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# The Effects of Nutrient Concentrations on Macroinvertebrate Distributions in Georgia

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# THE EFFECTS OF NUTRIENT CONCENTRATIONS ON MACROINVERTEBRATE DISTRIBUTIONS IN GEORGIA

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# The Effects of Nutrient Concentrations on Macroinvertebrate Distributions in Georgia

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by

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# ABSTRACT

Nutrients are considered the second largest nonpoint source pollutant in Georgia's running water ecosystems. Nutrients can naturally occur in low amounts and are typically increased in streams as a result of industry, agriculture, silviculture, and urbanization. In large amounts, nutrients can cause many problems to occur in the stream ecosystem. Macroinvertebrates have been established as good indicators for determining the level of impairment of a stream; however, not much research has been done on how nutrients affect macroinvertebrates in a stream. Approximately 225 reference and impaired streams were sampled over a 3 - year field season, (index period from September to February), using the EPA's rapid bioassessment protocol (RBP). The concentrations of nutrients were measured in mg/L and ranged as follows: nitrite and nitrite/nitrate <0.01 to >1.0, ammonia <0.03 to 3.0, and phosphorous <0.01 to 1.2. Analysis determined (1) the RBP has the potential to indicate nutrient loading; (2) nutrient parameters that were significantly different varied across the state, varied at ecoregion level and varied at subecoregion within an ecoregion; (3) the data indicated some correlations between nutrients and macroinvertebrate distribution, however the RBP was not a good indicator of nutrient loading in all ecoregions and subecoregions across the state; and (4) nutrient analysis should continue to be part of the rapid bioassessment protocol.

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# DEDICATION

I dedicate my thesis to my loving family and in memory of my father and grandfather. My father, Michael L. Pollock, always taught me to be the best I could be and to continue to strive toward higher goals. My grandfather, Paul Harrell, always showed a strong interest in this project and was very proud of the work I was doing.

## INTRODUCTION

Plant nutrients (*i.e.* various forms of nitrogen and phosphorus) are a major nonpoint source problem in running water ecosystems. Nutrients from non-point source loading are considered to have an important impact on streams in Georgia. Nutrient input can come from commercial fertilizer, runoff (*i.e.* agriculture and roads), livestock manure, decomposition of plant and animal matter, atmospheric inputs, soil erosion, organic matter, bacteria, silviculture, industry, and other human influences (Puckett 1994). Two important nonpoint sources of nitrogen are commercial fertilizer and animal manure, which are considered the largest of the quantifiable sources of nutrients (Puckett 1994).

Nitrate and orthophosphate are considered the necessary nutrients for the growth of algae and aquatic plants in streams (Boyd 1996). A major problem with overabundance of nitrogen and phosphorus in slow moving reaches of streams is eutrophication (Puckett 1994). Excessive concentrations of nutrients in streams can trigger algal booms and excessive aquatic plant growth leading to anoxia, which can result in fish kills and taste and odor problems (Boyd 1996). Eutrophication also can lead to aesthetic degradation, loss of pollution-sensitive invertebrate taxa through smothering of substrata by algae, clogging of water intake structures, and degradation of water quality (Biggs 2000). As a eutrophication control, the United States Environmental Protection Agency (U.S. EPA) has recommended that a total phosphate concentration in streams should not exceed 0.1 mg/l as phosphorus (Puckett 1994). For 19 invertebrate species, which represented 14 families and 16 genera, un-ionized ammonia was reported to be acutely toxic at 0.083 to 4.60 mg/L (EPA 1986). Levels below 90 mg/L for nitrate and below 5 mg/L for nitrite are the levels that protect most warmwater fish. For salmonid fish, nitrite levels should be below 0.60 mg/L to protect these fish.

Phosphorus- and nitrogen-based nutrients are also necessary for the life of macroinvertebrates and the supply may be potentially limiting to biological activity within stream ecosystems (Allan 1995). The principal forms found in streams are nitrate ($NO_3^-$), organic nitrogen, ammonia ($NH_4^+$), orthophosphate ($PO_4^{3-}$), and organic phosphorus (Puckett 1994). Ammonia, nitrate, and orthophosphate are the forms most readily assimilated by stream biota. The uptake, transformation, and release of nutrients are influenced by a number of abiotic and biotic processes (Allan 1995). In streams, nutrients do not cycle in one place, but are displaced downstream as the cycling is completed; a process called nutrient spiraling (Newbold *et al.* 1981). Nitrogen and phosphorus are critical to the maintenance of ecosystem function and the cycling of these nutrients affects the ability of an ecosystem to withstand and recover from perturbations (Newbold *et al.* 1981). Thus, nutrient availability may control important ecological processes like primary productivity and decomposition in rivers and streams (Hart *et al.*1992).

**<u>Nitrogen Compounds</u>**

Water quality and the health of fish and other aquatic organisms can be affected by the discharge of nitrogen to the receiving waters (Pauer and Auer 2000). Reduced nitrogen forms, like ammonia and organic nitrogen, can be oxidized in freshwater systems resulting in oxygen depletion (Pauer and Auer 2000). Oxygen depletion can seriously impair, or even kill, aquatic organisms. In addition, a number of factors interacting with ammonia, nitrite and nitrate can affect the toxicity of nitrogenous compounds to aquatic organisms. These factors include pH, dissolved oxygen concentration, temperature, calcium concentration, salinity, fluctuation or intermittency of exposures, and presence of other toxicants (Russo 1985).

Ammonia, nitrite, and nitrate are interrelated through the process of nitrification (Russo 1985). Nitrification is the biological oxidation of ammonia to nitrate, with nitrite being produced as an intermediate product. The oxidation of nitrite to nitrate is a rapid process, but the conversion of ammonia to nitrite is the rate-limiting step in the process.

Although nitrogenous compounds are naturally available in stream systems, two major exogenous sources of nitrogen are fertilizer and animal manure (Kronening and Stark 1997). Approximately 11.5 million tons of nitrogen is applied as commercial fertilizer for agricultural purposes throughout the United States. Manure that contains an estimated 6.5 million tons of nitrogen is produced from farm animals each year in the United States. Large amounts of nitrogen are distributed over the landscape when farm animals are allowed to roam. When farm animals are confined, the area then becomes a point-source problem. When manure is not properly handled or disposed, these nitrogenous wastes can be conveyed to lotic ecosystems.

An important external nitrogen source to upland ecosystems comes from atmospheric inputs (Reynolds and Edwards 1995). Industrialization and intensification of agriculture increase these atmospheric inputs of nitrogen (as waste emissions and aerosol application of fertilizers). Atmospheric inputs may surpass the retention capacity of soils and biota, which may cause a disruption of the nitrogen cycle and enhanced nitrate leaching. Disruption of the nitrogen cycle could then lead to nutrient enrichment and acidification of adjacent waters.

Nitrate is formed by complete oxidation of ammonia through the nitrification process (Russo 1985). Nitrate is considered to be less toxic to aquatic organisms than are ammonia and nitrite because it is very mobile, not readily retained by the soil and is highly soluble in water (Kronening and Stark 1997). Nitrate can be found in relatively high concentrations in surface waters, entering streams from runoff of agricultural fertilizer. Nitrate may increase the net productivity of the system and, to a point, may be beneficial to the aquatic community (Russo 1985). However, when nitrate concentration becomes excessive and other essential nutrient factors are present, eutrophication and associated algal blooms can become a problem.

Since nitrite is rapidly oxidized to nitrate, it is usually present only in trace amounts in most natural freshwater systems (Russo 1985). Nitrite ($NO_2^-$) can be extremely toxic to aquatic life, but does not usually occur in natural surface water systems at concentrations considered harmful to aquatic organisms. However, in natural waters, at high concentrations, nitrite may be detrimental to freshwater aquatic life. Nitrite inputs can impair the ability of blood to transport oxygen in fish (Russo 1985). Increased methemoglobin levels in fish blood can occur from increased nitrite concentration as low as 0.015 mg/l $NO_2$-N. Aquatic toxicity of nitrite is based upon the presence of other chemicals. For example, as nitrite toxicity decreases, concentrations of chloride ions increase (Russo 1985). Also, over the pH range 6.4 to 9.0, nitrite decreases as pH increases. Bromide, sulfate, phosphate and nitrate also inhibit nitrite toxicity. Increased calcium concentrations have also been shown to decrease the toxicity of nitrite.

Ammonia ($NH_3$) is relatively toxic in nature and its existence throughout surface water systems makes it one of the most important pollutants in the aquatic environment (Russo 1985). Ammonia enters streams from many sources, including industrial wastes, sewage effluents, alternative fuel conversion processes, and agricultural discharges. Toxicity of total ammonia solutions appear to be greater at higher pH values because increasing pH increases the concentration of $NH_3$ (Chipman 1934; Russo 1985; Wuhrmann *et al.* 1947; and Wuhrmann and Woker 1948). Elevated ammonia concentrations in streams may increase concentrations of un-ionized ammonia ($NH_3$), which can kill fish and other aquatic life (Puckett 1994). It is likely that ammonia has a different mode of action at high and low concentrations (Russo 1985). High ammonia concentrations can be toxic to fish causing loss of equilibrium, increased breathing, cardiac output and oxygen uptake, and, in severe cases, convulsions, coma and death; which are most likely the direct effect of ammonia on the central nervous system (EPA 1986). Elevated ammonia, in vertebrates, displaces potassium ions and depolarizes neurons, thus causing activation of N-methyl-D-aspartate type glutamate receptor, which then leads to an influx of excessive calcium ions and results in cell death in the central nervous system (Randall and Tsui 2002). At low concentrations, ammonia can still affect fish by reducing hatching success, reducing growth rate and morphological development, as well as pathologic changes in tissues of gills, livers, and kidneys. Among macroinvertebrates, lethal concentrations of 0.011 to 0.036 mg/l $NH_3$ have been reported to cause a reduction in ciliary beating rate in the fingernail clam (*i.e. Musculium transversum*) (Russo 1985).

An important factor in ammonia toxicity to aquatic life is the interaction between ammonia and dissolved oxygen (Russo 1985). Increases in ammonia discharges frequently results in a reduction of oxygen levels in the receiving waters. This reduction can occur as the result of the increased oxygen demand of the ammonia as it is converted by natural microbial oxidation to nitrite and nitrate, the chemical and biological oxygen demand of other chemicals that may be discharged along with ammonia, or the decreased oxygen-carrying capacity of the receiving water caused by a high temperature discharge (resulting in a greater fraction of total ammonia present as $NH_3$) (Russo 1985). Ammonia can act synergistically with other chemicals, resulting in toxic effects to aquatic organisms. There has been some evidence that a combination of ammonia and copper is more toxic than either toxicant individually (Russo 1985). Similarly, the combination of both ammonia and zinc was greater than that of each chemical separately. The combination of ammonia and nitrate were reported to have additive toxicity, except when ammonia-to-nitrate ratios were very low.

**Phosphorus Compounds**

Natural phosphorus comes from rocks and natural phosphate deposits, which are released through weathering, leaching, erosion and mining (EPA 1999b). Phosphorus inputs accelerate eutrophication in most freshwater systems (Sharpley *et al.* 1998). Phosphorus is often the limiting element and its control is of prime importance in reducing the accelerated eutrophication of fresh waters. Concentrations of no greater than 0.03–0.04 mg/L of total phosphorus are found in streams that are relatively unaffected by human activity (McMahon and Harned 1998). A concentration below 0.1 mg/L is recommended to prevent algal blooms in streams (McMahon and Harned 1998).

Studies with radiotracers have demonstrated that orthophosphate is assimilated and cycled rapidly through the food web in streams (Meyer and Likens 1979). Diverse physical, chemical, and biological factors, including phosphorus sorption by sediments, water flow turbulence and velocity, uptake by vegetation, solute concentration, light, and temperature all help control phosphorus assimilation in streams (Reddy *et al.* 1996). Since phosphorus is transported in headwater streams from the catchments to rivers, any net retention of phosphorus by the stream ecosystem, or any transformations that alter its availability to the biological community will have biological repercussions in rivers (Meyer and Likens 1979). Enriched streams increase invertebrate biomass, thus can alter invertebrate communities (Bourassa and Cattaneo 1998). Phosphorus can cause proliferation on algal masses and in the worse cases eutrophication can cause blooms of cyanobacteria, thus can lead to livestock deaths and concerns about impacts on humans (Bowling and Baker 1996).

