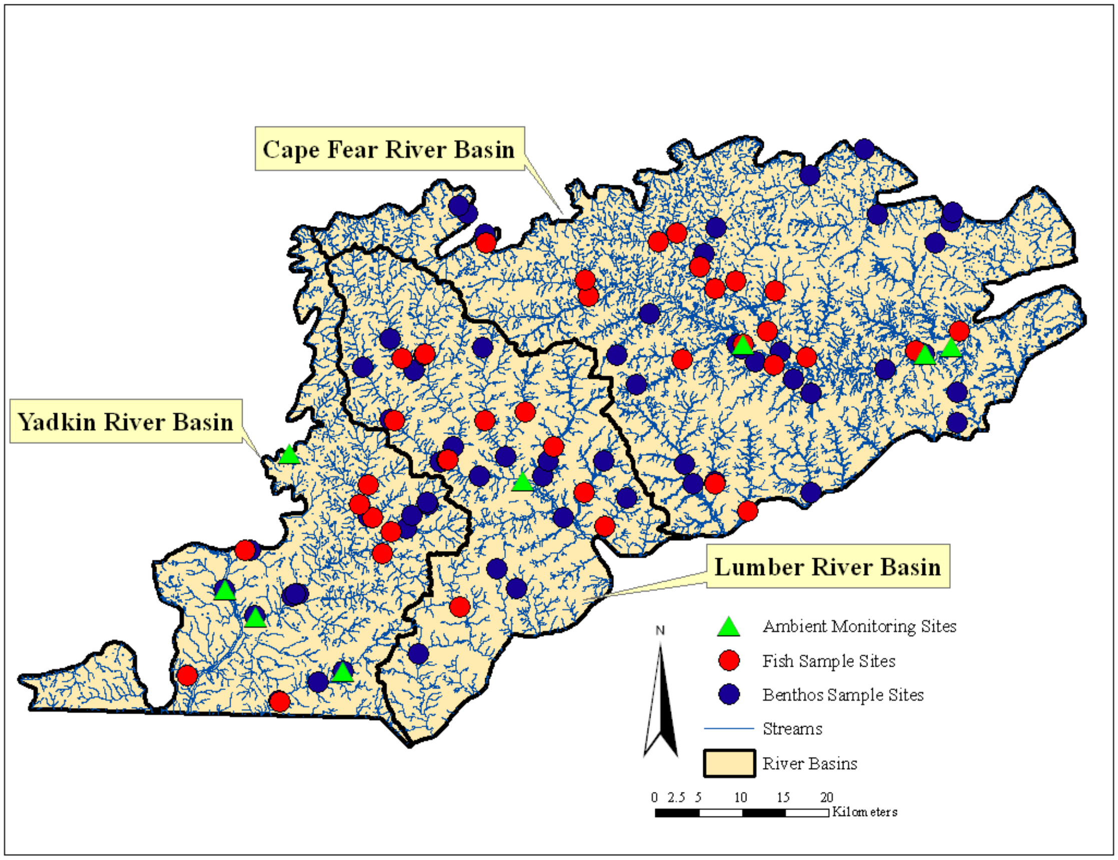

Figure 1.1. NCDWQ fish assemblage, benthos macroinvertebrate, and ambient monitoring sites within the Sandhills

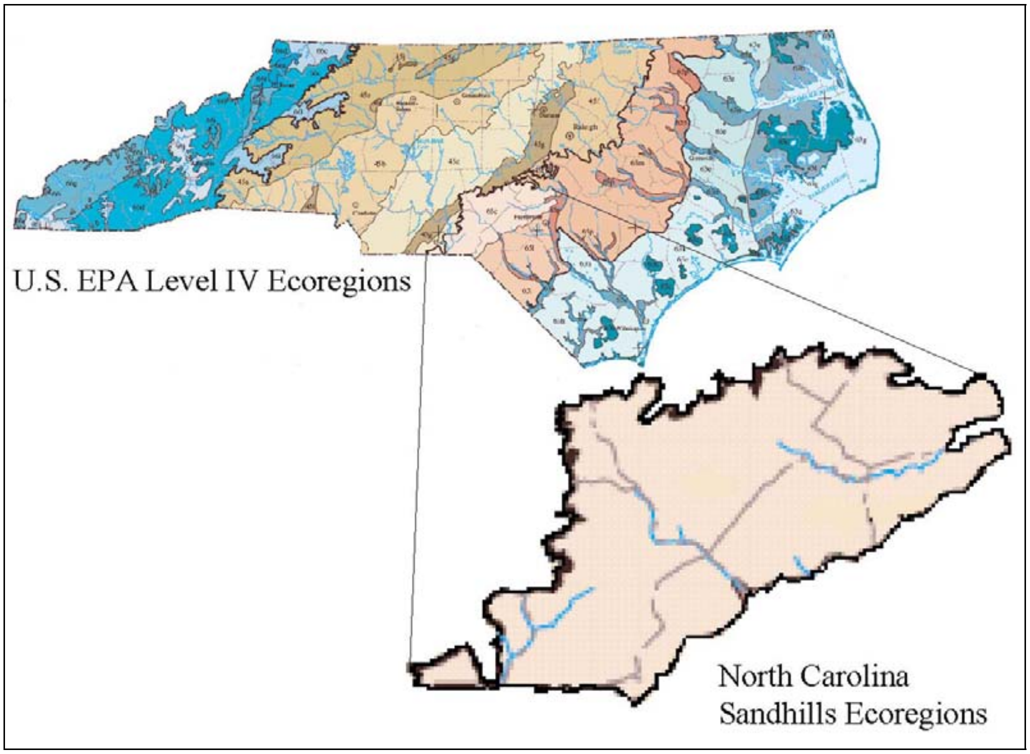

Figure 1.2. North Carolina and Sandhills Level-IV ecoregions as designated by U.S. EPA (Griffith et al. 2002)

2. A Fish-Based Index of Biotic Integrity for an Ecoregion with Low Natural Productivity and Biodiversity

Ernie F. Hain[1], Stacy A.C. Nelson, Halil I. Cakir

Center for Earth Observation and the Department of Forestry and Environmental Resources, North Carolina State University, Raleigh, NC 27695, USA

Bryn H. Tracy

Environmental Senior Specialist, North Carolina Division of Water Quality, Raleigh, NC 27699-1621, USA

Abstract. We describe a process for developing an index of biotic integrity (IBI) for resident fish assemblages in an ecoregion that exhibits low natural productivity and biodiversity. From 1990 to 2006, 55 fish community samples were collected by the North Carolina Division of Water Quality (NCDWQ) in the Cape Fear, Lumber, and Yadkin river basins within the Sandhills US EPA level IV ecoregion. Initial analyses of these data, using the 12 IBI metrics employed by the

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[1] E-mail: fhernst@ncsu.edu

NCDWQ in other regions of the state, failed to distinguish significant differences between reference and non-reference streams. To develop a more robust method of measuring responses to anthropogenic disturbance, we delineated contributing watersheds for each of the 36 sample sites using GIS, hydrologic modeling, and 20-foot resolution Digital Elevation Models (DEM) derived from Light Detection and Ranging (LiDAR) data. The 2001 National Land Cover Database (NLCD) and *in situ* habitat data were used to determine various land use/land cover and hydrologic variables within each watershed. These variables were then used to select the sample sites with absolute minimal anthropogenic impacts. We used the Kruskal-Wallis test to identify eleven fish community metrics, two chemical metrics, and nine individual species that were significantly different in reference and non-reference sites. Of these fifteen metrics, only three exhibited higher values in reference streams. Our results demonstrate that the abundance and diversity of the Sandhills fish fauna are greater in area more highly impacted by anthropogenic activities. By automating the process by which reference sites are chosen, we were able to produce a multi-metric IBI that reflects the varying levels of anthropogenic impacts on wadeable streams in the Sandhills ecoregion.

Key words: Bioindicators, Biomonitoring, Fish Ecology, Fisheries, Geographic Information Systems, IBI, Land Use, Landscape Management, LiDAR, Sandhills, Streams, Water Quality Monitoring

2.1. Introduction

Land conversions from development may ultimately degrade water quality and fish habitat within areas of high change by increasing impervious surfaces, storm water runoff, excess sediment, and environmental pollutants which then drain into adjacent waterways (Paul and Meyer 2001, Wang et al. 2001, Jennings and Jarnagin 2002). Increasing developmental pressures within the Sandhills region of North Carolina may conflict with the state's ability to develop baseline reference stream sites and robust indices for the geographically unique waters of this area. Thus, it is important that agencies such as the North Carolina Division of Water Quality (NCDWQ) continue to develop more efficient monitoring tools which account for escalating levels of human population growth and development, and their associated impacts on aquatic ecosystem health (Schueler 1994, Schueler and Clayton 1997). The development of effective biological classification criteria and the use of geospatial tools, such as geographic information systems (GIS) and remote sensing, in land use and freshwater monitoring can play a vital role in reducing the cost, labor, and time required to monitor these systems over large geographic areas (Nelson et al. 2003, Nelson et al. 2006).

The Federal Clean Water Act (FCWA, sections 305b and 303d) requires all states to identify natural waters that are impaired or otherwise too degraded to support their designated and existing uses. In addition to the environmental and recreational benefits (*fishable* and *swimable* waters as defined by the FCWA), these waters are also important to the economy of each state (Canfield et al. 2002). These resources provide a valuable source of water, critical linkages to commercial and sports fisheries, habitat support for fish and wildlife, local and regional hydroelectric power, as well as aesthetic and recreational values to the citizenry (Heiskary et al. 1994, Kerr et al. 1994, Obrecht et al. 1998, Canfield et al. 2002). In fact, the importance of these functions is so critical, that any reductions in stream function or ecosystem health could mean severe impacts, not only within the affected local region, but on a statewide level as well. Thus, routine and effective monitoring is important to measure changes in water quality and biota so appropriate management decisions can be made (Heiskary et al. 1994, Obrecht et al. 1998, Canfield et al. 2002). However, extensive monitoring and collection programs on a statewide level still remain costly and logistically prohibitive (Nelson et al. 2003). Thus, novel approaches are required to assess water quality and stream health over large geographic areas.

In recent decades numerous federal and state agencies, as well as volunteer organizations, have developed integrative approaches to efficiently monitor the health of our nation's flowing waters (Heiskary et al. 1994, Kerr et al. 1994, Obrecht et al. 1998). Traditionally, *in-situ* chemical and benthic macroinvertebrate monitoring have been the primary method of water quality and aquatic systems assessment (Hilsenhoff 1982, US EPA 1996). These methods have proven useful for reflecting local impairments to the sampled water bodies, over shorter time periods. However, both methods may be limited in providing a "*whole-systems approach*" in the assessment of environmental conditions on a larger spatial and temporal scale. Fish assemblage assessments, or fish indices of biotic integrity (IBI), have recently evolved as a prominent tool for monitoring stream ecosystem health (Zampella and Bunnell 1998, Karr and Chu 1997, Bozzetti and Schulz 2004).

Fish IBI's were first developed in the Midwestern United States as an innovative approach to assess the diminishing capability of freshwater systems to support stable biotic communities (Karr 1981). Significant correlation exists between fish community composition and habitat quality (Pirhalla 2004, Zampella and Bunnell 1998). The primary objective of a fish index of biotic integrity (IBI) is to first determine what fish community characteristics are typical, or can be expected in a given ecoregion (Bozzetti and Schulz 2004). In order to determine the biologic consequences of human actions, a baseline that estimates minimal human impact should be established (Karr and Chu 1997). Ideally, this baseline would represent a natural condition, absent of anthropogenic disturbance or alteration (Stoddard et al. 2006). However, in most regions, reference conditions may be based on least-disturbed conditions or best attainable conditions (Stoddard et al. 2006). Once a baseline is established, candidate community metrics may be chosen to describe reference conditions.

Many state monitoring and regulatory agencies have now adopted such techniques. The development of a fish IBI takes into account resident fish populations and assemblages by assessing the numbers and types of fish in comparison to populations within relatively undisturbed, or reference, waters. Fish population sizes, taxa richness, trophic structure, and species composition, as indicated by the IBI, coupled with benthic macroinvertebrate monitoring and chemical water quality monitoring, provide a much broader indication of the overall health of an ecosystem (Karr 1991). This is primarily a result of the biological communities sampled being able to provide a more accurate reflection of the long-term conditions occurring within the environment, as opposed to the "*snapshot*" conditions provided by the more traditional methods.

In addition to fish communities, the concept of an IBI has been used to assess ecological conditions based on invertebrates, birds, and zooplankton (Kerans and Karr 1994, O'Connell et al. 2000, Lougheed and Chow-Fraser 2002). The original fish IBI proposed by Karr (1981) incorporated species composition and richness as well as ecological factors such as trophic status and disease to discriminate between disturbed and non-disturbed sites (Karr 1981). This concept has been expanded in some studies to include behavioral and physiological factors such as migration, habitat usage, and nest placement (O'Connell et al. 2000). IBI's based on fish or other taxa have proven successful not only in assessing water quality, but also in predicting community assemblages and explaining ecological processes (Wallace et al. 1996, Hawkins et al. 2000).

Indices based on fish assemblages may show the effects of land conversions on water quality conditions more quickly than conventional methods of chemical and benthic macroinvertebrate sampling (Karr 1981, Karr 1991). Ambient water quality and benthic macroinvertebrate sampling by the NCDWQ within the Sandhills region of North Carolina (Figure 2.1) suggest excellent water quality conditions, despite the fact that the fish assemblage quality has not been rated (*NCDWQ personal communication 2006*). Many intolerant fish species have been collected, including the endemic pinewoods darter and Sandhills chub (NCDWQ 2002 and 2004). Any change in this level of species presence could suggest a potentially critical and ecologically important shift in the fish assemblages, and ultimately the region's water quality. Currently the NCDWQ has collected over 15 years of fish population and habitat data within the Sandhills regions, which coupled with land use and other geospatial analysis methods, may provide an exceptionally valuable dataset for assessing the future stream health and water quality changes associated with ever increasing anthropomorphic impacts.

Streams in the Sandhills region receive consistent flow throughout the year due to the sandy soils, ground water storage, and aquifer additions (Griffith et al. 2002, Winner and Coble 1996). This characteristic, along with low gradients and mild climates, has resulted in higher fish fauna diversity in the regions headwater streams than in other regions of the U.S. (Paller 1994). Paller et al. (1996) developed a fish IBI in the South Carolina Coastal Plain and Sandhills that was able to discriminate between reference and non-reference sites using six community metric categories (Paller et al. 1996). While the South Carolina IBI was more precise than a benthic macroinvertebrate multimetric index for the same region, identification of disturbed sites was most accurate when using both taxonomic groups (Paller 2001).

In order to produce robust criteria for designating reference sites that demonstrate minimal anthropogenic impacts, a database of detailed land use/land cover and habitat variables were produced for each site. To accomplish this, high-resolution digital elevation models (DEM) were developed from LiDAR data, obtained from the North Carolina Flood Plains Mapping Program. Using the hydrology tools in the Spatial Analyst Toolbox within ESRI's ArcGIS 9.2 (ESRI, ArcGIS 9.2, 2006), watersheds draining to each sample site were delineated. Land use, habitat variables, physical characteristics and hydrologic features collected with remote sensing and GIS technologies were then analyzed to determine anthropogenic impacts within each watershed. These data were then used to select the least impacted sites to be used in describing reference conditions. Quantitative statistics were then developed for the land use/land cover and habitat variables in each of these watersheds.

In order to develop a more robust method of measuring responses to anthropogenic disturbance, we created a model that delineates local water catchments using 20-foot resolution LiDAR data, and NCDWQ sample sites as the mouth of watershed catchments. This model extracts 2001 National Land Cover Dataset (NLCD) land use data from these catchments, and along with *in situ* data in the attribute table of the NCDWQ shapefiles and additional data stored in Microsoft Access, the model determines if each sample site meets reference conditions. Reference criteria data and fish assemblage data for each site were then exported to SAS for statistical analyses. We used the model to establish which community metrics are significantly different ($p<0.05$) between reference and non-reference sites. These metrics were used to create a scoring technique whereby sampled sites can be rated as either poor, fair, good, or excellent.

2.2. Methods

2.2.1. Study Area

The North Carolina Sandhills, designated as an U.S. EPA Level IV ecoregion (Figure 2.1), contains portions of seven state counties, as well as portions of three major river basins; the Cape Fear, Lumber and Yadkin River Basins (Figure 2.2). The Sandhills ecoregion is considered a transition zone between the Coastal Plain and Piedmont, with a landscape characterized by rolling, sandy hills and dense hydrologic drainage networks. Soils in this region typically consist of thick, droughty, and low-nutrient, Cretaceous-aged marine sands (Griffith et al. 2002). This ecoregion is home to urban areas such as Fayetteville and Southern Pines, as well as the Fort Bragg Military Reservation and Pope Air Force Base. This region’s forested areas have been drastically reduced as a result of logging and clearing, and fire suppression has altered the community properties (Landers et al. 1995). Additionally, within this area, numerous birds, mammals, mussels, insects, plants, and fish species, such as the endemic *Etheostoma mariae* (Pinewoods Darter) and *Semotilus lumbee* (Sandhills Chub), are now considered rare or endangered (Rohde and Arndt 1991, Rohde and Ross 1987, Snelson and Suttkus 1978).

Geologically, the Sandhills ecoregion is composed of dry, sandy ridgetops (Jacqmain et al. 1999). The ridgetops are commonly explained as being formed as dunes by the Cretaceous Atlantic shoreline (Griffith et al. 2002, Collins et al. 2006). This area is known as the Fall Line, and is the region that separates the Coastal Plain from the Piedmont Plateau. Rivers within this region flow across the Fall Line through a series of rapids and falls before entering the Coastal Plain. The Fall Line parallels the Atlantic Coastline from New Jersey to Georgia, and represents the range boundaries for many tree species (Shankman and Hart 2007). While the area was once dominated by *Pinus palustris* (Longleaf pine), *Pinus taeda* (Loblolly pine) and *P. echinata* (Shortleaf pine) are now also found alongside in a mixed pine matrix. Hardwood *Quercus* (oak) forests are also found in the Sandhills, especially in more fertile and moist soils (Skeen et al. 1993, Ware et al. 1993).

2.2.2. Data Collection

The Biological Assessment Unit (BAU) of the NCDWQ samples each of the state's 17 river basins on a five year rotation to support the Planning Section's Basinwide Water Quality Management Plans (NCDWQ 2006). The Cape Fear River basin was sampled in 1998, 2000, and 2003, the Lumber River basin was sampled in 1996, 2001, and 2006, and the Yadkin River basin was sampled in 1998 and 2003. Additional sampling by the BAU was conducted as early as 1990.

As part of the stream fish community assessment program, the BAU samples only streams that are wadeable from shoreline to shoreline for a distance of 600 feet (182.88 meters). A four-person team collects all fish at each site with a two-pass depletion technique using two backpack electrofishing units. Two team members operate the electrofishing units while two net fish. The first pass is conducted upstream. After allowing the water to clear, the second pass is conducted downstream. All habitat types are sampled, and a seine is used where necessary for riffles, although riffles are rare in the Sandhills. The first fifty specimens of each species are identified, measured, inspected for disease or deformities, and released (after the first fifty, specimens are just counted and released). Specimens not easily identified in the field are preserved in 10 percent formalin and returned to the BAU laboratory in Raleigh, NC.

The BAU has developed a Habitat Assessment Index for North Carolina streams. This index is used to evaluate the physical habitat of the visible watershed on a scale from 1-100, where a higher numbers indicates higher habitat quality. The Habitat Assessment Index is calibrated per physiographic region (Mountains, Piedmont, Sandhills, and Coastal Plain), and in the Sandhills uses seven habitat characteristics for evaluation. These characteristics include channel modification, amount of instream habitat, type of bottom substrate, pool variety, bank stability, light penetration, and riparian zone width (NCDWQ 2006).

Water quality analyses are conducted at the time of each fish community sample. Measurements include water temperature, specific conductance, pH, stream flow, water clarity, and dissolved oxygen. All field meters are calibrated daily before use and as needed on site (NCDENR 2006).

The BAU uses a Garmin GPS meter and digital camera to record latitude and longitude of the bridge crossing over the most downstream point of the sample reach and digital images of various points along the reach.

Seven criteria are used by the BAU to assess whether a site meets reference conditions (Table 2.1). These criteria include the Habitat Assessment score, presence of discharge permits, land cover, and riparian and channel characteristics. The land cover criteria for a reference site requires that the watershed land cover ≥ 70% forested and ≤ 10% urbanized (Table 2.1).

2.2.3. Data Management

For this study NCDWQ provided ArcGIS shapefile data for fish sampling locations, pollution discharge permit locations, and sub-basins for each of the three primary river basins in the Sandhills region. The fish community data contained road location, county, latitude and longitude, date of collection, name of stream, river basin, and an unique collection identification number. NCDWQ also provided fish community raw data (counts by species), fish distributions by basin, and fish tolerance and feeding data. All data are available to the public at http://h2o.enr.state.nc.us/esb/BAU.html (accessed 1 August 2008).

The 2001 National Land Cover Dataset (NLCD) land use/land cover data was downloaded from the Multi-Resolution Land Characteristics Consortium (MRLC) at http://www.mrlc.gov/index.php (accessed 1 August 2008). This dataset provides land cover and land use classifications based on a modified Anderson Level-II classification at a 30m resolution (Lillesand and Kiefer 1994). The value of using this dataset is that it is well suited for large area analyses and provides standardized classifications of LULC across the entire United States. This dataset also allows for subsequent land cover change analyses and spatial prediction models, incorporating future changes, to be based on similar datasets. Resulting models can be updated as future NLCD datasets become available.

Light Detection and Ranging (LiDAR) data was downloaded from the North Carolina Floodplain Mapping Program at www.ncfloodmaps.com (accessed 1 August 2008), as well as from the North Carolina Department of Transportation at http://www.ncdot.org/it/gis/DataDistribution/ContourElevationData/default.html (accessed 1 August 2008). Elevation data derived from LiDAR has been used in hydrologic modeling and to produce highly accurate hydrologic maps (Wehr and Lohr 1999, Colson 2007). The high resolution digital elevation models (DEM) produced from LiDAR data are ideal for topography-based watershed delineations (Thompson 2001).

All spatial data for this study were stored in an ArcGIS personal geodatabase. This structure serves as a user interface in ArcGIS for data manipulation, while storing all physical files in a Microsoft Access database. The advantage of the personal geodatabase is that it automatically updates topology features, such as area measurements, as data are manipulated. Additionally, it provides an organization structure for the data (Figure 2.3).