**<u>Sediment – Nutrient Interactions</u>**

A healthy sediment ecosystem might be defined as a satisfactorily functioning system that supports an active and diverse biological population (Maher *et al.* 1999). Sediments should not contain chemical constituents that impair the growth and function of their dependents. Sediments contain a mosaic of inorganic and organic materials such as rock and shell fragments, minerals, plant detritus and animal waste, along with anthropogenically derived substances. Sediment particles range from <63 mm (silt) to >1 mm (small rocks) in diameter, as defined by Maher *et al.* (1999). Fine sediment, in small amounts, can be suspended in most water bodies, while denser particles generally accumulate at the bottom. Bottom sediments may act both as a sink for contaminants and as a source that modifies the chemical composition of the overlying water, thus influencing water quality. Sediment contaminants are released by their dissolution into the sediment pore waters. This occurs when the concentration in the pore water is greater than the overlying water concentration. Benthic organisms can be exposed to containments in the pore water during feeding, ingestion of sediment particles, and dermal contact when burrowing.

Sediment can act as a sink where phosphorus may be stored and becomes a potential source to the overlying water and biota (Juracek 1998). Phosphorus in streams tends to be adsorbed on sediment particles. Phosphorus also readily sorbs to clay particles in the water column, which reduces the availability of uptake by algae, bacteria, and macrophytes (EPA 2000). Phosphorus in sediment is slow to recycle into the water column because exchanges across the sediment-water interface are regulated by mechanisms associated with mineral-water equilibria, sorption processes, redox iterations and the activities of bacteria, fungi, algae, and invertebrates.

Nutrients in sediment can be as significant an impairment as in the water column. A major route of nutrient exposure for many lotic species may be the direct transfer of chemicals from sediments. An increased number of tumors have been observed in many species of fish that have direct contact with sediments, due to nutrients (Chambers and Prepas 1994).

Sediment deposition constitutes a problem to invertebrates that are considered sediment intolerant species. For example if a stream's bank becomes unstable due to channelization or other human influences it becomes easily eroded, thus the input of fine sediment might cause a decline in species composition and might result in a change to species that are more tolerant of sediment (Hauer and Lamberti 1996).

Soil-derived phosphorus loading from runoff and erosion results in eutrophication of lakes and streams (Logan 2000). Since phosphorus is frequently the growth-limiting nutrient, phytoplankton in surface waters respond to the increase in phytoavailable phosphorus levels. Phytoplankton are able to utilize sediment bound phosphorus through desorption and dissolution processes, but respond most rapidly to dissolved orthophosphate. Bioassay or extraction techniques have demonstrated an average algal bioavailability of 20% to 40% for bound phosphorus (Logan 2000).

Sediment phosphorus can be a chronic source of phosphorus for aquatic biota (Sharpley *et al.* 1998). The characteristics of bottom sediments and the concentration in the water column control this long-term phosphorus retention (Reddy *et al.* 1996). Long-term retention is determined by sediment and site characteristics despite the fact that aquatic vegetation and periphyton provide short-term retention and facilitate long-term phosphorus storage through accumulation of organic matter.

## Geological Influences in Georgia

The state of Georgia has differing geology throughout the state. The Blue Ridge, Ridge and Valley, and Southwestern Appalachian ecoregions are dominated by bedrock and cobble streams. The Piedmont ecoregion has a mixture of cobble dominated streams.

The Southeastern Plains ecoregion streams have substrates dominated by a mix of sediment sizes. The Coastal Plains ecoregion streams have substrates that contain a mix of sediments, with some areas consisting mostly of silt, clay, and sand substrates. Above the Fall Line, a mix of igneous, metamorphic, and sedimentary materials dominates the area and below the Fall Line the area is characterized exclusively of sedimentary materials (refer to Appendix 1 for further information on geology of Georgia).

The macroinvertebrate community found in a stream is related to the stream's substrate, since large majorities of macroinvertebrates live in close association with the substrate (Allan 1995). Some organisms show some degree of substrate specialization, such as sand, stones, and moss. In stony substrates lithophilous species are found on gravel of all sizes. The larvae of the water penny (genus: *Psephenidae*) are mainly found underneath rocks and often under boulders in torrential flow. Due to instability and tight packing of sand grains, sand is a poor substrate for many macroinvertebrates. The tight packing of sand grains reduces the trapping of detritus and limits the availability of oxygen. However, psammophilous taxa (oligochaetes, early instar chironomids, nematodes and copepods) are specialists of this habitat. Burrowing taxa are sometimes specific to the particle size of substrate they inhabit. For example the mayflies *Ephemera danica* and *Ephemera simulans* burrow in gravel, but *Hexagenia limbata* does well in fine sediments.

With substrate stability and the presence of organic detritus, macroinvertebrate diversity and abundance increase (Allan 1995). Diversity and abundance also increases with increase of particle size, i.e. from sand to gravel substrates.

Likens and Borman (1974) study indicated that geology might play as great or greater role than land uses in determining the nutrient concentrations of unpolluted waters. In another study (Dillon and Kirchner 1975), it was determined that a larger amount of phosphorus is exported from watersheds draining rocks of sedimentary origin, than compared with those watersheds of igneous origin because sedimentary rocks contain larger amounts of phosphorus than igneous rocks. Phosphate and nitrate in stream water was substantially higher in sedimentary watersheds, than watersheds containing sandstone and shales as the main geologic types present (Thomas and Cruchfield 1974).

Particle size and types of rocks present are also important in the ability of a stream to process nutrients. Since the clay/silt particles, less than 63 micrometers, have a high specific surface area and because of surface coatings of iron and manganese oxides and natural organics, these particles are more likely to adsorb organic and trace metal contaminants (Maher *et al.* 1999). Thus clay and silt particles are most often associated with anthropogenic contaminants. These substrates allow phosphorus to be bound in the streams. The breakdown of rock and soil minerals introduces phosphorus, a mineral nutrient, into the biological components of the environment (EPA 2000). Streams containing phosphorus rich rocks, such as sedimentary or volcanic, can be enriched naturally, thus causing problems associated with increased phosphorus concentrations (EPA 1999b).

**Influence of Land Use**

The single greatest factor affecting aquatic resources is land use change (Hunsaker and Levine 1995). The physical and biological character of streams is affected when naturally vegetated landscapes are changed to urban or agricultural areas (Roth *et al.* 1996). These changes may result in habitat degradation, altered hydrology, and increased non-point source pollution by nutrient and sediment additions. Four significant land use changes in Georgia are due to agriculture, urbanization, silviculture, and removal of riparian vegetation.

The two most significant agricultural nonpoint sources are non-irrigated crop production and livestock, which account for 36% and 32% of nutrient loads, respectively (EPA 1992b). Livestock inputs are derived from feedlots, animal holdings or management areas, and pasture lands. Livestock grazing along stream banks can cause erosion, which alters habitat and allows more nutrient inputs into the stream. Studies of agricultural runoff from various agricultural activities have shown that the largest part of nutrients leaving croplands appears to be associated with sediment (Omernik *et al.* 1981). Additional pollutants generated by agricultural activities are suspended sediment from soil erosion, nutrients (*i.e.* nitrogen and phosphorus forms) from fertilizer and soil mineralization, bacteria and oxygen-demanding organic matter from animal production, and several kinds of pesticides (Brezonik *et al.* 1999).

Urbanization is creating an increasing number of impaired catchments throughout the United States and the world (Jones and Clark 1987). Urban sources represent 4% of the impaired river miles from nonpoint source pollution and a major cause is runoff, which contributes 70% of the urban runoff (EPA 1992a). Because of the complex nature of pollution sources (both surface runoff and waste discharge), it is difficult to access water quality in urban streams (Duda *et al.* 1982). Some potential sources of pollutants in urban streams are atmospheric fallout and washout of air pollutants, road surface and vehicular pollutants, street litter, animal wastes, and lawn and garden chemicals. Increased density of impervious surfaces in urban areas has resulted in the increase of direct runoff to catchments (Jones and Clark 1987). In some cases, phosphorus and nitrogen losses from urban watersheds may be two to ten times greater than those from forested watersheds. In highly insolated areas of urban streams, these increased nutrient concentrations can alter the aquatic food web in the stream. Jones and Clark (1987) concluded that changes in urbanized catchments resulted in changes in the taxonomic composition of stream insect communities. Tolerant taxa such as chironomid genera increased in abundance and intolerant taxa decreased or were eliminated. These urban stream communities contained a lower diversity of insects with genera representing fewer orders. Diptera and Trichoptera dominated these highly urbanized streams.

Nonpoint source contributions from silviculture depend upon site conditions and management activities (Currier *et al.* 1980). Silviculture may affect the hydrologic response of a stream, but this varies greatly from region to region. When logging activity, road building, fires or other unpredictable activities disturb forest environments; soil loss increases and becomes a major nonpoint pollutant. By removal of the canopy, streams may exhibit a change in temperature. Large temperature changes can affect the biota in the stream ecosystem (Currier *et al.* 1980). Increased temperatures can reduce dissolved oxygen after an area has been harvested for timber. Nutrient enrichment may also result from deforestation and lead to eutrophication, which affects the ultimate water quality of the stream.

The removal of riparian vegetation is likely to affect species diversity and composition of fish communities. The absence of woody debris causes a reduction in heterogeneity of depth, substrate, and current velocity, resulting in wide, shallow streams with little structural complexity and affording poor habitat for many aquatic species (Roth *et al.* 1996 and Gregory 1983). By removing native vegetation, the potential for overland and channel erosion is increased resulting in increased siltation of stream bottoms and obliterating the clean gravel surfaces, needed for spawning habitat by many species (Roth *et al.* 1996).

Nonpoint sources are also major contributors to estuary and coastal waters in the United States (EPA 1992a). These waters receive nonpoint pollution runoff from city streets, golf courses, suburban developments, parking lots, and farms located within coastal areas, as well as being the ultimate sink from the contributing catchment. Coastal ecosystems are of concern because these areas are highly productive, sensitive ecosystems, and provide habitat for commercial and recreation fish and shellfish, endangered birds, marine mammals, and other wildlife.

Historically, pollution control management in streams and rivers has focused on the increase of gross impacts from untreated domestic sewage and industrial discharges (Miltner and Rankin 1998). In the early 1970's, negative effects of nutrient enrichment on rivers and streams received attention with strategies to control the loading of primary nutrients. This was largely geared toward reducing eutrophication of lakes or estuaries. A program to control input of primary nutrients into lotic ecosystems as a means to maintain biotic integrity has not been widely implemented, even though a phosphorus-chlorophyll relationship, the nutrient limitations of periphyton, and bottom up control in streams has been demonstrated. In streams, light limitation, the frequency of flash flooding, grazing, rapid nutrient cycling, catchment area, input source and the variable nature of nutrient limitation in running water ecosystems have caused the control efforts to lag behind those for lakes (Miltner and Rankin 1998). Nutrient limitation in streams is most detectable at sites with near-pristine conditions, and suggests that relatively small increments in nutrient concentrations in streams should have measurable effects on biological communities (Newbold 1992).

In 1992, EPA summarized State estimates for 3.5 million miles of rivers and streams from 305 (b) reports (EPA 1994b). The rivers and streams ranged in size from the Mississippi River to small streams that only flow during wet conditions. Of the rivers and streams accessed, siltation was the leading cause of water quality impairment in 45% of the river miles accessed across the United States. Nutrient pollution was the second lead cause; with 37% of the river miles accessed being impaired (Richter *et al.* 1997). Agriculture practices contributed to the impairment of 72% of stream miles assessed. Municipal point sources (15%), urban runoff and storm sewer discharges (11%), resource extraction (11%), industrial point sources (7%), and silviculture (7%) also resulted in lotic ecosystem impairment. The United States Geological Survey (USGS) also reported that poor agricultural practices were the leading cause of nutrient enrichment in rivers and lakes of the United States (Richter *et al.* 1997) while municipal sources were additional impairments in many areas.

**Laws and Regulations**

The condition of surface waters in the Unites States is covered by a number of regulations regarding monitoring and control of identified pollutants, non-point sources of pollutants, the maximum load of both point and non-points pollutants, and the development of new and better monitoring strategies.