Tabular data, such as fish distributions, feeding type, tolerance values, and habitat assessment data were stored in a separate Microsoft Access database. The Microsoft Access database allowed for data for each sample site to be pulled from all original tables in order to analyze all habitat characteristics as well as to calculate fish community metrics. This was accomplished using a series of Query functions. An advantage of this database structure is that the original tabular data is not altered, rather new tables with the selected data and calculations can be created through the MakeTable Query function.

The Python Programming Language is a free, open source and object-oriented programming language that can integrate seamlessly with many software programs, including ArcGIS 9.2. The advantage of Python is that it does not require specialized computer programming skills to use (Karssenberg et al. 2007). It is also ideal for "looping" functions, where the same function needs to be applied multiple times, such as to each sample site. We used Python to program a large portion of our geoprocessing model (Appendix 1). Generally, Python called functions from ArcGIS and repeated the functions in a specified order on each sample site. Python was also used to organize and search through the ArcGIS personal geodatabase and Microsoft Windows folder structure. By scripting the geoprocessing model, as opposed to manually applying the functions, we were able to not only save time, but also preserve the details of the model in text format. This allows us to repeat the model if needed, and to adjust the details of the functions (see Appendix 1).

The geoproccessing model delineates contributing watersheds for each sample site and extracts the NLCD land use/land cover data for each watershed. The watershed delineations are produced using the hydrology tools in the spatial analyst toolbox of ArcToolbox 9.2, using 20-foot resolution LiDAR data. The model loops through each sample within a specified sub-basin, identifies all LiDAR files necessary to produce a digital elevation model (DEM) for the sub-basin, and creates the DEM. This DEM is used to delineate contributing watersheds for each sample site. The contributing watershed is converted to a polygon and used to extract the NLCD land use/land cover data, using the “extract by mask” function in the spatial analyst toolbox. We used the spatial join tool in the Analysis toolbox to join the watershed polygon to the sample site and any discharge permit site that may have been within the watershed. We manually exported the attribute table of this extracted land use/land cover data and used it as a parameter in establishing whether a sample site meets reference criteria or not. All new spatial data created by the model was outputted to the personal geodatabase.

The NCDWQ considered sites to meet reference conditions if they met seven criteria, including the watershed land use/land cover being ≥ 70% forested and ≤ 10% urban (Table 2.1). Because the Sandhills region is heavily forested, we used professional judgment to increase the land use criteria to being ≥ 75% forested and ≤ 10% urban. The NLCD data contains 16 land use classes for North Carolina, 15 of these occur in the Sandhills (Table 2.2). Forested area was calculated by combining the Deciduous Forest, Evergreen Forest, Mixed Forest, Shrub/Scrub, Grassland/Herbaceous, Woody Wetland, and Emergent Herbaceous Wetlands classes. Urbanized area was calculated by combining the Developed Open Space, Developed Low Intensity, Developed Medium Intensity, and Developed High Intensity classes.

Forty-three candidate fish metrics were calculated for each sample site. Candidate metrics were chosen based on their inclusion in Karr's original IBI (Karr 1981), the North Carolina IBI (IBI) currently used by the NCDWQ (2006), Paller's (1996) South Carolina Coastal Plain IBI, or found in a survey of the IBI literature (Karr 1981, NCDWQ 2006, Paller 1996, Zampell and Bunnell 1998, Pirhalla 2004, Karr 1991, Breine et al. 2004, McCormick et al. 2001, Whittier et al. 2007, Hughes et al. 2004).

2.2.4. Data Analyses

All statistical analyses were performed using SAS 9.1 (SAS Institute Inc., SAS 9.1.2, 2004). Summary statistics, including mean, standard deviation, median, and the 5th, 10th, 25th, 75th, and 90th percentiles were produced for all sites, as well as for only reference sites and only non-reference sites, using the Proc Means function (see Appendix 2). To identify fish community metrics, chemical variables, and individual fish species that differed between reference and non-reference sites, we used the Kruskal-Wallis test, a non-parametric one-way analysis of variance (ANOVA) (Paller 1996, Sokal and Rohlf 1995). This test was performed in SAS 9.1 using the NPAR1WAY procedure (see Appendix 3).

2.3. Results

2.3.1. Significant Metrics

We identified 11 fish community metrics that were significantly different ($p<0.05$) between reference and non-reference sites using the Kruskal-Wallis test (Table 2.3). The Kruskal-Wallis one-way analysis of variance (ANOVA) is a non-parametric method performed on ranked data and has been demonstrated to be useful for testing population medians among groups (Sokal and Rohlf 1995). Additionally, we identified two chemical variables, and nine fish species' counts that significantly differed ($p<0.05$) between reference and non-reference sites (Table 2.3). Eight of the community metrics found to be significantly different included metrics within the metric categories of species richness and composition (total number of fish species, number of tolerant individuals, number of tolerant species, number of Cyprinidae individuals, number of Cyprinidae species, proportion of individuals as tolerant, proportion of individuals as intolerant, and number of *Centrarchidae* individuals). Two trophic composition metrics were also found to show significant differences between reference and non-reference sites (number of insectivore individuals and number of insectivorous Cyprinidae individuals). The one fish abundance metric, total number of fish in sample, showed a significant difference between reference and non-reference sites at the 0.05 level. Of these metrics, only the proportion of individuals as intolerant was found to be a positive-scoring metric, which indicates a metric whose numbers would be expected to decline with increased watershed disturbance (McCormick et al. 2001). No other community metric tested as significant.

We found two chemical variables, pH and specific conductance, and nine individual fish species' abundances that were significantly different at the 0.05 level. The significant fish species included *Anguilla rostrata, Aphredoderus sayanus, Centrarchidae auritus, Etheostoma olmstedi, E. serrifer, Minytrema melanops, Ameiurus platycephalus, Centrarchidae macrochirus,* and *Enneacanthus gloriosus.* Of these species, only *A. sayanus* and *E. serrifer* were found to be positive-scoring. Over-parameterization of the model was avoided by including only the positive scoring species in the final IBI. This method also allowed our model development to remain consistent with IBI procedures which have traditionally focused on community metrics rather than individual species (Karr 1981). Our resulting model included all 11 community metrics as well as the two chemical variables, and the two positive scoring species (Table 2.4).

2.3.2. Metric Descriptions

Fish samples were analyzed by numbers, species, family, feeding type, and tolerance values. Feeding type and tolerance values were provided by the NCDWQ (http://h2o.enr.state.nc.us/esb/BAU.html) (see Appendix 4). Total numbers of individuals per species, number of species, number of families, and number of individuals within each family were calculated and cross-referenced with feeding type and tolerance values.

2.3.3. Development of the Index of Biotic Integrity and Scoring Technique

The final IBI included 11 community metrics, two chemical variables, and two individual species. Of these metrics, three were positive scoring and 12 were negative scoring (McCormick et al. 2001). Our IBI scale ranged from 0-100, so as to follow the more familiar decimal system, and provide easier comparison to other biologic and habitat evaluations, as opposed to Karr's original 12-60 scale (Karr 1981, Minns et al. 1994, Hughes et al. 1998). Scores for our individual metrics were produced following McCormick et al. (2001). For positive scoring metrics, a given site received a score of 0 if its value was less then the 5th percentile of non-reference sites. A site received a score of 10 if its value was above the 50th percentile of reference sites. Negative scoring metrics received a 0 if its value was greater than the 90th percentile of non-reference sites and a 10 if less than the 50th percentile of reference sites (McCormick et al. 2001, Whittier et al. 2007, Minns et al. 1994, Hughes et al. 1998). For both positive and negative scoring metrics, the values of 0 and 10 were linearly interpolated for each metric. This provided a scale of metric values that corresponded to each whole number between 0 and 10 (Table 4). Scores for each metric were then assigned based on each sample site's metric values. Since there are 15 metrics in the IBI, the final score for each metric is multiplied by a coefficient (0.667) so that the final score is within a range of 0-100.

Final IBI scores ranged from 15.3 to 99.3. The median score was a 68 and the mean score was 65.67 with a standard deviation of 20.04. When used to analyze the original 55 samples, final IBI scores were found to differ significantly between reference and non-reference sites ($P<0.0001$).

IBI classes were established by summarizing the final scores of all sites ($N=55$). Any site with a final score ≥ the 90th percentile of all sites (87.3) was rated as "Excellent". Final scores between the 50th and 90th percentile of all sites (68-87.3) were rated as "Good", and scores between the 10th and 50th percentiles of all sites (36.6-67.9) were rated as “Fair”. All final scores lower than the 10th percentiles (36.6) were rated as “Poor.” Previous IBI’s have used only reference sites for final classes (McCormick 2001). However, since we had a small number of reference sites, we decided to use the entire data set. This system resulted in six “Excellent” sites, 22 “Good” sites, 21 “Fair” sites, and six “Poor” sites (Figure 2.4) (see Appendix 5).

2.3.4. Sample Scores

The Cape Fear River basin contained five sites that received a rating of “Excellent,” eight sites that received a rating of “Good,” five sites that received a rating of “Fair,” and four sites received a rating of “Poor.” The Yadkin River basin contained one site that received a rating of “Excellent,” three sites that received a rating of “Good,” four sites that received a rating of “Fair,” and two sites that received a rating of “Poor.” The Lumber River basin contained no sites that received a rating of “Excellent,” eleven sites received a rating of “Good,” twelve sites received a rating of “Fair,” and no sites received a rating of “Poor.” All “Poor” and “Excellent” sites were located in either the Yadkin or Cape Fear basins. Appendix 5 summarizes these findings. No “Poor” or “Excellent” rated sites were located in the Lumber basin, which lies between the Yadkin and Cape Fear (Figure 2.5).

2.4. Discussion

2.4.1. Condition of Sand Hills’ Streams

We developed a fish index of biotic integrity for the Sandhills region of North Carolina using 55 fish community samples collected by the BAU of the NCDWQ from 1990 to 2006. Using 15 fish metrics that tested significantly ($p<0.05$) between reference and non-reference sites, we produced IBI scores for all 55 samples on a scale from 0-100. There is no indication that any of the three river basins in the region consistently rated high or low, although the Lumber River did not receive exceptionally high or low ratings. We found no individual streams whose rating fluctuated drastically between different sample sites along the stream or repeated samples at the same location. However, a few individual streams did stand out. Crane Creek in Moore County and the Cape Fear Basin scored consistently low at four different sample sites and five total samples (see Appendix 5). Buffalo Creek, also in Moore County and the Cape Fear basin, rated as "Excellent" twice, once in 1998 and once in 2003. These two samples were the highest rated sites in the dataset (99.33 and 95.33, respectively). Marks Creek in Richmond County and the Yadkin basin received the lowest rating (15.33) in 2006.

2.4.2. Advantages of Model Automation

The NCDWQ dataset contained 55 samples at 36 sample sites, of which only 15 were considered reference samples (11 out of 36 sample sites). As the NCDWQ will continue to sample the river basins in this ecoregion on a five year rotation (NCDWQ 2006), they will presumably increase the number of reference sites sampled. By automating a large portion of the reference stream identification process, this model provides an increased opportunity for monitoring agencies, such as the NCDWQ, to be able to select reference sites based on larger datasets in the future. This is valuable because the ability to identify reference sites from larger datasets helps to ensure future area-specific statistical analyses are based on comprehensive representative samples. Additionally, changes in land use, development, stream restorations etc. may affect the fish assemblages in Sandhills streams. The availability of an automated model will allow the NCDWQ to choose whether to rate streams based on the results from the original 55 samples, or the results from a larger database after additional sampling has occurred. Ideally, both analyses could be produced, allowing NCDWQ to track changes in reference conditions, such as increased or decreased homogeneity between reference and non-reference sites, or the disappearance of reference sites due to land conversions.