Section 101(a) of the Clean Water Act (CWA) states that the primary objective of the Act is to restore and maintain the chemical, physical, and biological integrity of the nation's waters. Section 305 (b) of the CWA requires states to regularly report the condition of their waters (EPA 1997). This is accomplished by conducting ambient water monitoring to determine changes in water quality over time, designating the sources of water quality problems, and determining if pollution control programs are working.

A point source is defined as any discernible, confined, and discrete conveyance, including but not limited to any pipe, ditch, channel, tunnel, conduit, well, discrete fissure, container, rolling stock, concentrated animal feeding operation, or vessel or other floating craft, from which pollutants are or may be discharged (EPA 1992a). According to section 502(14) of the CWA, nonpoint sources are defined as sources of water pollution that do not meet the legal definition of a "point source". Section 319 of the 1987 CWA indicates that states are "(1) required to conduct statewide assessments of their waters to identify those that were either impaired (did not fully support state water quality standards) or threatened (presently meet water quality standards but are likely not to continue to meet water quality standards fully) because of Nonpoint Source Programs (NPS's); (2) required to develop NPS management programs to address the impaired or threatened waters identified in their nonpoint assessments; and (3) entitled to receive annual grants from the EPA to assist them in implementing their NPS management programs once the EPA has approved the assessments and programs" (EPA 1997).

Total maximum daily loads (TMDLs) allocate allowable loads among different pollutant sources (both point- and nonpoint-) so that appropriate control actions can be taken, water quality standards achieved, and human health and aquatic resources protected (EPA 1994a). TMDLs are a significant issue throughout the nation and it is important to understand the relationship between nonpoint sources and biological assessments and criteria. The total maximum daily load (TMDL) program is designed to identify those waters that do not meet non-point source water quality standards, required by section 303 (d) of CWA (EPA 1997). States are required to develop TMDL's for each chemical parameter and a priority ranking for those waters not meeting water quality standards. The TMDL program helps to identify and establish controls to reduce nonpoint source pollution.

In 1990, approximately 37% of the United States River miles that were tested still did not fully support the uses designated by the states (Puckett 1994). The main reason the rivers were still polluted in 1990 was that the focus had been on point sources and not on nonpoint-source pollution. These problems caused the EPA to propose NPS guidelines for streams and rivers. Chemical-specific, *in situ* and toxicity–based water quality criteria has been widely developed (EPA 1985a,b). However, the use of biological criteria (biocriteria) has been largely ignored or is of recent consideration. The EPA has recommended monitoring plans that emphasize the acceleration of the development of biological sampling as a component of surface water programs (EPA 1987a,b).

Biocriteria for impacted waters are to be identified and evaluated for nonpoint sources of pollution as provided by state Water Quality Standards.

Bioassessments are ideally suited to identify aquatic life use impairments and, thus, the identification of pollutants. Biological monitoring of streams is important to the determination of the extent of anthropogenic perturbation. If only chemical and physical samples are analyzed, only the conditions at the particular time the sample was collected are considered (Hauer and Lamberti 1996).

One major component of biological monitoring is the examination of the community structure of aquatic macroinvertebrates to determine the level of impairment in streams. Aquatic macroinvertebrates are good indicators of contamination because they are resident monitors of pollution and are less able to migrate from the impairment. Since macroinvertebrates must persist in the contaminant field, they indicate past conditions as well as current conditions (Hauer and Lamberti 1996).

In a lotic ecosystem, invertebrate communities consist of several hundred species from numerous phyla including arthropods, mollusks, annelids, nematodes, and platyhelminthes. Benthic macroinvertebrates are most often recommended for biological monitoring and mainly include aquatic insects, mites, molluscs, crustaceans, and annelids (Hauer and Lamberti 1996). Some of the advantages of using benthic macroinvertebrates are: large number of species offering a spectrum of responses to perturbations; the sedentary nature of many species allowing spatial analysis of disturbance effects; well developed qualitative sampling and analysis and simple inexpensive equipment requirements; responses of many common species to different types of pollution have been established; macroinvertebrates are well suited to experimental studies of perturbation; and the taxonomy of many groups is well known and identification keys are available (Hauer and Lamberti 1996). One advantage of sampling macroinvertebrates is their generally restricted mobility and often multi-year life cycles, which allows the effects of both chemical and physical perturbations over time to be integrated. Changes in nutrient enrichment, toxic contamination and morphological and habitat changes caused by erosion and sedimentation in streams will be reflected by changes of the macroinvertebrate community structure and function.

The Rapid Bioassesment Protocol (RBP) is the method that is recommended for analysis of biocriteria in the state of Georgia (EPA 1999b). Once reference and impaired sites are characterized by land use, physical, and chemical data; a macroinvertebrate multimetric index of impairment for each ecoregion and subecoregion can be developed. Metrics are defined as "calculated terms or enumerated values representing some aspects of biological assemblage structure, function, or other measurable characteristic that change in predictable ways with increased human influence" (Barbour *et al.* 1999). The multimetric indices make biological data more understandable and are an integral part of the state water quality management process (Yoder and Rankin 1998). Indeed, Barbour *et al.* (1999) predicted that the multimetric indices should be able to identify sources and intensities of impairments, both chemical and physical.

Ecoregions are intended to provide a spatial framework for ecosystem assessment, research, inventory, monitoring, and management (Omernik and Bailey 1997). Ecoregion classification divides the landscape into variously sized ecosystem units, which have significance, both for development of resources and for conservation. It might be possible to predict the behavior of an unvisited stream, by observing the behavior of the different kinds of systems within a region. By grouping basins into ecoregions having similar geology, topography, soil, and vegetation, a comparison of streams of similar size across a relatively homogeneous area can be provided (Bryce and Clarke 1996). The ecoregions are further subdivided into subecoregions in the same manner as the ecoregion level. Subecoregionalization allows streams to be compared with other streams that are of similar size across a relatively homogeneous area. Comparing similarities and differences between ecoregions and/or subecoregions can be helpful in maintaining and determining the quality of streams as well as determining sources of impairment.

**Objectives**

My research was part of a multi-phased project to develop biological criteria for wadeable streams and rivers in the state, based on scientifically defensible set of standards; a study initiated by Columbus State University in July 2000 (Gore *et al.* 2004). The ultimate goal of the study is to create a numerical index of impairment for all wadeable streams in the state of Georgia and to recommend methods for incorporating this index into the state regulatory structure. The assessment of baseline biological and chemical conditions in each of the ecoregions of the state was the initial step for biological criteria development. Georgia's ecoregions were refined in February 2001 through the National Resources Conservation Service (NRCS) (Griffith *et al.* 2001). Ecoregion delineation categorized the state based on logical units of similar geology, physiography, soils, vegetation, land use/land cover, and water quality (Gore 2000).

My research goals were to determine: (1) if there was a difference between impaired and reference site nutrient concentrations: (2) if there was a difference in nutrient concentrations and related impairment between ecoregions and/or subecoregions; and (3) if the Rapid Bioassessment Protocol (RBP) could detect different nutrient concentrations and related impairment in stream ecosystems; specifically does nutrients affect the distribution of macroinvertebrates as reflected by macroinvertebrate metric scores. The primary objective of my research was to determine if there was a distinctive nutrient characteristic for wadeable streams in each ecoregion and possibly subecoregion in wadeable streams in Georgia.

## MATERIALS AND METHODS

### Site Selection

The Rapid Bioassessment Protocol (RBP) (Barbour *et al.* 1999) was followed for sampling and analysis in this study. The Rapid Bioassessment Protocol is a guide for conducting cost-effective biological assessments of lotic systems. The RBP is an integrated assessment, which compares habitat, water quality and biological measures to define a reference condition.

A listing of potential reference and impaired streams for each of the ecoregions and subecoregions was determined for the state of Georgia by using Geographical Information Systems (GIS) (Olson 2002). Since little historical data were available for the State of Georgia, the term "reference condition" refers to the least impaired stream in an ecoregion or subecoregion. Stream sites and catchments were ranked, based upon the criteria in table 1, other land use data, GIS, and groundtruthing. Sites were visited prior to field season, habitat assessments were conducted, and land use inspected for noticeable changes, for groundtruthing. The highest-ranking sites were selected as candidate reference sites. Impaired sites were selected and ranked as sub-optimal (near reference condition), medium, and high land use stress. Five reference sites and five impaired sites, with different intensities of impairment, were sampled, when possible, for each subecoregion. In order to obtain a more representative sample, additional impaired sites were also sampled in larger subecoregions.

For Level IV evaluation, Georgia was divided into six ecoregions and twenty-five subecoregions (Figure 1 and Table 2). Some of the subecoregions were combined together because of the small size and small number of streams available to sample.

| Table 1: Criteria for Reference Conditions (Gore 2000) | | | |
|---|---|---|---|
| **Step** | **Criteria** | **Action** | **Means of Evaluation** |
| 1 | % Urban land use | Screen out sites with > 15% | |
| 2 | % Agriculture | Screen out sites with > 50% | GIS evaluation of MRLC data |
| 3 | Road Density | Select lowest Density | Evaluation of DOT GIS data |
| 4 | Minimum Riparian Zone | Screen out sites with < 15m width | GIS evaluation of MRLC |
| 5 | Channel Alteration | Screen out sites with any alteration | Evaluation of map/aerial photo |
| 6 | Impoundments | Select lowest Density | Evaluation of USGS lake data |
| 7 | Point Source Discharges | Screen out sites with any discharges | EPA NPDES permits |
| 8 | % Silviculture | Select lowest Density | GIS evaluation of MRLC data |

Thus, six different ecoregions and twenty-three subecoregions were analyzed (For complete descriptions of the ecoregions and subecoregions, refer to Appendix 1.). Sampling was conducted over three seasons (or index periods): (1) September 2000 to February 2001, (2) September 2001 to February 2002, and (3) September 2002 to February 2003. For this research, 106 reference sites and 119 impaired sites were analyzed (Table 3). Physical parameters and habitat assessments were also analyzed and evaluated at each stream (Gore *et al.* 2004). For each sample, nutrients in the form of nitrite, nitrate-nitrite, ammonia and total phosphorous as well as macroinvertebrate composition were analyzed. Of those sites, 182 were analyzed for total phosphorus in sediment.

The sampling was performed in a 100-meter stream segment, which had no major tributaries in the assessment area (Gore 2000). The area of study was located at least 100 meters upstream from any road or bridge crossing, to minimize the impacts of velocity, depth, and overall habitat alterations from the structure.

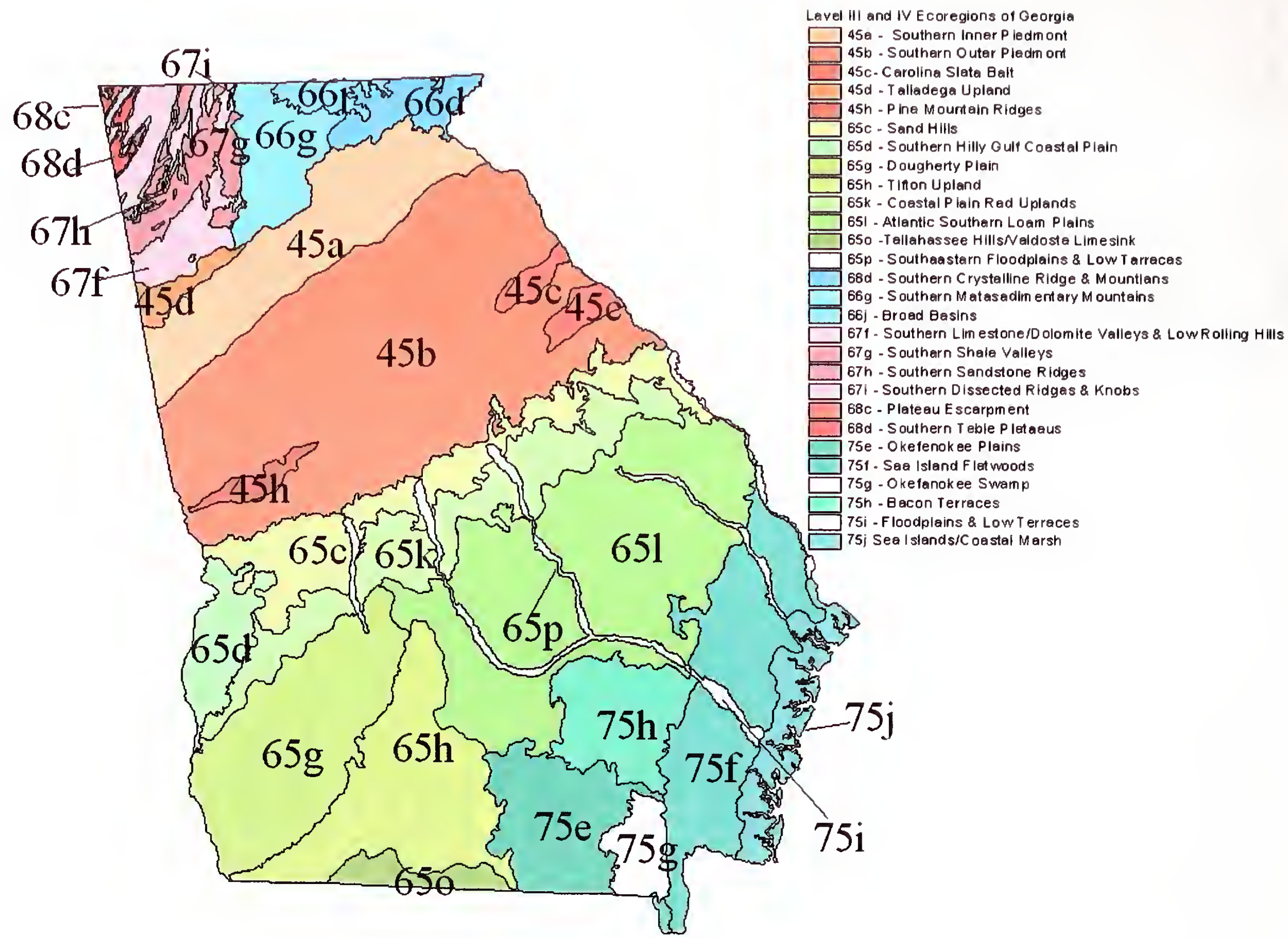


Figure 1: Level III and IV Ecoregions Map for the State of Georgia (Griffith *et al.* 2001)

**Table 2: List of Ecoregions and Subecoregions for the State of Georgia (Gore 2000 and Griffith *et al.* 2001)**

| Ecoregion # | Ecoregion Type | Subecoregion # | Subecoregion Type |
|---|---|---|---|
| 45 | Piedmont | a | Southern Inner Piedmont |
| | | b | Southern Outer Piedmont |
| | | c | Carolina Slate Belt |
| | | d | Talladega Upland |
| | | h | Pine Mountain Ridge |
| 65 | Southeastern Plains | c | Sand Hills |
| | | d | Southern Hilly Gulf Coastal Plain |
| | | g | Dougherty Plain |
| | | h | Tifton Upland |
| | | k | Coastal Plain Red Uplands |
| | | l | Atlantic Southern Loam Plains |
| | | o | Tallahassee Hills/ Valdosta Limesink |
| 66 | Blue Ridge | d | Southern Crystalline Ridges and Mountains |
| | | g | Southern Metasedimentary Mountains |
| | | j | Broad Basins |
| 67 | Ridge and Valley | f | Southern Limestone /Dolomite Valleys and Low Rolling Hills |
| | | g | Southern Shale Valleys |
| | | h | Southern Sandstone Ridges |
| | | i | Southern Dissected Ridges and Knobs |
| 68 | Southwestern Appalachians | c | Plateau Escarpment |
| | | d | Southern Table Plateaus |
| 75 | Southern Coastal Plains | e | Okefenokee Plains |
| | | f | Sea Island Flatwoods |
| | | h | Bacon Terraces |
| | | j | Sea Islands/Coastal Marsh |

| Table 3: Number of Reference and Impaired Sites per Subecoregion Analyzed | | | |
|---|---|---|---|
| **Subecoregion** | **Subecoregion Name** | **Reference Sites** | **Impaired Sites** |
| 45a | Southern Inner Piedmont | 5 | 5 |
| 45b | Southern Outer Piedmont | 5 | 6 |
| 45c | Carolina Slate Belt | 5 | 5 |
| 45d | Talladega Upland | 5 | 5 |
| 45h | Pine Mountain Ridge | 5 | 5 |
| 65c | Sand Hills | 5 | 7 |
| 65d | Southern Hilly Gulf Coastal Plain | 5 | 5 |
| 65g | Dougherty Plain | 5 | 10 |
| 65h | Tifton Upland | 5 | 5 |
| 65k | Coastal Plain Red Uplands | 5 | 5 |
| 65l | Atlantic Southern Loam Plains | 5 | 5 |
| 65o | Tallahassee Hills/Valdosta Limesink | 4 | 5 |
| 66d | Southern Crystalline Ridges and Mountains | 5 | 5 |
| 66g | Southern Metasedimentary Mountains | 5 | 7 |
| 66j | Broad Basins | 5 | 5 |
| 67f&I | Southern Limestone /Dolomite Valleys and Low Rolling Hills & Southern Dissected Ridges and Knobs | 5 | 5 |
| 67g | Southern Shale Valleys | 5 | 5 |
| 67h | Southern Sandstone Ridges | 4 | 2 |
| 68c&d | Plateau Escarpment & Southern Table Plateaus | 4 | 5 |
| 75e | Okefenokee Plains | 5 | 5 |
| 75f | Sea Island Flatwoods | 4 | 6 |
| 75h | Bacon Terraces | 5 | 6 |
| 75j | Sea Islands/Coastal Marsh | 11 | 10 |
| Total | | 106 | 119 |

## **Habitat Assessment**

A habitat assessment was conducted throughout the 100-meter reach, prior to chemical and biological sampling (Gore 2000). Each parameter was assessed on a zero- to twenty-point subjective scale. In high gradient streams, epifaunal substrate/available cover, embeddedness, velocity/depth regime, sediment deposition, channel flow status, channel alternation, frequency of riffles (or bends), bank stability, vegetation protection, and riparian vegetative zone width were similarly assessed (refer to Appendix 2 for Habitat Assessment form). In low gradient streams, epifaunal substrate/available cover, pool variability, pool substrate characterization, sediment deposition, channel flow status, channel alteration, channel sinuosity, bank stability, vegetation protection, and riparian vegetative zone width were subjectively assessed (refer to Appendix 2 for Habitat Assessment form).

**Benthic Macroinvertebrates**

A single benthic macroinvertebrate sample was collected from each stream. The sampling technique for collecting macroinvertebrates was the 20-jab method using a D-frame net with a mesh size of 595 to 600 microns (Gore 2000). The jabs were taken in various habitats depending on whether the stream was classified as a high or low gradient stream (Table 4). Sampling was conducted from downstream to upstream by jabbing the D-frame net into productive and stable habitats 20 times. "Jabbing" is forcefully thrusting the net into a productive habitat for a linear distance of one meter (Gore 2000). If habitats were not present, then a strict reallocation was done. The priority list for reallocation of unavailable habitat types (Table 4) was followed in sequence until all unallocated habitat jabs were reallocated. Macrophytes were not used in reallocation. The samples were preserved with 95% ethyl alcohol and properly labeled in the field; then returned to the laboratory.

A 200-organism subsample was randomly selected in the laboratory. The samples were separated onto a tray that was divided into grids (Caton 1991). Grid squares were

**Table 4: Macroinvertebrate Habitat Types Sampled using D-frame Net (Gore 2000)**

| **HIGH GRADIENT STREAMS** | | |
|---|---|---|
| **Priority** | **Habitat Type** | **Number of Jabs** |
| 1 | Fast Riffle | 3 |
| 2 | Slow Riffle | 3 |
| 3 | Snags | 5 |
| 4 | Undercut Banks/Rootwads | 3 |
| 5 | Leaf Packs (handfuls) | 3 |
| 6 | Sand | 3 |
| 0 | Macrophytes (when present) | 3 |
| **LOW GRADIENT STREAMS** | | |
| **Priority** | **Habitat Type** | **Number of Jabs** |
| 1 | Woody debris/Snags | 8 |
| 2 | Undercut Banks/Rootwads | 6 |
| 3 | Leaf Packs (handfuls) | 3 |
| 4 | Sand | 3 |
| 0 | Macrophytes (when present) | 3 |

chosen at random and then the contents were sorted. When 200 organisms had been collected from the squares, the subsample was complete. At least four squares had to be sorted in order to obtain a target number of 200 (+/- 40) individuals. If the four squares contained more than 240 individuals, the individuals were returned to the original composite and that sample was subsampled again. All selected squares were completely sorted. When there were not enough individuals in the first four squares, additional squares were sampled, at random, until the target of 200 (+/- 40) individuals was reached. Ten percent of the sort residues were selected for quality control (additional sorting), to insure macroinvertebrates were not missed.

Macroinvertebrates were identified to the lowest possible taxonomic level, which was usually to genus, and species when possible (refer to Appendix 3 for taxonomic keys list). The life-stage (adult, larval, or nymphal) and number of individuals, for all taxa, were recorded for each site. The larval or nymphal stages were identified for all taxonomic groups. Adult stages of Coleopterans, Hemipterans, Crustaceans, Mollusks, and Gastropodas were identified, as well. Pupae, emergent and damaged individuals were recorded, but not identified. Only individuals that could be identified to family were counted in the total, with the exceptions of Oligochaeta, Polychaeta, Nemata, Hirudina, Cladocera, Ostrocoda, and Neoloricata. CMCP-10©, a high viscosity mounting and clearing medium, was used to mount larval Chironomidae prior to identification.

## Chemical Parameters

A Hydrolab H2O® Water Quality Multiprobe with a Water Quality Multiprobe/Scout 2 Display unit was used for *in-situ* chemical analysis. Water temperature, air temperature, pH, conductivity, dissolved oxygen levels, depth, turbidity, and percent dissolved oxygen were recorded for each site.

Polyethylene bottles were used for water sample collection and were tested for contamination using de-ionized water prior to sampling. Water samples were then collected in those uncontaminated polyethylene bottles by the grab method at the beginning of the 100 meter reach, for each stream (refer to Appendix 4 for procedure) (Gore 2000). Nitrite, nitrate-nitrite, ammonia, and total phosphorous water samples were preserved with sulfuric acid, since samples could not be analyzed immediately (refer to Appendix 4 for procedure). The samples were properly labeled and transported in a cooler to the laboratory. The samples were stored at 4° C until they could be analyzed for nitrate-nitrite, nitrite, ammonia, and total phosphorus (Table 5). Three replicates for each stream and parameter were analyzed. For quality control, de-ionized water blanks were

| Table 5: Handling and Preservation Specifications for Chemical Parameters (Gore 2000) | | | | |
|---|---|---|---|---|
| **Parameter** | **Sampling Container** | **Sample Volume** | **Preservation** | **Maximum Storage Time** |
| Nitrate-Nitrite | Plastic | 1 L | < 4° C or frozen | 4 days |
| Nitrite | Plastic | 1 L | < 4° C or frozen | 4 days |
| Ammonia | Plastic | 1 L | freeze (-20°C) or pH < 2 with $H_2SO_4$ | 28 days |
| Total Phosphorous | Plastic | 1 L | pH < 2 with $H_2SO_4$ | 28 days |

also analyzed to assure that the glassware and procedure were not causing contamination. These chemical parameters were analyzed using EPA approved methods (Table 6).

| Table 6: Nutrient Analysis Procedures (EPA 1999a) | | | |
|---|---|---|---|
| **Parameter** | **Method** | **Procedure** | **Detection Limit (mg/L)** |
| Nitrate-Nitrite | EPA 353.2 | Spectrophotometric, Cadmium Reduction | 0.01 to 1.0 |
| Nitrite | EPA 354.1 | Spectrophotometric | 0.01 to 1.0 |
| Total Phosphorous | EPA 365.3 | Colorimetric, Ascorbic Acid | 0.01 to 1.2 |
| Ammonia | EPA 350.3 | Ion Selective Electrode | 0.03 to 1400 |

EPA method 353.3 (refer to Appendix 5 for complete procedure) was used for analysis of nitrogen, nitrate-nitrite determination by cadmium column reduction (EPA 1999a). The water samples were passed through a column containing granulated copper-cadmium to reduce nitrate to nitrite. Nitrogen, nitrite was analyzed by EPA method 354.1 (refer to Appendix 6 for complete procedure). The nitrite was determined by diazotizing with sulfanilamide and coupling with N-(1-naphthyl)-ethylenediamine dihydrochloride to form a highly colored azo dye, which was measured colorimetrically (EPA 1999a).