This model provides an additional benefit to monitoring agencies in that the automated procedure can be extrapolated to other regions of North Carolina for assisting in the development of region-specific IBI's. The North Carolina IBI (NCIBI) has been used by NCDWQ to successfully rate streams in the Piedmont and Mountains. High elevation cold water trout streams and Coastal Plain streams have yet to be rated (NCDWQ Personal Communication 2006). Each unrated region possesses its own unique set of obstacles for developing an IBI, but an automated analysis would prove useful in streamlining this process, as it has in the Sandhills. Further, the model could be applied to the Piedmont dataset to compare and contrast the NCIBI to the results from the procedures presented here.

2.4.3. Implications for Diversity

The development of a fish index of biotic integrity for the Sandhills region of North Carolina has demonstrated that the region exhibits a lower abundance of fish fauna in undisturbed streams than in disturbed streams. Resource additions related to anthropogenic impact have resulted in increased fish abundance as well as modified fish assemblages in the Sandhills. Low natural biodiversity and resource availability may have played a role in preventing large numbers of colonizing species from establishing populations in the region's undisturbed streams (Levine 2000, Tilman 2004, Maron and Marler 2008). Twelve of the 15 significant ($p<0.05$) metrics presented in this study were negative scoring (Table 4), where we would expect to find lower numbers in less disturbed streams (McCormick et al. 2001). Thus in the Sandhills region, a fish assemblage exhibiting low abundance and diversity is an indication of high biologic integrity. This result is contrary to Karr's original IBI (Karr 1981), and supports the NCDWQ's assertion that the Piedmont-derived IBI does not accurately evaluate Sandhills fish communities.

2.4.4. Recommendations for NCDWQ

Further validation of this research may include repeating the Kruskal-Wallis non-parametric ANOVA on additional datasets as they becomes available (Paller 1996). Also, numerous studies have shown than fish IBI's are most useful when used in conjunction with ratings based on other taxonomic groups (Paller 2001, Johnson et al. 2006, Paavola 2003, O'Connor 2000). The BAU of NCDWQ also sample benthic macroinvertebrates, often at the same sample sites as fish. A detailed comparison of fish and benthic macroinvertebrate communities may assist in further refinements to both rating systems, as well as provide insights into particular environmental stressors affecting the Sandhills (Paller 2001, O'Connor 2000).

A customized user interface that allows the model to be accessed from a series of customized menus or a dedicated toolbar in a GIS would facilitate repeated use of the model. The toolbar could consists of control buttons that open a “pop-up” menu. The “pop-up” menu would allow the user to choose specific data to run each of the major model functions. The custom model interface could be created using readily available object-oriented programming languages, such as Microsoft Visual Basic (VBA). The job of the toolbar would be to call and run the IBI model functions written in the Python programming language. Both VBA and Python communicate seamlessly with ESRI’s ArcMap (ESRI, ArcGIS 9.2, 2006).

2.4.5. Future Work

One area of IBI development and validation not covered by this study is the quantitative analysis of metric responsiveness across a gradient of anthropogenic disturbance. This level of validation has become more common in IBI development in the past decade (Whittier et al. 2007, McCormick et al. 2001, Hughes et al. 2004). Measuring metric responsiveness to anthropogenic disturbance first requires quantifying disturbance levels. In this study, we tested metric significance by categorizing sites as either reference or non-reference. Recommendations for future work include a comprehensive analysis of all anthropogenic disturbance variables and the development of a continuous ranking of stream disturbance for sample sites. A disturbance gradient could be developed from the Habitat Assessment Index currently employed by the NCDWQ (NCDWQ 2006). This type of disturbance gradient can then be used to test correlation of each metric to disturbance (Teels and Adamus 2002).

Several statistical techniques have been used to test candidate metrics across a gradient of anthropogenic disturbance. Spearman correlations and scatter plots can be used to test the responsiveness of metrics to disturbance (McCormick et al. 2001). This method can also be used to test metrics against watershed and in-stream variables such as pH, specific conductance, substrate type, canopy, etc. in order to eliminate metrics strongly associated with natural gradients (Hughes et al. 2004, McCormick et al. 2001). Spearman correlations can also be used to test redundancy, thereby limiting the IBI to only the most informative metrics (Whittier 2007). Repeatability or precision of metric values can be measured by a signal to noise test, where the signal is the variance among sites and the noise is the variance of repeated samples from the same site (Whittier et al. 2007, McCormick et al. 2001, Kaufmann et al. 1999). This method helps to eliminate metrics that vary within a single site as much as across multiple sites (Whittier et al. 2007). Feature Analysis, Principal Component Analysis, and Cluster Analysis have also been effectively used to test correlations with disturbance gradients and overlapping among metrics (Breine et al. 2004, Hughes et al. 2004).

2.4.6. Conclusion

Using a standardized, and partially automated index of biotic integrity model, we were able to classify NCDWQ fish assemblage samples on a scale of 0-100 and as either “Excellent”, “Good”, “Fair”, or “Poor”. Even though we were unable in this study to assess the signal to noise ratio, redundancy, and correlation to natural and anthropogenic disturbance gradients, we believe that we have selected metrics that are relevant in the assessment of water quality in the

Sandhills region of North Carolina based on resident fish assemblages. The final scores of the IBI were shown to distinguish between reference and non-reference sites in the original dataset. In addition to the more stringent data analysis recommended above, we recommend that validation be repeated on additional samples as they become available. The model approach taken here will prove valuable in developing additional indices across the ecoregions of North Carolina as well as with other taxonomic groups. The results of this study should be used in addition to other water quality indices in order to achieve to most valuable assessment of the state’s flowing waters.

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Table 2.1.NCDWQ Reference Criteria (NCDWQ 2006)

Criterion	Qualification
1 -- Habitat	Total habitat score ≥ 65
2 – NPDES dischargers	No NPDES dischargers ≥ 0.01 MGD above the site or if there are small dischargers (~≤ 0.01 MGD), the dischargers are more than one mile upstream
3 – Percent urbanization	< 10% of the watershed is urban or residential areas
4 – Percent forested	≥ 70% of the watershed is forested or in natural vegetation
5 – Channel incision	At the site, the stream is not incised beyond natural conditions
6 – Riparian zone integrity	No breaks in the riparian zones or, if there are breaks, the breaks are rare
7 – Riparian zone width	Coastal Plain/Sandhill streams – width of the riparian zone along both banks is ≥ 18 m
Exception 1	If the site satisfied Criteria 1 - 6, except one of the two riparian widths was less than one unit optimal, then the site still qualified as a reference site
Exception 2	If the site satisfied Criteria 1 - 3 and 5 - 7, but the percentage of the watershed in forest or natural vegetations was ≥ 60% (rather than ≥ 70%), then the site still qualified as a reference site.

Table 2.2. NLCD Class Descriptions (Homer et al. 2004)

NLCD Class	Description
11	Open Water
21	Developed, Open Space
22	Developed, Low Intensity
23	Developed, Medium Intensity
24	Developed, High Intensity
31	Barren Land
41	Deciduous Forest
42	Evergreen Forest
43	Mixed Forest
52	Shrub/Scrub
71	Grasslands Herbaceous
81	Pasture/Hay
82	Cultivated Crops
90	Woody Wetlands
95	Emergent Herbaceous Wetlands

Table 2.3. Significant metrics with mean and median by reference and probability

	Metric	Reference Median	Non-Reference Median	Reference Mean	Non-Reference Mean	Probability[a]
Species Richness and Composition						
	Total Species	10	13.5	10.533	13.425	0.013
	Number of Tolerant Individuals	3	6.5	4.133	17.575	0.003
	Number of Tolerant Species	1	2	1.133	2.05	0.009
	Number of Cyprinidae Species	1	2	1.067	2.05	0.002
	Number of Cyprinidae Individuals	10	27.5	17.733	44.225	0.028
	Proportion of Individuals as Tolerant	5.882	8.805	7.071	13.991	0.031
	Proportion of Individuals as Intolerant	6.154	2.853	11.138	5.941	0.031
	Number of *Centrarchidae* Individuals	7	16.5	13.067	34.3	0.048
Trophic Composition						
	Number of Insectivore Individuals	49	73.5	46.667	95.675	0.034
	Number of Insectivorous Cyprinidae Individuals	10	22	15.467	39.45	0.017
Fish Abundance						
	Total Number of Fish in Sample	54	86.5	58.267	111.675	0.029
Chemical Variables						
	Specific Conductance	21	30	19.933	36.45	0.000
	pH	5.4	6	5.307	5.945	0.002
Fish Species						
	Anguilla rostrata	0	2.5	1	2.8	0.007
	Aphredoderus sayanus	4	1	6.267	3.45	0.008
	Centrarchidae auritus	1	5	3	11.025	0.010
	Etheostoma olmstedi	0	3.5	2.467	5.55	0.012
	Etheostoma serrifer	0	0	1.733	0.35	0.012
	Minytrema melanops	0	0	0.133	1.425	0.015
	Ameiurus platycephalus	0	0	0.067	0.725	0.017
	Centrarchidae macrochirus	0	2	5.6	15.925	0.023
	Enneacanthus gloriosus	1	0	1.067	1.5	0.043
Index of Biotic Integrity						
	Final IBI Scores	80	58.667	82.356	59.417	<0.0001

[a] **Determined by Kruskal-Wallis Test**

Table 2.4. Metric Scores

Negative Metrics	**0 if greater than**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10 if less than**
Total Number of Fish in Sample	251	229	207	185	163	141	120	98	76	54	54
Total Species	18	17	16	15	14	14	13	12	11	10	10
Number of Tolerant Individuals	45	40	36	31	26	22	17	12	8	3	3
Number of Tolerant Species	4	3	3	3	2	2	2	2	1	1	1
Number of Cyprinidae Species	4	4	3	3	3	2	2	2	1	1	1
Number of Cyprinidae Individuals	96	86	77	67	58	48	39	29	20	10	10
Percent of Individuals as Tolerant	34	31	28	25	22	18	15	12	9	6	6
Number of *Centrarchidae* Individuals	84	75	67	58	50	41	33	24	16	7	7
Number of Insectivore Individuals	224	204	185	165	146	127	107	88	68	49	49
Number of Insectivorous Cyprinidae Individuals	89	80	71	63	54	45	36	28	19	10	10
Specific Conductance	60	55	51	47	42	38	34	30	25	21	21
pH	6.70	6.56	6.41	6.27	6.12	5.98	5.83	5.69	5.54	5.40	5.40

Positive Metrics	**0 if less than**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10 if greater than**
Proportion of Individuals as Intolerant	0.00	0.68	1.37	2.05	2.74	3.42	4.10	4.79	5.47	6.15	6.15
Aphredoderus sayanus	0.00	0.44	0.89	1.33	1.78	2.22	2.67	3.11	3.56	4.00	4.00
Etheostoma serrifer	0	-	-	-	-	-	-	-	-	-	0