EPA method 350.3 was used for analyzing nitrogen, ammonia (refer to Appendix 7 for complete procedure). This method determined ammonia potentiometrically using an ion selective ammonia electrode (EPA 1999a). The probe uses a gas-permeable membrane to measure the ammonia concentration.

Total phosphorous was analyzed using EPA method 365.3 (refer to Appendix 8 for complete procedure). In this method, an acid medium with dilute solutions of phosphorus reacted with ammonium molybdate and antimony potassium tartrate to form an antimony-phospho-molybdate complex (EPA 1999a). Ascorbic acid was used as a complex and the complex was reduced to an intensely blue-color. The color was proportional to the phosphorus concentration.

**Sediment**

Sediment samples were collected with a polypropylene scoop throughout the 100 meter reach and transferred to plastic bags. Each scoop was collected from the top five centimeters of substrate. Samples were taken systematically; for every tenth pebble counted during the Wolman Pebble count (described below), a sediment scoop was collected. In areas of large boulders and/or bedrock, the scoops were collected randomly. If ten scoops could not be taken (where bedrock substrates dominated), the maximum number possible was collected. Areas of benthic algal growth were avoided while collecting the sample. The sediment was stored at 4° C, to reduce microbial activity. During analysis, the sediment was air dried and then sieved through a number ten sieve

(2mm diameter). Sieving removed most of the organic matter, larger pebbles, and organisms that were collected in the sample.

Total phosphorus was analyzed for each sediment sample, employing the Mehlich No. 3 method (Soil and Plant Analysis Council, Inc. 1992). This method was designed to be applicable across a wide range of soil properties ranging in reaction from acid to basic. The Mehlich-3 extracting solution was added to the samples and then samples were put on a reciprocating shaker (refer to Appendix 9 for complete procedure). The sample extracts were analyzed with a Shimadzu spectrophotometer (UV – visible recording spectrophotometer, UV160U). For quality control, de-ionized water blanks were analyzed to make sure the glassware did not cause interference or contamination.

**Physical parameters**

After chemical sampling, and during biological sampling, cross-sectional area, mean velocity, and substrate composition, using the Wolman Pebble count were recorded. A 100 meter reach was marked off at the 0 m, 50 m, 100 m points. At either the zero- or 50-meter mark, cross-sectional profile and mean velocity were recorded (refer to Appendix 10 for field data sheet). The time of travel of a half-water-filled tennis ball over a certain distance in the reach was recorded as the typical current velocity.

A Modified Wolman Pebble Count was used to assess substrate composition (Rosgen 1996 and Harelson *et al.* 1994). The Wolman Pebble count divides substrate types into six categories: silt/clay, sand, gravel, cobble, boulder, and bedrock (refer to Appendix 11 for field data sheet). The sand, gravel, cobble, and boulder categories are further broken down by size increments. The pebble count was performed along a series of diagonal transects, moving from downstream to upstream. The width of the stream channel determined the number of transects that were created. Each time a person took a step; the sand, pebble, or rock directly in front of the foot was measured and recorded (refer to Appendix 11 for field data sheet and complete procedure). For sand grains, a sand card (McCollough 1984) was used to compare textures and, for pebbles and rocks, calipers were used to determine the grain size.

**Preliminary Site Analysis**

Before metric analyses were completed, land use, physical parameters, habitat assessments, and water chemistry data were used to determine if candidate reference sites should be considered as reference sites for developing the multi-metric index (Hughes 2005). The candidate reference stream must have met the preliminary reference condition criteria, which was established for each ecoregion or subecoregion level, to have been considered for use in defining the biological condition (metrics) of the reference stream. A few reference sites were excluded for various reasons (Table 7).

**Table 7: Reference Sites Not Used to Develop Index (Hughes 2005)**

| Reference Sites Removed | Reason |
|---|---|
| 45c-18 | Phosphorus value > 2SD from mean |
| 45d-8 | Crosses ecoregion 67 |
| 65g-82 | 100% silt, Dissolved Oxygen < 2.0 mg/l |
| 65g-83 | Channelized |
| 67g-2 | Channelized |
| 75j-29 | Channelized |

Metrics attempt to quantify aspects of the structure and function of the benthic community and, in turn, ambient water quality conditions (Hughes 2005). For each of the ecoregions and subecoregions in Georgia, multi-metric indices were developed (Hughes 2005 and Gore *et al.* 2004). Metrics were divided into five major groups: taxonomic richness, composition, tolerance/intolerance, functional feeding group, and habit (Table 8). Increasing taxonomic richness correlates with increasing water quality and stream health (Hughes 2005 and Gore *et al.* 2004). Composition metrics indicate the number or percentage of specific taxa and can serve as a tolerance/intolerance metric as certain families or genera have an established tolerance to organic pollution (Gerritsen *et al.* 2000). Tolerance metrics are based upon tolerance classes, average tolerance values, and sometimes-weighted averages. Not all taxa have been assigned tolerance values, habits, or functional feeding groups; thus based on current information, a protocol was developed to assign values to those unknown taxa (refer to Appendix 12 for Tolerance Value Protocol).

**Table 8: Five Major Groups of Metrics (Hughes 2005 & Barbour *et al.* 1999)**

| Metric Group | Example of Metrics |
|---|---|
| Taxonomic richness | Total Taxa<br>EPT Taxa<br>Diptera Taxa |
| Composition | % Chironomus sp &<br>Cricotopus sp./Total Chironomidae |
| Tolerance/Intolerance | Hilsenhoff Biotic Index (Hilsenhoff 1987)<br>North Carolina Biotic Index (Lenat 1993) |
| Functional Feeding Group | Scraper Taxa<br>Predator %<br>Shredder Taxa |
| Habit | Swimmer Taxa<br>% Sprawlers |

Specialized feeding groups are more or less sensitive to disturbance and pollution than generalized feeders. Habit describes the movement and position of benthic organisms as they forage within the benthic community.

The Ecological Data Application System (EDAS) version 3.3.2k was used for metric analysis (EDAS 2001). Metric values were exported to Microsoft Excel© for each site at ecoregion and subecoregion level. Within STATISTICA (StatSoft, Inc. 2003), a Pearson-product-moment correlation analyses revealed those metrics that were redundant (Hughes 2005). Those metrics that did not differentiate reference from impaired streams were removed from consideration for the multi-metric index (Hughes 2005). Redundant metrics were also removed from consideration. Individual metrics that best differentiated reference and impaired streams from each metric category were used in the creation of the final index.

Candidate metrics were standardized according to their response to stress (refer to Appendix 13 for metric stress response). The standardized metrics from each metric category were combined to make candidate indices for each ecoregion and subecoregion level. The candidate indices contained five to seven metrics on 100-point scale. The candidate indices were, then, averaged to determine an index score for each site at both ecoregion and subecoregion level (Hughes 2005).

To determine which indices were best for both the ecoregion and subecoregion level, discrimination efficiency was calculated and box and whisker plot created for purpose of comparison. The discrimination efficiency is the number of impaired streams that meet the criteria (criteria depends upon metric stress response) divided by the total number of impaired streams (Hughes 2005). The box and whisker plots were used to determine how similar a group of reference or impaired sites are to each other and the degree of separation between the two groups.

**Analysis**

The Mann-Whitney U-Test, cluster analysis, Spearman's Rank Correlation Coefficient test and metric analysis were used to determine if the RBP could detect changes in nutrient characteristics and/or if nutrients were correlated with the distribution of macroinvertebrates. The Man-Whitney U-Test is a nonparametric rank sum test, an alternative to the student t-test (Glantz 1992). Spearman rank correlation coefficient, a nonparametric test, is a test that determines the probability associated with the occurrence of a correlation, which the null hypothesis states the variables are unrelated in population (Siegel 1956). STATISTICA software was used to facilitate analysis of the difference in nutrient concentrations between reference and impaired sites and correlations between nutrient concentrations and index scores.

Cluster analysis (using Ward's method (Krebs 1998) with Euclidean distances) was used to determine differences in benthic macroinvertebrate communities in reference and impaired streams. If the reference and impaired condition clustered separately, a significant correlation with sources of impairment might be possible.

**RESULTS**

**Preliminary Analysis**

For each ecoregion and subecoregion, the best metrics that discriminated between reference and impaired sites were determined (Hughes 2005 and Gore *et al.* 2004). These metrics varied between ecoregions and among subecoregions. The metrics determined for each index are contained in appendix 14. Index scores were calculated on a 100-point scale for each ecoregion and subecoregion and varied throughout the state. Metric values for the various ecoregions and subecoregions are listed in appendices 15 through 42.

The pebble counts indicated the variability of substrate particle sizes throughout the state and are summarized in table 9. North of the Fall Line a mix of gravel, cobble, boulder and bedrock dominated substrates. The impaired sites north of the Fall Line tend to have an increase in sand and silt as compared with the reference sites. Substrates south of the Fall Line are dominated by sand and silt/clay. The coastal plain ecoregion was heavily dominated by silt and clay particles.

**Table 9: Particle Size Ranges (percentage) for Ecoregions**

| | Blue Ridge - 66 | | Ridge and Valley - 67 | |
|---|---|---|---|---|
| **Particle Size** | **Impaired Sites** | **Reference Sites** | **Impaired Sites** | **Reference Sites** |
| Silt/Clay | 0-19 | 0-12 | 0-75 | 0-14 |
| Sand | 5-60 | 0-28 | 0-33 | 2-14 |
| Gravel | 10-65 | 13-69 | 5-97 | 28-86 |
| Cobble | 6-58 | 4-54 | 0-46 | 0-40 |
| Boulder | 0-27 | 0-33 | 0-24 | 0-20 |
| Bedrock | 0-19 | 0-8 | 0-26 | 0-19 |

| Table 9 (Continued): Particle Size Ranges (percentage) for Ecoregions | | | | |
|---|---|---|---|---|
| | **Southwestern Appalachians - 68** | | **Piedmont - 45** | |
| **Particle Size** | **Impaired Sites** | **Reference Sites** | **Impaired Sites** | **Reference Sites** |
| Silt/Clay | 0-34 | 0 | 0-19 | 0-42 |
| Sand | 5-78 | 4-22 | 10-90 | 3-89 |
| Gravel | 0-44 | 8-22 | 2-65 | 2-79 |
| Cobble | 0-42 | 18-58 | 0-36 | 0-52 |
| Boulder | 13-49 | 13-94 | 0-7 | 0-33 |
| Bedrock | 1-14 | 1-14 | 0-12 | 0-21 |
| | **Southeastern Plains - 65** | | **Southern Coastal Plains - 75** | |
| **Particle Size** | **Impaired Sites** | **Reference Sites** | **Impaired Sites** | **Reference Sites** |
| Silt/Clay | 0-96 | 0-100 | 0-100 | 0-100 |
| Sand | 4-99 | 0-100 | 0-100 | 0-100 |
| Gravel | 0-73 | 0-30 | 0-18 | 0-4 |
| Cobble | 0-12 | 0-16 | 0-2 | 0 |
| Boulder | 0-5 | 0-2 | 0 | 0 |
| Bedrock | 0-30 | 0-2 | 0 | 0 |

Land use was determined from the 1998 national land cover data, for most sites (Institute of Ecology 2001 and Gore *et al.* 2004). For those streams that crossed into surrounding states, land use was determined from the 1994 national land cover data set (Vogelmann *et al.* 2001 and United States Geological Survey 1999). As might be expected, land use varied across ecoregions and is summarized in table 10.

| Table 10: Land use Ranges (percentage) for Ecoregion Level | | | | |
|---|---|---|---|---|
| **Land use %** | **Blue Ridge - 66** | | **Ridge and Valley - 67** | |
| | **Reference Sites** | **Impaired Sites** | **Reference Sites** | **Impaired Sites** |
| % Natural | 33.07 - 98.66 | 29.85 - 94.72 | 65.45 - 97.12 | 27.27 - 98.13 |
| % Urban | 1.34 - 8.96 | 1.03 - 28.66 | 2.41 - 9.75 | 0.00 - 53.74 |
| % Forested | 28.15 - 96.41 | 32.89 - 98.66 | 64.63 - 95.32 | 25.26 - 94.13 |
| % Barren | 0.00 - 12.00 | 0.00 - 4.00 | 0.38 - 17.83 | 0.0 - 9.39 |
| % Agricultural Total | 0.0 - 11.68 | 0.46 - 25.59 | 0.07 - 30.39 | 0.01 - 46.94 |
| % Agricultural Pasture | 0.30 - 25.59 | 0.0 - 11.68 | 0.07 - 30.40 | 0.01 - 46.95 |
| % Agriculture Row Crop | 0.00 - 0.00 | 0.0 - 9.05 | 0.00 - 0.00 | 0.00 - 0.00 |

| Table 10 (Continued): Land use Ranges (percentage) for Ecoregion Level | | | | |
|---|---|---|---|---|
| **Land use %** | **Southwestern Appalachians - 68** | | **Piedmont - 45** | |
| | **Reference Sites** | **Impaired Sites** | **Reference Sites** | **Impaired Sites** |
| % Natural | 67.65 - 80.76 | 0.94 - 83.82 | 29.82 - 97.12 | 16.89 - 92.79 |
| % Urban | 3.02 - 9.66 | 0.30 - 8.41 | 0.00 - 17.46 | 4.50 - 81.12 |
| % Forested | 65.01 - 79.38 | 0.41 - 80.28 | 25.91 - 91.17 | 14.78 - 89.11 |
| % Barren | 0.63 - 2.64 | 0.11 - 3.27 | 0.56 - 18.70 | 0.015 - 13.40 |
| % Agricultural Total | 9.58 - 25.17 | 0.00 - 43.37 | 0.0 - 29.83 | 0.34 - 61.43 |
| % Agricultural Pasture | 9.58 - 25.17 | 0.0 - 43.37 | 0.0 - 27.43 | 0.32 - 52.35 |
| % Agriculture Row Crop | 0.00 - 0.00 | 0.00 - 0.00 | 0.00 - 2.41 | 0.00 - 10.03 |
| **Land use %** | **Southeastern Plains - 65** | | **Southern Coastal Plains - 75** | |
| | **Reference Sites** | **Impaired Sites** | **Reference Sites** | **Impaired Sites** |
| % Natural | 14.05 - 94.76 | 13.85 - 86.92 | 64.55 - 95.90 | 14.67 - 91.38 |
| % Urban | 1.48 - 11.80 | 1.10 - 81.92 | 4.10 - 20.53 | 3.92 - 84.65 |
| % Forested | 10.86 - 85.19 | 8.46 - 77.42 | 24.00 - 80.92 | 2.73 - 72.80 |
| % Barren | 0.15 - 24.84 | 0.74 - 21.46 | 0.24 - 34.98 | 0.14 - 6.63 |
| % Agricultural Total | 0.66 - 46.15 | 7.35 - 81.06 | 0.00 - 27.12 | 0.00 - 38.38 |
| % Agricultural Pasture | 0.08 - 13.84 | 0.00 - 27.22 | 0.00 - 7.22 | 0.00 - 12.86 |
| % Agriculture Row Crop | 0.02 - 36.95 | 3.41 - 80.58 | 0.00 - 25.99 | 0.00 - 33.10 |

**Nutrients**

Nutrient concentrations varied between ecoregions and subecoregions throughout the state. Nutrient concentrations ranged from below detection to levels above detection limits for the testing method (Table 6, above).

Approximately 1300 different macroinvertebrate taxa occurred throughout the state of Georgia. The relationship between macroinvertebrates and nutrients are presented by ecoregion and subecoregion.

**Ecoregion 66 (Blue Ridge)**

The Blue-ridge ecoregion is divided into three subecoregions: Southern

Crystalline Ridges and Mountains, Southern Metasedimentary Mountains, and Broad Basins (refer to Appendix 15 for map). A total of 15 reference sites and 17 impaired sites were analyzed for this ecoregion. Thirty of these sites were analyzed for total phosphorus in sediment. The ranges and means for the nutrient concentrations are in table 11.

**Table 11: Nutrient Concentration Ranges and Means (mg/L) Ecoregion 66**

| **Blue Ridge - 66 Ecoregion** | | | **Means*** | |
|---|---|---|---|---|
| Nutrient Parameter | Impaired Sites | Reference Sites | Impaired Sites | Reference Sites |
| Ammonia | <0.03 - 0.09 | <0.03 - 0.08 | 0.04 | 0.04 |
| Nitrite | <0.01 - 0.12 | <0.01 - 0.13 | 0.06 | 0.05 |
| Nitrite-Nitrate | 0.04 - 0.60 | <0.01 - >1.00 | 0.24 | 0.19 |
| Total Phosphorus | <0.01 - 0.21 | <0.01 - 0.14 | 0.05 | 0.03 |
| Total Phosphorus Sediment | 0.062 - 0.373 | 0.039 - 0.348 | 0.152 | 0.108 |

(*Means were calculated by changing below and above detection concentrations to the lower and upper limits, respectively.)

**<u>Subecoregion 66d (Southern Crystalline Ridges and Mountains)</u>**

For the Southern Crystalline Ridges and Mountains, five reference and five impaired sites were analyzed. The ranges and means for the nutrient concentrations are in table 12. (Refer to Appendix 16 for map, nutrient concentrations, and complete macroinvertebrate taxa list.)

**Table 12: Nutrient Concentration Ranges and Means (mg/L) Subecoregion 66d**

| **Southern Crystalline Ridges and Mountains - 66d Subecoregion** | | | **Means*** | |
|---|---|---|---|---|
| Nutrient Parameter | Impaired Sites | Reference Sites | Impaired Sites | Reference Sites |
| Ammonia | <0.03 - 0.09 | 0.04 - 0.06 | 0.05 | 0.05 |
| Nitrite | 0.07 - 0.09 | <0.01 - 0.05 | 0.08 | 0.03 |
| Nitrite-Nitrate | 0.10 - 0.49 | <0.01 - 0.06 | 0.23 | 0.04 |
| Total Phosphorus | 0.05 - 0.21 | <0.01 - 0.14 | 0.12 | 0.04 |
| Total Phosphorus Sediment | 0.089 - 0.272 | 0.067 - 0.348 | 0.156 | 0.147 |

(*Means were calculated by changing below and above detection concentrations to the lower and upper limits, respectively.)

**Subecoregion 66g (Southern Metasedimentary Mountains)**

For the Southern Metasedimentary Mountains, five reference and seven impaired sites were analyzed. The ranges and means for the nutrient concentrations are in table 13. (Refer to Appendix 17 for map, nutrient concentrations, and complete macroinvertebrate taxa list.)

**Table 13: Nutrient Concentration Ranges and Means (mg/L) Subecoregion 66g**

| **Southern Metasedimentary Mountains - 66g Subecoregion** | | | **Means*** | |
|---|---|---|---|---|
| Nutrient Parameter | Impaired Sites | Reference Sites | Impaired Sites | Reference Sites |
| Ammonia | <0.03 - 0.06 | <0.03 - 0.08 | 0.04 | 0.05 |
| Nitrite | <0.01 - 0.12 | <0.01 - 0.13 | 0.04 | 0.07 |
| Nitrite-Nitrate | 0.05 - 0.60 | <0.01 - >1.0 | 0.30 | 0.45 |
| Total Phosphorus | <0.01 - <0.01 | <0.01 - 0.08 | 0.01 | 0.05 |
| Total Phosphorus Sediment | 0.062 - 0.373 | 0.039 - 0.086 | 0.137 | 0.063 |

(*Means were calculated by changing below and above detection concentrations to the lower and upper limits, respectively.)

**Subecoregion 66j (Broad Basins)**

For the Broad Basins, five reference and five impaired sites were analyzed. The ranges and means for the nutrient concentrations are in table 14. (Refer to Appendix 18 for map, nutrient concentrations, and complete macroinvertebrate taxa list.)

**Table 14: Nutrient Concentration Ranges and Means (mg/L) Subecoregion 66j**

| **Broad Basins - 66j Subecoregion** | | | **Means*** | |
|---|---|---|---|---|
| Nutrient Parameter | Impaired Sites | Reference Sites | Impaired Sites | Reference Sites |
| Ammonia | <0.03 - 0.03 | <0.03 - 0.05 | 0.03 | 0.03 |
| Nitrite | 0.05 - 0.10 | <0.01 - 0.09 | 0.06 | 0.06 |
| Nitrite-Nitrate | 0.04 - 0.25 | 0.06 - 0.15 | 0.15 | 0.10 |
| Total Phosphorus | <0.01 - 0.07 | <0.01 - <0.01 | 0.04 | 0.01 |
| Total Phosphorus Sediment | 0.093 - 0.242 | 0.058 - 0.232 | 0.165 | 0.113 |

(*Means were calculated by changing below and above detection concentrations to the lower and upper limits, respectively.)

**Ecoregion 67 (Ridge and Valley)**

The Ridge and Valley ecoregion is divided into three subecoregions: Southern Limestone/Dolomite Valleys and Low Rolling Hills & Southern Dissected Ridges and Knobs, Southern Shale Valleys, and Southern Sandstone Ridges (refer to Appendix 19 for map). A total of 14 reference sites and 12 impaired sites were analyzed. Twenty-three of these sites were analyzed for total phosphorus in sediment. The ranges and means for the nutrient concentrations are in table 15.

**Table 15: Nutrient Concentration Ranges and Means (mg/L) Ecoregion 67**

| Ridge and Valley - 67 Ecoregion | | | Means* | |
|---|---|---|---|---|
| Nutrient Parameter | Impaired Sites | Reference Sites | Impaired Sites | Reference Sites |
| Ammonia | <0.03 - 0.04 | <0.03 - 0.03 | 0.40 | 0.04 |
| Nitrite | <0.01 - 0.12 | <0.01 - 0.04 | 0.05 | 0.01 |
| Nitrite-Nitrate | 0.05 - >1.00 | <0.01 - 0.68 | 0.50 | 0.22 |
| Total Phosphorus | <0.01 - 0.95 | <0.01 - 0.10 | 0.15 | 0.02 |
| Total Phosphorus Sediment | 0.029 - 0.858 | 0.029 - 0.191 | 0.186 | 0.088 |

(*Means were calculated by changing below and above detection concentrations to the lower and upper limits, respectively.)

**Subecoregion 67f&I (Southern Limestone/Dolomite Valleys and Low Rolling Hills & Southern Dissected Ridges and Knobs)**

For the Southern Limestone/Dolomite Valleys and Low Rolling Hills & Southern Dissected Ridges and Knobs, five reference and five impaired sites were analyzed. The ranges and means for the nutrient concentrations are in table 16. (Refer to Appendix 20 for map, nutrient concentrations, and complete macroinvertebrate taxa list.)

**Subecoregion 67g (Southern Shale Valleys)**

For the Southern Shale Valleys, five reference and five impaired sites were analyzed. The ranges and means for the nutrient concentrations are in table 17. (Refer to

Appendix 21 for map, nutrient concentrations, and complete macroinvertebrate taxa list.)

**Table 16: Nutrient Concentration Ranges and Means (mg/L) Subecoregion 67f&i**

| **Southern Limestone/Dolomite Valleys and Low Rolling Hills & Southern Dissected Ridges and Knobs - 67f&i Subecoregion** | | | **Means*** | |
|---|---|---|---|---|
| Nutrient Parameter | Impaired Sites | Reference Sites | Impaired Sites | Reference Sites |
| Ammonia | <0.03 - 3.04 | <0.03 - 0.06 | 0.85 | 0.04 |
| Nitrite | <0.01 - 0.03 | <0.01 - 0.04 | 0.02 | 0.02 |
| Nitrite-Nitrate | <0.01 -0.55 | 0.08 - 0.43 | 0.56 | 0.22 |
| Total Phosphorus | <0.01 - 0.17 | <0.01 - <0.01 | 0.04 | 0.01 |
| Total Phosphorus Sediment | 0.055 - 0.254 | 0.044 - 0.191 | 0.113 | 0.097 |

(*Means were calculated by changing below and above detection concentrations to the lower and upper limits, respectively.)