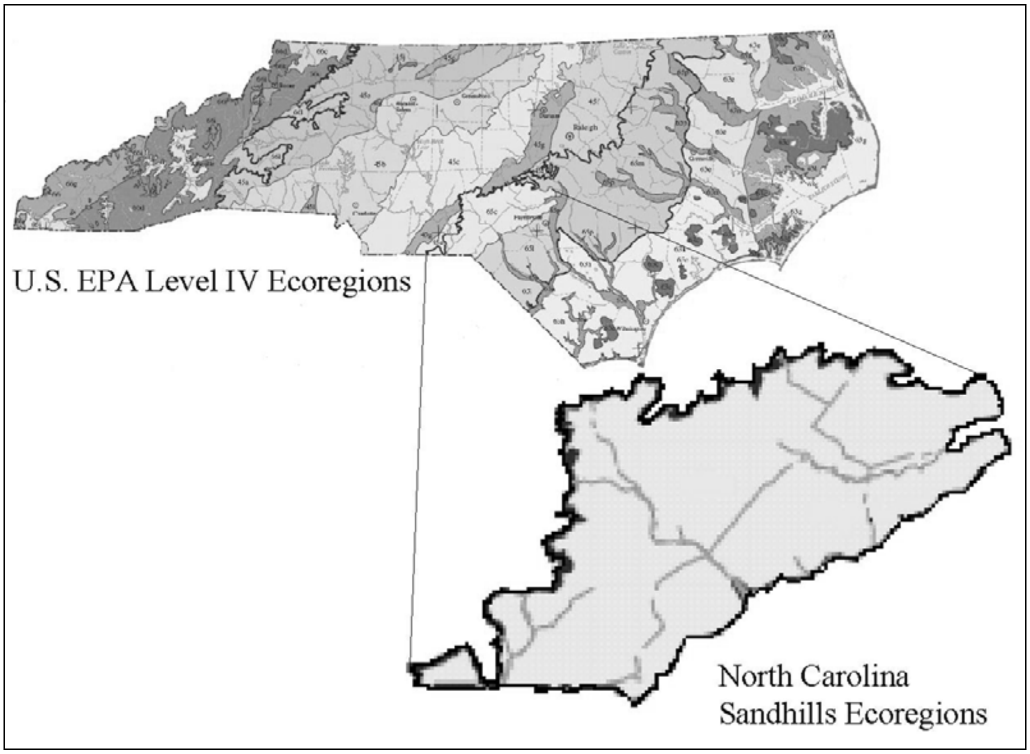


Figure 2.1. North Carolina and Sandhills Level-IV ecoregions as designated by U.S. EPA (Griffith et al. 2002)

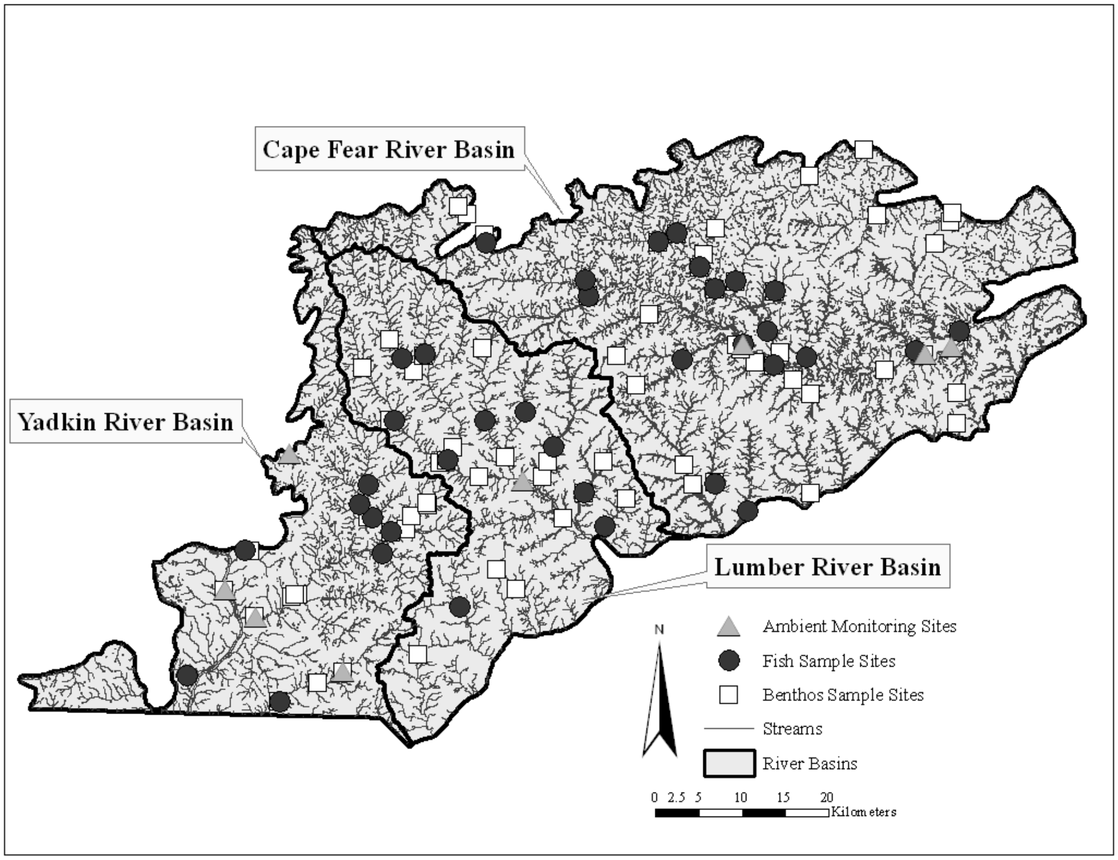
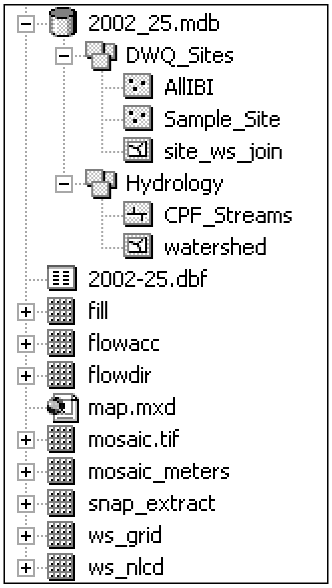


Figure 2.2. Location of Fish Community Sample Sites, Benthos Sample Sites and Ambient Monitoring Sites within the Sandhills



- 2002_25.mdb
  - DWQ_Sites
    - AllIBI
    - Sample_Site
    - site_ws_join
  - Hydrology
    - CPF_Streams
    - watershed
- 2002-25.dbf
- fill
- flowacc
- flowdir
- map.mxd
- mosaic.tif
- mosaic_meters
- snap_extract
- ws_grid
- ws_nlcd

Figure 2.3. ArcGIS Personal Geodatabase with Fish Sample Site Data

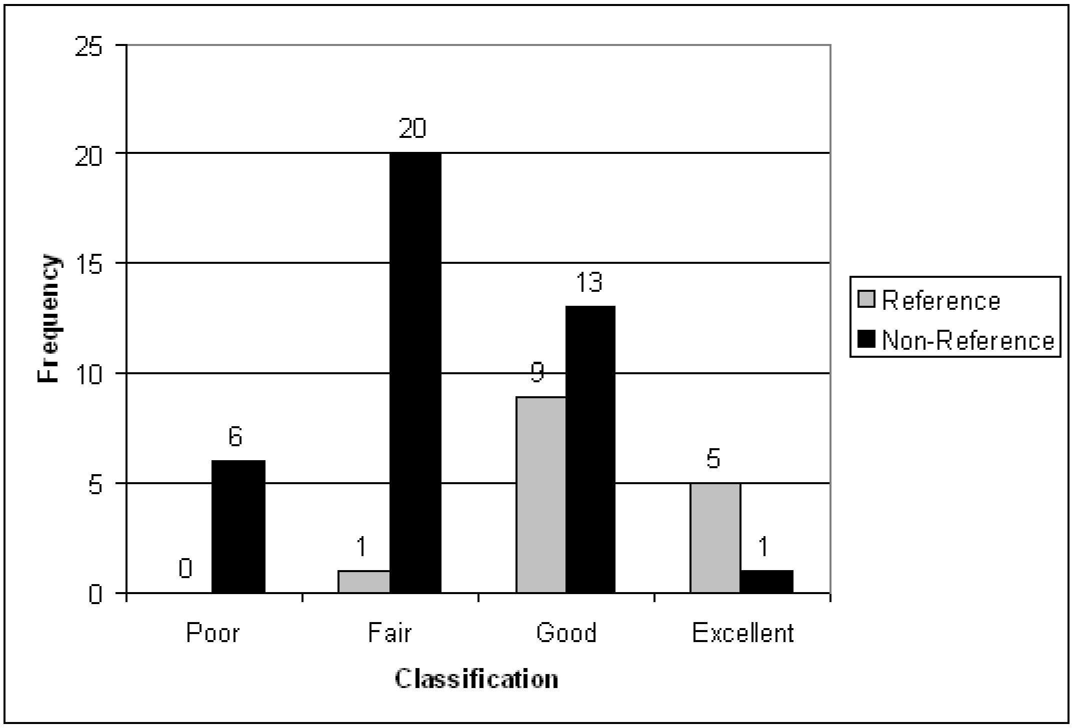


Figure 2.4. Histogram of Sample Sites' IBI Classification Grouped by Reference/Non-Reference

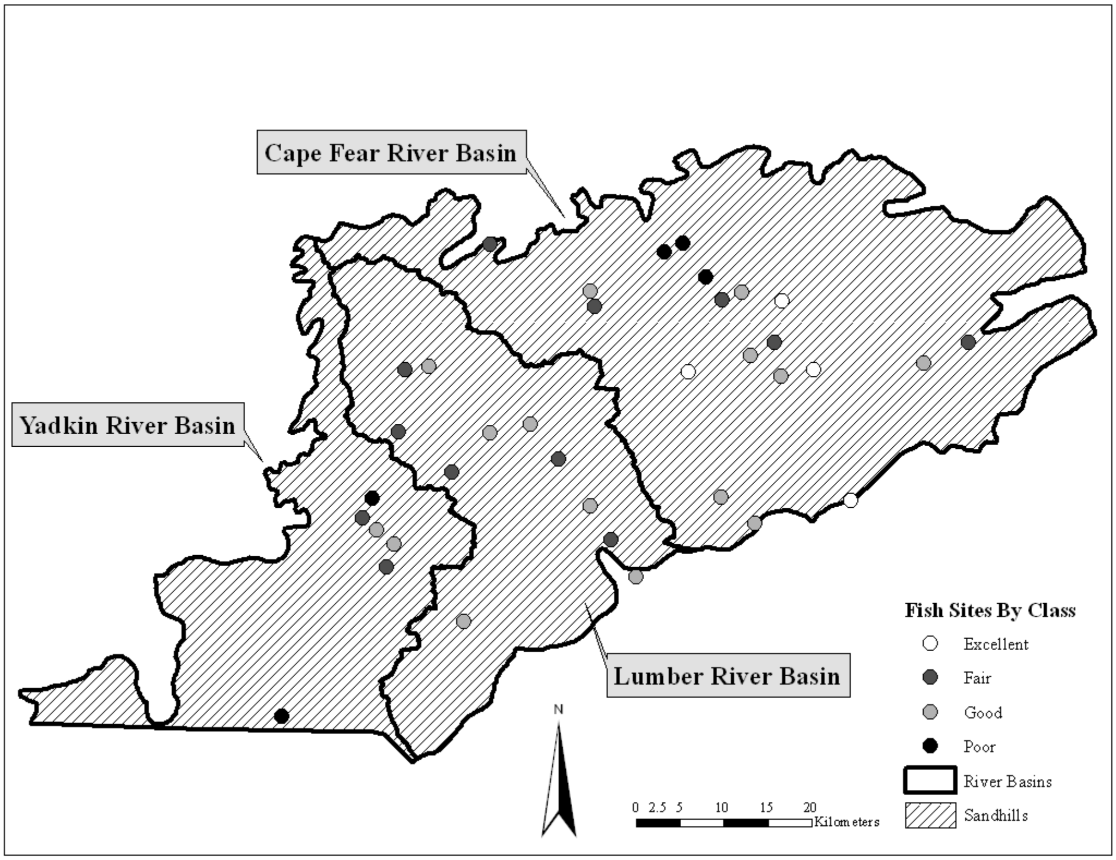


Figure 2.5. Fish Community Sample Sites by IBI Classification

3. CONCLUSION

3.1. Overview

The research described herein presents a method for developing a statistically defensible and effective fish index of biotic integrity (IBI) for the Sandhills region of North Carolina. The work is presented in two chapters. The first chapter serves as an introduction to the Sandhills region and the use of IBI's by the North Carolina Division of Water Quality (NCDWQ) and others. The second chapter is a stand-alone manuscript intended for submission for peer-reviewed publication. This second chapter describes the process by which the Sandhills IBI was developed. Following is a short summary of the work presented in each chapter.