**Table 17: Nutrient Concentration Ranges and Means (mg/L) Subecoregion 67g**

| **Southern Shale Valleys - 67g Subecoregion** | | | **Means*** | |
|---|---|---|---|---|
| Nutrient Parameter | Impaired Sites | Reference Sites | Impaired Sites | Reference Sites |
| Ammonia | <0.03 - 0.05 | <0.03 - 0.08 | 0.03 | 0.05 |
| Nitrite | 0.02 - 0.11 | <0.01 - 0.03 | 0.06 | 0.02 |
| Nitrite-Nitrate | 0.09 - 0.84 | 0.02 - 0.68 | 0.48 | 0.34 |
| Total Phosphorus | 0.08 - 0.95 | <0.01 - 0.10 | 0.27 | 0.03 |
| Total Phosphorus Sediment | 0.029 - 0.858 | 0.048 - 0.123 | 0.308 | 0.084 |

(*Means were calculated by changing below and above detection concentrations to the lower and upper limits, respectively.)

**Subecoregion 67h (Southern Sandstone Ridges)**

For the Southern Sandstone Ridges, four reference and two impaired sites were analyzed. The ranges and means for the nutrient concentrations are in table 18. (Refer to Appendix 22 for map, nutrient concentrations, and complete macroinvertebrate taxa list.)

| Table 18: Nutrient Concentration Ranges and Means (mg/L) Subecoregion 67h | | | | |
|---|---|---|---|---|
| Southern Sandstone Ridges - 67h Subecoregion | | | Means* | |
| Nutrient Parameter | Impaired Sites | Reference Sites | Impaired Sites | Reference Sites |
| Ammonia | <0.03 - 0.36 | <0.03 - 0.059 | 0.19 | 0.04 |
| Nitrite | 0.06 - 0.12 | <0.01 - 0.01 | 0.09 | 0.01 |
| Nitrite-Nitrate | 0.35 - 0.40 | <0.01 - 0.11 | 0.38 | 0.05 |
| Total Phosphorus | 0.11 - 0.12 | <0.01 - <0.01 | 0.11 | 0.01 |
| Total Phosphorus Sediment | 0.030 - 0.030 | 0.029 - 0.159 | 0.030 | 0.084 |

(*Means were calculated by changing below and above detection concentrations to the lower and upper limits, respectively.)

**Ecoregion 68 & Subecoregion 68c&d (Plateau Escarpment and Southern Table Plateaus)**

For the Plateau Escarpment & Southern Table Plateaus, four reference and five impaired sites were analyzed. This ecoregion has only one subecoregion in Georgia (68c&d) (refer to Appendix 23 for map). The ranges and means for the nutrient concentrations are in table 19. (Refer to Appendix 23 for map, nutrient concentrations, and complete macroinvertebrate taxa list.)

| Table 19: Nutrient Concentration Ranges and Means (mg/L) Subecoregion 68c&d | | | | |
|---|---|---|---|---|
| Plateau Escarpment and Southern Table Plateaus - 68c&d Subecoregion | | | Means* | |
| Nutrient Parameter | Impaired Sites | Reference Sites | Impaired Sites | Reference Sites |
| Ammonia | <0.03 - 1.07 | <0.03 - 0.04 | 0.24 | 0.04 |
| Nitrite | <0.01 - 0.06 | <0.01 - 0.01 | 0.02 | 0.01 |
| Nitrite-Nitrate | 0.05 - 0.66 | 0.08 - 0.20 | 0.22 | 0.13 |
| Total Phosphorus | <0.01 - 0.10 | <0.01 - <0.01 | 0.06 | 0.01 |
| Total Phosphorus Sediment | 0.014 - 0.100 | 0.021 - 0.071 | 0.049 | 0.040 |

(*Means were calculated by changing below and above detection concentrations to the lower and upper limits, respectively.)

**Ecoregion 45 (Piedmont)**

The Piedmont ecoregion is divided into five subecoregions: Southern Inner

Piedmont, Southern Outer Piedmont, Carolina Slate Belt, Talladega Upland, and Pine Mountain Ridge (refer to Appendix 24 for map). A total of 25 reference sites and 26 impaired sites were analyzed. Forty-seven of these sites were analyzed for total phosphorus in sediment. The ranges and means for the nutrient concentrations are in table 20.

**<u>Subecoregion 45a (Southern Inner Piedmont)</u>**

For the Southern Inner Piedmont, five reference and five impaired sites were analyzed. The ranges and means for the nutrient concentrations are in table 21. (Refer to Appendix 25 for map, nutrient concentrations, and complete macroinvertebrate taxa list.)

**Table 20: Nutrient Concentration Ranges and Means (mg/L) Ecoregion 45**

| Piedmont - 45 Ecoregion | | | Means* | |
|---|---|---|---|---|
| Nutrient Parameter | Impaired Sites | Reference Sites | Impaired Sites | Reference Sites |
| Ammonia | <0.03 - 1.09 | <0.03 - 0.97 | 0.15 | 0.08 |
| Nitrite | <0.01 - 0.08 | <0.01 - 0.08 | 0.04 | 0.02 |
| Nitrite-Nitrate | <0.01 - >1.00 | <0.01 - 0.25 | 0.29 | 0.05 |
| Total Phosphorus | <0.01 - 0.18 | <0.01 - 1.17 | 0.07 | 0.08 |
| Total Phosphorus Sediment | <0.010 - 0.267 | <0.010 - 0.189 | 0.090 | 0.083 |

(*Means were calculated by changing below and above detection concentrations to the lower and upper limits, respectively.)

**Table 21: Nutrient Concentration Ranges and Means (mg/L) Subecoregion 45a**

| Southern Inner Piedmont - 45a Subecoregion | | | Means* | |
|---|---|---|---|---|
| Nutrient Parameter | Impaired Sites | Reference Sites | Impaired Sites | Reference Sites |
| Ammonia | <0.03 - 0.04 | <0.03 - 0.07 | 0.03 | 0.04 |
| Nitrite | 0.05 - 0.07 | <0.01 - 0.01 | 0.06 | 0.01 |
| Nitrite-Nitrate | 0.12 - 0.79 | <0.01 - 0.05 | 0.52 | 0.02 |
| Total Phosphorus | <0.01 - 0.16 | <0.01 - <0.01 | 0.07 | 0.01 |
| Total Phosphorus Sediment | 0.048 - 0.177 | 0.041 - 0.145 | 0.100 | 0.080 |

(*Means were calculated by changing below and above detection concentrations to the lower and upper limits, respectively.)

**Subecoregion 45b (Southern Outer Piedmont)**

For the Southern Outer Piedmont, five reference and six impaired sites were analyzed. The ranges and means for the nutrient concentrations are in table 22. (Refer to Appendix 26 for map, nutrient concentrations, and complete macroinvertebrate taxa list.)

| Table 22: Nutrient Concentration Ranges and Means (mg/L) Subecoregion 45b | | | | |
|---|---|---|---|---|
| **Southern Outer Piedmont - 45b Subecoregion** | | | **Means*** | |
| Nutrient Parameter | Impaired Sites | Reference Sites | Impaired Sites | Reference Sites |
| Ammonia | <0.03 - 1.09 | <0.03 - 0.97 | 0.31 | 0.23 |
| Nitrite | <0.01 - 0.08 | <0.01 - 0.02 | 0.03 | 0.01 |
| Nitrite-Nitrate | <0.01 - 0.54 | 0.01 - 0.16 | 0.52 | 0.08 |
| Total Phosphorus | <0.01 -0.10 | <0.01 - 0.04 | 0.03 | 0.02 |
| Total Phosphorus Sediment | <0.010 - 0.140 | 0.028 - 0.103 | 0.080 | 0.065 |

(*Means were calculated by changing below and above detection concentrations to the lower and upper limits, respectively.)

**Subecoregion 45c (Carolina Slate Belt)**

For the Carolina Slate Belt, five reference and five impaired sites were analyzed. The ranges and means for the nutrient concentrations are in table 23. (Refer to Appendix 27 for map, nutrient concentrations, and complete macroinvertebrate taxa list.)

| Table 23: Nutrient Concentration Ranges and Means (mg/L) Subecoregion 45c | | | | |
|---|---|---|---|---|
| **Carolina Slate Belt - 45c Subecoregion** | | | **Means*** | |
| Nutrient Parameter | Impaired Sites | Reference Sites | Impaired Sites | Reference Sites |
| Ammonia | 0.04 - 1.04 | <0.03 - 0.05 | 0.26 | 0.04 |
| Nitrite | <0.01 - 0.08 | <0.01 - 0.08 | 0.03 | 0.04 |
| Nitrite-Nitrate | <0.01 - 0.19 | <0.01 - 0.25 | 0.09 | 0.06 |
| Total Phosphorus | <0.01 - 0.13 | <0.01 - 1.17 | 0.04 | 0.33 |
| Total Phosphorus Sediment | 0.041 - 0.136 | 0.058 - 0.189 | 0.083 | 0.120 |

(*Means were calculated by changing below and above detection concentrations to the lower and upper limits, respectively.)

**Subecoregion 45d (Talladega Upland)**

For the Talladega Upland, five reference and five impaired sites were analyzed. The ranges and means for the nutrient concentrations are in table 24. (Refer to Appendix 28 for map, nutrient concentrations, and complete macroinvertebrate taxa list.)

**Table 24: Nutrient Concentration Ranges and Means (mg/L) Subecoregion 45d**

| **Talladega Upland - 45d Subecoregion** | | | **Means*** | |
|---|---|---|---|---|
| Nutrient Parameter | Impaired Sites | Reference Sites | Impaired Sites | Reference Sites |
| Ammonia | 0.03 - 0.12 | <0.03 - 0.10 | 0.09 | 0.05 |
| Nitrite | 0.03 - 0.08 | <0.01 - 0.04 | 0.06 | 0.02 |
| Nitrite-Nitrate | 0.10 - 0.32 | <0.01 - 0.07 | 0.21 | 0.03 |
| Total Phosphorus | 0.07 - 0.18 | <0.01 - 0.19 | 0.14 | 0.05 |
| Total Phosphorus Sediment | 0.069 - 0.267 | <0.010 - 0.147 | 0.136 | 0.076 |

(*Means were calculated by changing below and above detection concentrations to the lower and upper limits, respectively.)

**Subecoregion 45h (Pine Mountain Ridge)**

For the Pine Mountain Ridge, five reference and five impaired sites were analyzed. The ranges and means for the nutrient concentrations are in table 25. (Refer to Appendix 29 for map, nutrient concentrations, and complete macroinvertebrate taxa list.)

**Table 25: Nutrient Concentration Ranges and Means (mg/L) Subecoregion 45h**

| **Pine Mountain Ridge - 45h Subecoregion** | | | **Means*** | |
|---|---|---|---|---|
| Nutrient Parameter | Impaired Sites | Reference Sites | Impaired Sites | Reference Sites |
| Ammonia | <0.03 - 0.04 | <0.03 - 0.06 | 0.03 | 0.05 |
| Nitrite | <0.01 - 0.04 | <0.01 - <0.01 | 0.02 | 0.01 |
| Nitrite-Nitrate | 0.06 - 0.11 | 0.02 - 0.12 | 0.07 | 0.05 |
| Total Phosphorus | <0.01 - 0.12 | <0.01 - <0.01 | 0.06 | 0.01 |
| Total Phosphorus Sediment | 0.021 - 0.080 | 0.045 - 0.145 | 0.042 | 0.081 |

(*Means were calculated by changing below and above detection concentrations to the lower and upper limits, respectively.)

**Ecoregion 65 (Southeastern Plains)**

The Southeastern Plains ecoregion is divided into seven subecoregions: Sand Hills, Southern Hilly Gulf Coastal Plain, Dougherty Plain, Tifton Upland, Coastal Plain Red Uplands, Atlantic Southern Loam Plains, and Tallahassee Hills/Valdosta Limesink (refer to Appendix 30 for map). A total of 34 reference sites and 42 impaired sites were analyzed. Forty-eight of these sites were analyzed for total phosphorus in sediment. The ranges and means for the nutrient concentrations are in table 26.

| **Table 26: Nutrient Concentration Ranges and Means (mg/L) Ecoregion 65** | | | | |
|---|---|---|---|---|
| **Southeastern Plains - 65 Ecoregion** | | | **Means*** | |
| Nutrient Parameter | Impaired Sites | Reference Sites | Impaired Sites | Reference Sites |
| Ammonia | <0.03 - 2.22 | <0.03 - 0.09 | 0.31 | 0.05 |
| Nitrite | <0.01 - 1.0 | <0.01 - 0.06 | 0.03 | 0.01 |
| Nitrite-Nitrate | <0.01 - >1.00 | <0.01 - 0.81 | 0.28 | 0.12 |
| Total Phosphorus | <0.01 - 0.85 | <0.01 - 0.21 | 0.06 | 0.03 |
| Total Phosphorus Sediment | <0.010 - 0.202 | <0.010 - 0.236 | 0.057 | 0.058 |

(*Means were calculated by changing below and above detection concentrations to the lower and upper limits, respectively.)