3.2. Summary of Chapters

3.2.1. Literature Review and Introduction.

This chapter describes the mission of the NCDWQ Biological Assessment Unit as assessing the water quality conditions of streams and rivers of North Carolina and report their findings to the Basinwide Planning Unit. The fish community program works with the benthic macroinvertebrate and ambient monitoring systems programs to gauge the impacts of escalating levels of anthropogenic activities on aquatic ecosystem health. The fish community program uses an IBI to assess stream health based on resident fish assemblages. The IBI has become a popular monitoring approach throughout the country as well as internationally. While the NCDWQ has successfully incorporated a modified IBI (NCIBI) in assessing streams in other physiographic regions throughout the state, the Sandhills have proven difficult to assess. The problem arises from the low natural abundance and diversity of fish communities in streams within this region.

The use of geographic information systems (GIS) and remote sensing as tools for streamlining the IBI development process are discussed in this chapter. Also, a discussion of the unique ecological attributes of the Sandhills argues for prioritizing the region for more efficient aquatic monitoring protocols. The chapter presents the research objectives of this study as to work with the NCDWQ to produce a highly automated GIS-based data management model and statistical analysis for the fish community data in the Sandhills. The result of this model is a suggested protocol for developing fish IBI's in the Sandhills or any other region of the state and beyond.

3.2.2. Manuscript: A GIS Approach to Developing a Fish IBI for the North Carolina Sandhills

This chapter describes a process for developing an IBI for resident fish assemblages in an ecoregion that exhibits low natural productivity and biodiversity. To develop a more robust and automated method of measuring responses to anthropogenic disturbance, we delineated contributing watersheds for each of the 36 sample sites in the study, using GIS, hydrologic modeling, and 20-foot resolution Light Detection and Ranging (LiDAR) data. 2001 National Land Cover Database (NLCD) and *in situ* habitat data were also used to determine whether sample sites met reference conditions or not.

After determining whether sample sites met reference conditions or not, the Kruskal-Wallis test was conducted on more than 43 community metrics, as well as chemical and fish species variables, to determine which variables were significantly different between reference and non-reference sites at the 0.05 level. Eleven community metrics, 2 chemical variables (pH and specific conductance), and 9 fish species were found to be significant (p<0.05) in distinguishing anthropogenic impact. We used the 11 community metrics, both chemical variables, and the two fish species whose abundance decreased in more disturbed streams to develop a 15-metric IBI scoring system. We evaluated all 55 samples in our study on an IBI scale, each receiving a score between 0 and 100. Final IBI scores ranged from 15.3 to 99.3. The median score was a 68 and the mean score was 65.67 with a standard deviation of 20.04. We also developed a system for classifying the streams, based on their final IBI score, as either Excellent, Good, Fair, or Poor.

3.3. Recommendations for Future Work

The research described herein presents an automated protocol for developing a region-specific IBI from raw fish community data. There are numerous opportunities to improve upon the model presented, as well as numerous implications for extrapolating the model to other regions or taxonomic groups. The following recommendations summarize and prioritize possible future work:

- Validation of the modeling approach presented is necessary to ensure the effectiveness of the IBI. As additional data are collected by the NCDWQ within the Sandhills region, the Kruskal-Wallis non-parametric ANOVA should be run to determine if the IBI is differentiating between reference and non-reference sites.

- A detailed comparison of fish and benthic macroinvertebrate community data may assist in further refinements to both the fish and benthic rating systems, as well as provide insights into particular environmental stressors affecting the Sandhills region. This type of comparison may serve to validate both systems as well as identify sites where fish and benthic community ratings differ. Assessment of water quality based on resident biota are most informative when used in conjunction with other taxonomic groups.
- The usability of the geoprocessing model can be further refined by developing an IBI toolbar, or user interface, in ArcGIS. The toolbar could assist in the repeated use of the model on future Sandhills data or data from other regions.
- Using the protocols presented here on the NC piedmont streams where the NCDWQ has been successful in applying IBI assessments may help to validate or refine our approach.
- In an effort to refine our IBI approach and incorporate techniques used in other studies, a quantified analysis of metric responsiveness across a gradient of anthropogenic disturbance should be conducted. If a gradient of human disturbance can be developed, Spearman correlations and scatter plots can be used to test the responsiveness of metrics to disturbance. This type of study can eliminate metrics that are strongly associated with natural gradients, and those that are redundant or overlap with other

metrics. Eliminating these metrics may reduce the number of parameters in the IBI model.

The work presented in this thesis, coupled with the recommendations above, present an opportunity for continued collaboration between North Carolina State University and the NCDWQ. This collaboration could assist in continued improvements in the monitoring protocols of our states waters. By developing automated models and protocols, we can provide the state's monitoring agencies with effective and time-saving tools for developing indices across the ecoregions of North Carolina and incorporating numerous taxonomic groups. We have shown how GIS and remote sensing can be used to help protect our state's flowing waters and aquatic ecosystems in the face of escalating human population growth and developmental pressures.

APPENDICIES

Appendix 1. Python Script for Geoprocessing Model.

```
#______________________________________________________________________________
______________________________________
#
#Title: extract_lidar.py
#
#Description: Locates, unzips ascii, converts to raster, defines projection as state pland NC feet,
and reprojects to state plane meters for all LiDAR
#           tiles within a given polygon.
#
#Directions: sys.argv[1] = working directory
#           sys.argv[2] = polygon shapefile (subbasins, counties etc.)
#           sys.argv[3] = expression to choose which record in polygon file to base selection on (ex.
"[SUBBASIN_N] = 'YAD1'")
#           sys.argv[4] = grid file (10000m floodplane grid)
#           sys.argv[5] = directory where ascii files are located
#           sys.argv[6] = samples
#           sys.argv[7] = nlcd
#Requirements: ArcGIS 9.2
#
#Author: Ernie Hain, Center for Earth Observation, North Carolina State University. email:
fhernst@ncsu.edu
#
#Date Written: 18 March 2008
#
#______________________________________________________________________________
___________________________________


import sys, string, os, arcgisscripting, glob, zipfile, traceback, shutil, time, datetime, os.path,
unzip2, mhlib

# Create the Geoprocessor object
gp = arcgisscripting.create()

# Check out any necessary licenses
gp.CheckOutExtension("spatial")

#set script to overwrite existing data:
gp.overwriteoutput = 1
```

```
# Load required toolboxes...
gp.AddToolbox("C:/Program Files/ArcGIS/ArcToolbox/Toolboxes/Conversion Tools.tbx")
gp.AddToolbox("C:/Program Files/ArcGIS/ArcToolbox/Toolboxes/Data Management Tools.tbx")
gp.AddToolbox("C:/Program Files/ArcGIS/ArcToolbox/Toolboxes/Spatial Analyst Tools.tbx")
gp.AddToolbox("C:/Program Files/ArcGIS/ArcToolbox/Toolboxes/Analysis Tools.tbx")


cwd = sys.argv[1]
print cwd

# Local variables...
select_out = cwd + "\\select.shp"
clip_out = cwd + "\\grid.shp"
selection_feature = sys.argv[2]
Expression = sys.argv[3]
print Expression
grid = sys.argv[4]
print grid

# Process: Select...
try:
    gp.Select_analysis(selection_feature, select_out, Expression)
except:
    print "error in select process, line 54" + gp.getmessages()

# Process: Clip...
try:
    gp.Clip_analysis(grid, select_out, clip_out)
except:
    print "error in clip process, line 60" + gp.getmessages()

#Create folders for outputs
ascii_dir = cwd + "\\ascii"
if os.path.exists(ascii_dir):
     answer = raw_input("ascii folder already exists. Overwrite? y/n?")
else:
    answer = "Make Folders"
    os.makedirs(cwd + "\\ascii")
if answer == "n" or answer == "N":
    print "Quitting program.  Rename, move, or delete current model file."
```

```
    exit = raw_input("Press 'Enter' to close program")
    sys.exit()

#delete existing directories
if answer == "y" or answer == "Y":
    tree = os.walk(ascii_dir)
    for root, dirs, files in os.walk(ascii_dir, topdown=False):
        for name in files:
            print "files = ",name
            os.remove(os.path.join(root, name))
        for name in dirs:
            print "dirs = ",name
            os.rmdir(os.path.join(root, name))

os.mkdir(cwd + "\\ascii\\final")
os.mkdir(cwd + "\\ascii\\output")
os.mkdir(cwd + "\\ascii\\unzip")

datapoints = cwd + "\\datapoints"
if os.path.exists(datapoints):
     answer = raw_input("datapoints folder already exists. Overwrite? y/n?")
else:
    answer = "Make Folders"
    os.makedirs(cwd + "\\datapoints")
if answer == "n" or answer == "N":
    print "Quitting program.  Rename, move, or delete current model file."
    exit = raw_input("Press 'Enter' to close program")
    sys.exit()

#delete existing directories
if answer == "y" or answer == "Y":
    tree = os.walk(datapoints)
    for root, dirs, files in os.walk(datapoints, topdown=False):
        for name in files:
            print "files = ",name
            os.remove(os.path.join(root, name))
        for name in dirs:
            print "dirs = ",name
            os.rmdir(os.path.join(root, name))

raster = cwd + "\\raster"
if os.path.exists(raster):
```

```
     answer = raw_input("raster folder already exists. Overwrite? y/n?")
else:
    answer = "Make Folders"
    os.makedirs(cwd + "\\raster")
if answer == "n" or answer == "N":
    print "Quitting program.  Rename, move, or delete current model file."
    exit = raw_input("Press 'Enter' to close program")
    sys.exit()

#delete existing directories
if answer == "y" or answer == "Y":
    tree = os.walk(raster)
    for root, dirs, files in os.walk(raster, topdown=False):
        for name in files:
            print "files = ",name
            os.remove(os.path.join(root, name))
        for name in dirs:
            print "dirs = ",name
            os.rmdir(os.path.join(root, name))
databases = cwd + "\\databases"
if os.path.exists(databases):
     answer = raw_input("databases folder already exists. Overwrite? y/n?")
else:
    answer = "Make Folders"
    os.makedirs(cwd + "\\databases")
if answer == "n" or answer == "N":
    print "Quitting program.  Rename, move, or delete current model file."
    exit = raw_input("Press 'Enter' to close program")
    sys.exit()

#delete existing directories
if answer == "y" or answer == "Y":
    tree = os.walk(databases)
    for root, dirs, files in os.walk(databases, topdown=False):
        for name in files:
            print "files = ",name
            os.remove(os.path.join(root, name))
        for name in dirs:
            print "dirs = ",name
            os.rmdir(os.path.join(root, name))
```

```
# The following script takes the output from a model and finds the zipped ascii files in a
directory that correspond to grid
# Create update cursor for feature class
rows = gp.UpdateCursor(clip_out) #the grid file is the 10000m tile floodplains tile grid clipped
to AOI
row = rows.Next()

files = os.listdir(sys.argv[5])
in_dir = sys.argv[5]
output_dir = cwd + "\\ascii\\"

while row:
    rownum = str(row.TERRAINEXP)
    for file in files:
        if file == ("D2" + rownum + "WC20040812.zip"): #This will need to be adjusted so that the
TERRAINEXP field value matches the naming convention on the ftp site
            print file
            unzip2.unzipper(in_dir + "\\" + file, output_dir)



    row = rows.Next()

#answer = raw_input("Click 'Enter' to exit")
# The following script converts each ascii file to a raster, defines the projection, and reprojects
them
new_dir = cwd + "\\ascii\\unzip"
Out = cwd + "\\ascii"
asciis = os.listdir(new_dir)
print new_dir
print Out

for ascii in asciis:
    Output = Out + "\\" + "output\\r" + ascii[2:12] +".tif"
    input = new_dir + "\\" + ascii
    print Output
    print input


    #Convert ascii to raster
    try:
```

```
        gp.ASCIIToRaster_conversion(input, Output, "FLOAT")
    except:
        print "error in convert ascii to raster, line 106" + gp.getmessages()

    # Process: Define Projection...
    try:
        gp.DefineProjection_management(Output,
"PROJCS['NAD_1983_StatePlane_North_Carolina_FIPS_3200_Feet',GEOGCS['GCS_North_A
merican_1983',DATUM['D_North_American_1983',SPHEROID['GRS_1980',6378137.0,298.25
7222101]],PRIMEM['Greenwich',0.0],UNIT['Degree',0.0174532925199433]],PROJECTION['La
mbert_Conformal_Conic'],PARAMETER['False_Easting',2000000.002616666],PARAMETER['
False_Northing',0.0],PARAMETER['Central_Meridian',-
79.0],PARAMETER['Standard_Parallel_1',34.33333333333334],PARAMETER['Standard_Paral
lel_2',36.16666666666666],PARAMETER['Latitude_Of_Origin',33.75],UNIT['Foot_US',0.3048
006096012192]]")
    except:
        print "error in define projection, line 111" + gp.getmessages()
    final_out = Out + "\\final\\r" + ascii[2:12] + "f"

    # Process: Project...
    try:
        gp.ProjectRaster_management(Output, final_out,
"PROJCS['NAD_1983_StatePlane_North_Carolina_FIPS_3200',GEOGCS['GCS_North_Americ
an_1983',DATUM['D_North_American_1983',SPHEROID['GRS_1980',6378137.0,298.257222
101]],PRIMEM['Greenwich',0.0],UNIT['Degree',0.0174532925199433]],PROJECTION['Lambe
rt_Conformal_Conic'],PARAMETER['False_Easting',609601.22],PARAMETER['False_Northin
g',0.0],PARAMETER['Central_Meridian',-
79.0],PARAMETER['Standard_Parallel_1',34.33333333333334],PARAMETER['Standard_Paral
lel_2',36.16666666666666],PARAMETER['Latitude_Of_Origin',33.75],UNIT['Meter',1.0]];IsHi
ghPrecision", "BILINEAR", "", "", "", "")
    except:
        print "error in project process, line 119" + gp.getmessages()

#The following script mosaics the new rasters

#Make list of rasters

RasList = ""
rasdir = os.listdir(cwd + "\\ascii\\final")

for i in rasdir:
    if i[-4:] != ".aux" and i[-4:] != "info":
```

```
        #print i
        RasList = RasList + cwd + "\\ascii\\final" + "\\" + i + ";"


print RasList

# Script arguments...
mosaic_tif = cwd + "\\raster\\mosaic.tif"

Input_Rasters = RasList

print Input_Rasters

# Local variables...
raster = cwd + "\\raster"

#Set Spatial Reference
sr =
"PROJCS['NAD_1983_StatePlane_North_Carolina_FIPS_3200',GEOGCS['GCS_North_Americ
an_1983',DATUM['D_North_American_1983',SPHEROID['GRS_1980',6378137.0,298.257222
101]],PRIMEM['Greenwich',0.0],UNIT['Degree',0.0174532925199433]],PROJECTION['Lambe
rt_Conformal_Conic'],PARAMETER['False_Easting',609601.22],PARAMETER['False_Northin
g',0.0],PARAMETER['Central_Meridian',-
79.0],PARAMETER['Standard_Parallel_1',34.33333333333334],PARAMETER['Standard_Paral
lel_2',36.16666666666666],PARAMETER['Latitude_Of_Origin',33.75],UNIT['Meter',1.0]]"


# Process: Mosaic To New Raster...
try:
    gp.MosaicToNewRaster_management(Input_Rasters, raster, "mosaic.tif", sr,
"32_BIT_FLOAT", "", "1", "MEAN", "FIRST")
except:
    print "error in mosaic to new raster process, line 154" + gp.getmessages()

#The following script incorporates hydrology tools from the spatial analyst toolbox

#Local variables
mosaic_meters = cwd + "\\raster\\mosaic_meters"

# Process: Single Output Map Algebra...
try:
    gp.SingleOutputMapAlgebra_sa(cwd + "\\raster\\mosaic.tif *  0.304801", mosaic_meters, "")
```

```
except:
    print "error in single output map algebra proccess, line 165" + gp.getmessages()

#New script as of 11-08-08


#Local Variables
dem_fill = cwd + "\\raster\\Fill_SingleO1"
FlowDir = cwd + "\\raster\\FlowDir_Fill1"
flowdrop = ""
flowacc = cwd + "\\raster\\FlowAcc_Flow1"
streams_grid = cwd + "\\raster\\SingleOutput2"
stream_link = cwd + "\\raster\\StreamL_Sing1"

#Processes
# Process: Fill...
try:
    gp.Fill_sa(mosaic_meters, dem_fill, "")
except:
    print "error in fill process, line 183" + gp.getmessages()

# Process: Flow Direction...
try:
    gp.FlowDirection_sa(dem_fill, FlowDir, "NORMAL", flowdrop)
except:
    print "error in flow dir process, line 188" + gp.getmessages()

# Process: Flow Accumulation...
try:
    gp.FlowAccumulation_sa(FlowDir, flowacc, "", "FLOAT")
except:
    print "error in flow acc process, line 195" + gp.getmessages()

setnull = "setnull (" + cwd + "\\raster\\FlowAcc_Flow1 < 1000 , 1)"
# Process: Single Output Map Algebra (2)...
try:
    gp.SingleOutputMapAlgebra_sa(setnull, streams_grid, flowacc)
except:
    print "error in single output map algebra, line 201" + gp.getmessages()

# Process: Stream Link...
try:
```

```
    gp.StreamLink_sa(streams_grid, FlowDir, stream_link)
except:
    print "error in stream link process, line 208" + gp.getmessages()

#Create Geodatabase for subbasin
#variables
DB_work = cwd + "\\databases"
DB = "subbasin"

#Process: Create Personal Geodatabase
try:
    PGDB = gp.CreatePersonalGDB_management(DB_work, DB)
except:
    print "Error in 'Create Personal Geodatabase'"


# Process: Create Basins Feature Dataset...
try:
    basins = gp.CreateFeatureDataset_management(PGDB, "basins", sr)
except:
    print "Error in 'Create Basins Feature Dataset'"
try:
    basins = gp.CreateFeatureDataset_management(PGDB, "watershed", sr)
except:
    print "Error in 'Create watershed Feature Dataset'"
try:
    basins = gp.CreateFeatureDataset_management(PGDB, "ws_streams", sr)
except:
    print "Error in 'Create ws_streams Feature Dataset'"
try:
    basins = gp.CreateFeatureDataset_management(PGDB, "nlcd", sr)
except:
    print "Error in 'Create nlcd Feature Dataset'"

#variables
sub_Buffer = PGDB + "\\basins\\Buffer"
##
samples = sys.argv[6]
sub_samples = PGDB + "\\basins\\sub_samples"

# Process: Clip...
try:
```

```
    gp.Clip_analysis(samples, select_out, sub_samples)
except:
    print "error in clip process, line 250" + gp.getmessages()

#The following script buffers the sample sites within the subbasin and uses the buffer file to split
the sample feature class into multiple feature classes
# Process: Buffer...
try:
    gp.Buffer_analysis(sub_samples, sub_Buffer, "2 Meters", "FULL", "ROUND", "NONE", "")
except:
    print "error in buffer process, line 257" + gp.getmessages()

# Process: Add Field...
try:
    gp.AddField_management(sub_Buffer, "split", "TEXT", "", "", "", "", "NULLABLE",
"NON_REQUIRED", "")
except:
    print "error in add field process, line 263" + gp.getmessages()

#variables
#sub_Buffer_2 = PGDB + "\\basins\\Buffer"

# Process: Calculate Field...
try:
    gp.CalculateField_management(sub_Buffer, "split", "[OBJECTID]", "VB", "")
except:
    print "error in calculate field process, line 272" + gp.getmessages()

#variables
data__2 = cwd + "\\datapoints"

# Process: Split...
try:
    gp.Split_analysis(sub_samples, sub_Buffer, "split", data__2, "")
except:
    print "error in split process, line 281" + gp.getmessages()

#loop through samples-create watershed and extract land cover data

gp.Workspace = cwd + "\\datapoints"

fcList = gp.ListFeatureClasses ("*","All")
```

```
fcList.reset()
fc = fcList.Next()

while fc:
    work = cwd + "\\datapoints\\"
    single_point = work + fc
    #print "arg1: = ",single_point
    fcname = fc.rstrip(".shp")
    pour = work + "Snap" + fcname
    #print "arg3: = ", pour
    flowacc = cwd + "\\raster\\flowacc_flow1"
    #print "arg2: = ", flowacc
    #print flowacc
    distance = "50"
    #Process: Snap Pour Point...
    try:
        gp.SnapPourPoint_sa(single_point, flowacc, pour, distance)
    except:
        print gp.getmessages()

    watershed_grid = cwd + "\\raster\\ws_grid" + fcname
    try:
        # Process: Watershed (2)...
        gp.Watershed_sa(FlowDir, pour, watershed_grid, "VALUE")

    except:
        print gp.getmessages()

    watershed = PGDB + "\\watershed\\ws" + fcname
    try:
        # Process: Raster to Polygon...
        gp.RasterToPolygon_conversion(watershed_grid, watershed, "SIMPLIFY",
"VALUE")
    except:
        print gp.getmessages()

    StreamT_SingleO1_shp = cwd + "\\raster\\streaml_sing1"
    ws_streams = PGDB + "\\ws_streams\\ws_streams" + fcname

    nlcd_nc__2_ = sys.argv[7]
    nlcd_ws = PGDB + "\\nlcd\\nlcd" + fcname
    try:
```

```
        # Process: Extract by Mask...
        gp.ExtractByMask_sa(nlcd_nc__2_, watershed, nlcd_ws)
    except:
        print gp.getmessages()

    fc = fcList.next()

answer = raw_input("Click 'Enter' to exit")
```

Appendix 2. SAS script for producing summary statistics

```
ods html body='Score_Sum';
PROC IMPORT OUT= WORK.scorerefsum
        DATAFILE= "E:\Sandhills\data\scores\Score.xls" /*Replace with location

                                of data*/
        DBMS=EXCEL REPLACE;
    SHEET="Score"; /*Excel sheet containing data*/
    GETNAMES=YES;
    MIXED=NO;
    SCANTEXT=YES;
    USEDATE=YES;
    SCANTIME=YES;
RUN;


PROC MEANS DATA=WORK.scorerefsum

        FW=12
        PRINTALLTYPES
        CHARTYPE
        QMETHOD=OS
        VARDEF=DF

                MEAN
                STD
                MIN
                MAX
                N
                P5
                P10
                Q1
                MEDIAN
                Q3
                P90     ;
        VAR FINAL_SCORE;

RUN;
```

```
ods html close;
```

Appendix 3. SAS script for producing Kruskal-Wallis scores

```
ods html body='IBI_KW';
PROC IMPORT OUT= WORK.IBI
        DATAFILE= "E:\Sandhills\data\significant\IBI_Scores.xls" /*Location of data
to be analyzed*/
        DBMS=EXCEL REPLACE;
    SHEET="tblIBI_Scores"; /*Excel sheet containing data*/
    GETNAMES=YES;
    MIXED=NO;
    SCANTEXT=YES;
    USEDATE=YES;
    SCANTIME=YES;
RUN;

PROC NPAR1WAY DATA=WORK.IBI WILCOXON EDF;

        CLASS REFERENCE; /*Column heading that represents categories to split data
by*/
        var FINAL_SCORE; /*Variable to be analyzed*/
        OUTPUT
OUT=SASUSER.NP1WNonParOneWayIMPW_0004(LABEL="NPAR1WAY output data set
for SASUSER.IMPW_0004")
                WILCOXON;
RUN; QUIT;

Proc Export data = SASUSER.NP1WNonParOneWayIMPW_0004
                                outfile = "E:\Sandhills\data\significant\IBI_Scores.txt" /*location
to export results text to*/
                                dbms = csv;

run;
ods html close;
```

Appendix 4. Table of family, feeding type and tolerance values for North Carolina fish (obtained from North Carolina Division of Water Quality)

ScientificName	**Common Name**	**Family**	**Pollution Tolerance**	**Feeding Type**
Petromyzon marinus	Sea Lamprey	Petromyzontidae	Intermediate	Parasitic
Amia calva	Bowfin	Amiidae	Tolerant	Piscivore
Anguilla rostrata	American Eel	Anguillidae	Intermediate	Piscivore
Dorosoma cepedianum	Gizzard Shad	Clupeidae	Intermediate	Omnivore
Clinostomus funduloides	Rosyside Dace	Cyprinidae	Intermediate	Insectivore
Cyprinella analostana	Satinfin Shiner	Cyprinidae	Tolerant	Insectivore
Cyprinella sp. cf. zanema	Thinlip Chub	Cyprinidae	Intolerant	Insectivore
Luxilus albeolus	White Shiner	Cyprinidae	Intermediate	Insectivore
Nocomis leptocephalus	Bluehead Chub	Cyprinidae	Intermediate	Omnivore
Notemigonus crysoleucas	Golden Shiner	Cyprinidae	Tolerant	Omnivore
Notropis altipinnis	Highfin Shiner	Cyprinidae	Intermediate	Insectivore
Notropis amoenus	Comely Shiner	Cyprinidae	Intermediate	Insectivore
Notropis chalybaeus	Ironcolor Shiner	Cyprinidae	Intolerant	Insectivore
Notropis chiliticus	Redlip Shiner	Cyprinidae	Intermediate	Insectivore
Notropis cummingsae	Dusky Shiner	Cyprinidae	Intermediate	Insectivore
Notropis hudsonius	Spottail Shiner	Cyprinidae	Intermediate	Omnivore
Notropis maculatus	Taillight Shiner	Cyprinidae	Intolerant	Insectivore
Notropis petersoni	Coastal Shiner	Cyprinidae	Intermediate	Insectivore
Notropis scepticus	Sandbar Shiner	Cyprinidae	Intermediate	Insectivore
Semotilus atromaculatus	Creek Chub	Cyprinidae	Tolerant	Insectivore
Semotilus lumbee	Sandhills Chub	Cyprinidae	Intolerant	Insectivore
Erimyzon oblongus	Creek Chubsucker	Catostomidae	Intermediate	Omnivore
Erimyzon sucetta	Lake Chubsucker	Catostomidae	Intermediate	Insectivore
Minytrema melanops	Spotted Sucker	Catostomidae	Intermediate	Insectivore
Moxostoma collapsum	Notchlip Redhorse	Catostomidae	Intermediate	Insectivore
Ameiurus brunneus	Snail Bullhead	Ictaluridae	Intermediate	Insectivore
Ameiurus natalis	Yellow Bullhead	Ictaluridae	Tolerant	Omnivore

Ameiurus nebulosus	Brown Bullhead	Ictaluridae	Tolerant	Omnivore
Ameiurus platycephalus	Flat Bullhead	Ictaluridae	Tolerant	Insectivore
Noturus gyrinus	Tadpole Madtom	Ictaluridae	Intermediate	Insectivore
Noturus insignis	Margined Madtom	Ictaluridae	Intermediate	Insectivore
Esox americanus	Redfin Pickerel	Esocidae	Intermediate	Piscivore
Esox niger	Chain Pickerel	Esocidae	Intermediate	Piscivore
Umbra pygmaea	Eastern Mudminnow	Umbridae	Intermediate	Insectivore
Aphredoderus sayanus	Pirate Perch	Aphredoderidae	Intermediate	Insectivore
Chologaster cornuta	Swampfish	Amblyopsidae	Intermediate	Insectivore
Labidesthes sicculus	Brook Silverside	Atherinidae	Intermediate	Insectivore
Fundulus lineolatus	Lined Topminnow	Fundulidae	Intermediate	Insectivore
Fundulus rathbuni	Speckled Killifish	Fundulidae	Intermediate	Insectivore
Gambusia holbrooki	Eastern Mosquitofish	Poecilidae	Tolerant	Insectivore
Acantharchus pomotis	Mud Sunfish	Centrarchidae	Intermediate	Insectivore
Centrarchus macropterus	Flier	Centrarchidae	Intermediate	Insectivore
Enneacanthus chaetodon	Blackbanded Sunfish	Centrarchidae	Intermediate	Insectivore
Enneacanthus gloriosus	Bluespotted Sunfish	Centrarchidae	Intermediate	Insectivore
Enneacanthus obesus	Banded Sunfish	Centrarchidae	Intermediate	Insectivore
Centrarchidae auritus	Redbreast Sunfish	Centrarchidae	Tolerant	Insectivore
Centrarchidae cyanellus	Green Sunfish	Centrarchidae	Tolerant	Insectivore
Centrarchidae gibbosus	Pumpkinseed	Centrarchidae	Intermediate	Insectivore
Centrarchidae gulosus	Warmouth	Centrarchidae	Intermediate	Insectivore
Centrarchidae macrochirus	Bluegill	Centrarchidae	Intermediate	Insectivore
Centrarchidae marginatus	Dollar Sunfish	Centrarchidae	Intermediate	Insectivore
Centrarchidae microlophus	Redear Sunfish	Centrarchidae	Intermediate	Insectivore
Centrarchidae punctatus	Spotted Sunfish	Centrarchidae	Intermediate	Insectivore
Micropterus punctulatus	Spotted bass	Centrarchidae	Intermediate	Piscivore
Micropterus salmoides	Largemouth Bass	Centrarchidae	Intermediate	Piscivore
Pomoxis nigromaculatus	Black Crappie	Centrarchidae	Intermediate	Piscivore
Etheostoma flabellare	Fantail Darter	Percidae	Intermediate	Insectivore
Etheostoma fusiforme	Swamp Dater	Percidae	Intermediate	Insectivore
Etheostoma mariae	Pinewoods Darter	Percidae	Intolerant	Insectivore

Etheostoma olmstedi	Tessellated Darter	Percidae	Intermediate	Insectivore
Etheostoma serrifer	Sawcheek Darter	Percidae	Intolerant	Insectivore
Perca flavescens	Yellow Perch	Percidae	Intermediate	Piscivore
Percina crassa	Piedmont Darter	Percidae	Intolerant	Insectivore
Elassoma evergladei	Everglades Pygmy Sunfish	Elassomatidae	Intermediate	Insectivore
Elassoma zonatum	Banded Pygmy Sunfish	Elassomatidae	Intermediate	Insectivore

Appendix 5. Study sample sites with IBI scores and class ratings.

cc-num	Basin	Reference	Final IBI Score	Class
2001-01	YAD	No	76.667	Good
2001-02	YAD	No	63.333	Fair
2001-04	YAD	No	39.333	Fair
2001-51	LBR	Yes	76.667	Good
2001-52	LBR	No	70.000	Good
2001-53	LBR	No	51.333	Fair
2001-54	LBR	No	81.333	Good
2001-55	LBR	No	58.000	Fair
2001-56	LBR	Yes	69.333	Good
2001-57	LBR	No	57.333	Fair
2001-58	LBR	No	52.000	Fair
2001-59	LBR	No	82.000	Good
2002-25	CPF	Yes	93.333	Excellent
2002-26	CPF	Yes	80.000	Good
2002-27	CPF	No	54.000	Fair
2002-28	CPF	No	22.667	Poor
2002-29	CPF	No	25.333	Poor
2002-30	CPF	No	17.333	Poor
2003-54	CPF	No	68.000	Good
2003-55	CPF	No	82.667	Good
2003-56	CPF	Yes	95.333	Excellent
2003-57	CPF	No	90.000	Excellent
2003-58	CPF	Yes	79.333	Good
2003-59	CPF	Yes	85.333	Good
2003-64	CPF	No	66.000	Fair
2003-67	CPF	Yes	94.000	Excellent
2003-68	CPF	No	86.667	Good
2003-70	CPF	Yes	74.000	Good
2006-20	YAD	No	57.333	Fair
2006-21	YAD	No	72.667	Good
2006-22	YAD	Yes	87.333	Excellent
2006-23	YAD	No	15.333	Poor
2006-24	YAD	No	64.000	Fair
2006-54	LBR	No	80.000	Good
2006-55	LBR	Yes	77.333	Good
2006-56	LBR	No	50.667	Fair
2006-57	LBR	No	58.667	Fair
2006-58	LBR	No	58.667	Fair
2006-59	LBR	No	68.000	Good
2006-60	LBR	No	82.000	Good
2006-61	LBR	No	49.333	Fair
2006-64	LBR	Yes	86.667	Good

90-09	LBR	No	56.667	Fair
90-10	YAD	No	25.333	Poor
94-11	CPF	No	56.667	Fair
94-12	CPF	No	72.000	Good
96-01	LBR	No	82.667	Good
96-02	LBR	No	58.667	Fair
96-13	YAD	Yes	76.000	Good
96-64	CPF	No	63.333	Fair
96-65	LBR	No	66.000	Fair
96-66	LBR	No	58.000	Fair
98-29	CPF	Yes	61.333	Fair
98-34	CPF	Yes	99.333	Excellent
98-35	CPF	No	36.667	Poor