**Subecoregion 65c (Sand Hills)**

For the Sand Hills, five reference and seven impaired sites were analyzed. The ranges and means for the nutrient concentrations are in table 27. (Refer to Appendix 31 for map, nutrient concentrations, and complete macroinvertebrate taxa list.)

| **Table 27: Nutrient Concentration Ranges and Means (mg/L) Subecoregion 65c** | | | | |
|---|---|---|---|---|
| **Sand Hills -65c Subecoregion** | | | **Means*** | |
| Nutrient Parameter | Impaired Sites | Reference Sites | Impaired Sites | Reference Sites |
| Ammonia | <0.03 - 0.23 | <0.03 - 0.07 | 0.09 | 0.05 |
| Nitrite | <0.01 - 0.06 | <0.01 - <0.01 | 0.03 | 0.01 |
| Nitrite-Nitrate | <0.01 - 0.49 | 0.07 - 0.47 | 0.36 | 0.18 |
| Total Phosphorus | <0.01 - 0.14 | <0.01 - <0.01 | 0.05 | 0.01 |
| Total Phosphorus Sediment | <0.010 - 100 | <0.010 - 0.035 | 0.045 | 0.015 |

(*Means were calculated by changing below and above detection concentrations to the lower and upper limits, respectively.)

**Subecoregion 65d (Southern Hilly Gulf Coastal Plain)**

For the Southern Hilly Gulf Coastal Plain, five reference and five impaired sites were analyzed. The ranges and means for the nutrient concentrations are in table 28. (Refer to Appendix 32 for map, nutrient concentrations, and complete macroinvertebrate taxa list.)

**Table 28: Nutrient Concentration Ranges and Means (mg/L) Subecoregion 65d**

| **Southern Hilly Gulf Coastal Plain -65d Subecoregion** | | | **Means*** | |
|---|---|---|---|---|
| Nutrient Parameter | Impaired Sites | Reference Sites | Impaired Sites | Reference Sites |
| Ammonia | <0.03 - 1.47 | <0.03 - 0.06 | 0.33 | 0.04 |
| Nitrite | <0.01 - 0.09 | <0.01 - 0.01 | 0.03 | 0.01 |
| Nitrite-Nitrate | 0.01 - 0.73 | <0.01 - 0.19 | 0.20 | 0.07 |
| Total Phosphorus | <0.01 - 0.16 | <0.01 - <0.01 | 0.11 | 0.01 |
| Total Phosphorus Sediment | <0.010 - 0.073 | <0.010 -0.236 | 0.026 | 0.061 |

(*Means were calculated by changing below and above detection concentrations to the lower and upper limits, respectively.)

**Subecoregion 65g (Dougherty Plain)**

For the Dougherty Plain, five reference and ten impaired sites were analyzed. The ranges and means for the nutrient concentrations are in table 29. (Refer to Appendix 33 for map, nutrient concentrations, and complete macroinvertebrate taxa list.)

**Table 29: Nutrient Concentration Ranges and Means (mg/L) Subecoregion 65g**

| **Dougherty Plain - 65g Subecoregion** | | | **Means*** | |
|---|---|---|---|---|
| Nutrient Parameter | Impaired Sites | Reference Sites | Impaired Sites | Reference Sites |
| Ammonia | <0.03 - 2.13 | 0.04 - 0.07 | 0.28 | 0.05 |
| Nitrite | 0.01 - 0.07 | <0.01 - 0.06 | 0.03 | 0.02 |
| Nitrite-Nitrate | <0.01 - 0.62 | 0.01 - 0.25 | 0.40 | 0.15 |
| Total Phosphorus | <0.01 - 0.07 | <0.01 - <0.01 | 0.02 | 0.01 |
| Total Phosphorus Sediment | 0.202 - 0.202 | 0.020 - 0.136 | 0.202 | 0.069 |

(*Means were calculated by changing below and above detection concentrations to the lower and upper limits, respectively.)

**Subecoregion 65h (Tifton Upland)**

For the Tifton Upland, five reference and five impaired sites were analyzed. The ranges and means for the nutrient concentrations are in table 30. (Refer to Appendix 34 for map, nutrient concentrations, and complete macroinvertebrate taxa list.)

| Table 30: Nutrient Concentration Ranges and Means (mg/L) Subecoregion 65h | | | | |
|---|---|---|---|---|
| **Tifton Upland - 65h Subecoregion** | | | **Means*** | |
| Nutrient Parameter | Impaired Sites | Reference Sites | Impaired Sites | Reference Sites |
| Ammonia | <0.03 - 2.22 | <0.03 - 0.06 | 0.91 | 0.04 |
| Nitrite | <0.01 - 0.10 | <0.01 - 0.01 | 0.03 | 0.01 |
| Nitrite-Nitrate | <0.01 - 0.47 | 0.02 - 0.37 | 0.15 | 0.16 |
| Total Phosphorus | <0.01 - 0.02 | <0.01 - <0.01 | 0.01 | 0.01 |
| Total Phosphorus Sediment | 0.043 - 0.087 | 0.059 - 0.126 | 0.067 | 0.082 |

(*Means were calculated by changing below and above detection concentrations to the lower and upper limits, respectively.)

**Subecoregion 65k (Coastal Plain Red Uplands)**

For the Coastal Plain Red Uplands, five reference and five impaired sites were analyzed. The ranges and means for the nutrient concentrations are in table 31. (Refer to Appendix 35 for map, nutrient concentrations, and complete macroinvertebrate taxa list.)

| Table 31: Nutrient Concentration Ranges and Means (mg/L) Subecoregion 65k | | | | |
|---|---|---|---|---|
| **Coastal Plain Red Uplands - 65k Subecoregion** | | | **Means*** | |
| Nutrient Parameter | Impaired Sites | Reference Sites | Impaired Sites | Reference Sites |
| Ammonia | <0.03 - 0.24 | <0.03 - 0.09 | 0.07 | 0.06 |
| Nitrite | <0.01 - 0.08 | <0.01 - <0.01 | 0.03 | 0.01 |
| Nitrite-Nitrate | <0.01 - 0.89 | <0.01 - 0.81 | 0.63 | 0.19 |
| Total Phosphorus | <0.01 - 0.85 | <0.01 - <0.01 | 0.21 | 0.01 |
| Total Phosphorus Sediment | 0.024 - 0.076 | 0.035 - 0.127 | 0.050 | 0.069 |

(*Means were calculated by changing below and above detection concentrations to the lower and upper limits, respectively.)

**Subecoregion 65l (Atlantic Southern Loam Plains)**

For the Atlantic Southern Loam Plains, five reference and five impaired sites were analyzed. The ranges and means for the nutrient concentrations are in table 32. (Refer to Appendix 36 for map, nutrient concentrations, and complete macroinvertebrate taxa list.)

| Table 32: Nutrient Concentration Ranges and Means (mg/L) Subecoregion 65l | | | | |
|---|---|---|---|---|
| **Atlantic Southern Loam Plains - 65l Subecoregion** | | | **Means*** | |
| Nutrient Parameter | Impaired Sites | Reference Sites | Impaired Sites | Reference Sites |
| Ammonia | <0.03 - 0.95 | <0.03 - 0.07 | 0.27 | 0.05 |
| Nitrite | <0.01 -0.05 | <0.01 - 0.02 | 0.03 | 0.01 |
| Nitrite-Nitrate | <0.01 - 0.16 | <0.01 - 0.36 | 0.06 | 0.09 |
| Total Phosphorus | <0.01 - <0.01 | <0.01 - 0.18 | 0.01 | 0.05 |
| Total Phosphorus Sediment | <0.010 - 0.019 | <0.010 - 0.031 | 0.019 | 0.019 |

(*Means were calculated by changing below and above detection concentrations to the lower and upper limits, respectively.)

**Subecoregion 65o (Tallahassee Hills/Valdosta Limesink)**

For the Tallahassee Hills/Valdosta Limesink, four reference and five impaired sites were analyzed. The ranges and means for the nutrient concentrations are in table 33. (Refer to Appendix 37 for map, nutrient concentrations, and complete macroinvertebrate taxa list.)

| Table 33: Nutrient Concentration Ranges and Means (mg/L) Subecoregion 65o | | | | |
|---|---|---|---|---|
| **Tallahassee Hills/Valdosta Limesink - 65o Subecoregion** | | | **Means*** | |
| Nutrient Parameter | Impaired Sites | Reference Sites | Impaired Sites | Reference Sites |
| Ammonia | <0.03 - 1.41 | <0.03 - 0.05 | 0.31 | 0.04 |
| Nitrite | <0.01 - 0.02 | <0.01 - 0.02 | 0.01 | 0.01 |
| Nitrite-Nitrate | <0.01 - 0.17 | <0.01 - 0.04 | 0.05 | 0.02 |
| Total Phosphorus | <0.01 - 0.08 | 0.05 - 0.21 | 0.02 | 0.10 |
| Total Phosphorus Sediment | 0.133 - 0.133 | 0.040 - 0.150 | 0.133 | 0.099 |

(*Means were calculated by changing below and above detection concentrations to the lower and upper limits, respectively.)

**Ecoregion 75 (Southern Coastal Plains)**

The Southern Costal Plains ecoregion is divided into 4 subecoregions: Okefenokee Plains, Sea Island Flatwoods, Bacon Terraces, and Sea Islands/Coastal Marsh (refer to Appendix 38 for map). A total of 25 reference sites and 26 impaired sites were analyzed. Twenty-two of these sites were analyzed for total phosphorus in sediment. The ranges and means for the nutrient concentrations are in table 34.

**Table 34: Nutrient Concentration Ranges and Means (mg/L) Ecoregion 75**

| **Southern Coastal Plains - 75 Ecoregion** | | | **Means*** | |
|---|---|---|---|---|
| Nutrient Parameter | Impaired Sites | Reference Sites | Impaired Sites | Reference Sites |
| Ammonia | <0.03 - 2.81 | <0.03 - 48.92 | 0.49 | 2.82 |
| Nitrite | <0.01 - >1.00 | <0.01 - 0.12 | 0.06 | 0.03 |
| Nitrite-Nitrate | <0.01 - >1.00 | <0.01 - >1.00 | 0.15 | 0.14 |
| Total Phosphorus | <0.01 - 0.83 | <0.01 - 0.32 | 0.19 | 0.05 |
| Total Phosphorus Sediment | <0.010 - 1.093 | 0.020 - 0.832 | 0.542 | 0.189 |

(*Means were calculated by changing below and above detection concentrations to the lower and upper limits, respectively.)

**Subecoregion 75e (Okefenokee Plains)**

For the Okefenokee Plains, five reference and five impaired sites were analyzed. The ranges and means for the nutrient concentrations are in table 35. (Refer to Appendix 39 for map, nutrient concentrations, and complete macroinvertebrate taxa list.)

**Subecoregion 75f (Sea Island Flatwoods)**

For the Sea Island Flatwoods, four reference and six impaired sites were analyzed. The ranges and means for the nutrient concentrations are in table 36. (Refer to Appendix 40 for map, nutrient concentrations, and complete macroinvertebrate taxa list.)

| Table 35: Nutrient Concentration Ranges and Means (mg/L) Subecoregion 75e | | | | |
|---|---|---|---|---|
| **Okefenokee Plains – 75e Subecoregion** | | | **Means*** | |
| Nutrient Parameter | Impaired Sites | Reference Sites | Impaired Sites | Reference Sites |
| Ammonia | <0.03 – 2.81 | <0.03 – 0.08 | 0.79 | 0.05 |
| Nitrite | 0.01 – 0.03 | 0.02 – 0.04 | 0.02 | 0.03 |
| Nitrite-Nitrate | <0.01 – 0.39 | <0.01 – 0.33 | 0.10 | 0.08 |
| Total Phosphorus | <0.01 – 0.51 | <0.01 – 0.04 | 0.18 | 0.02 |
| Total Phosphorus Sediment | <0.010 – 0.681 | 0.024 – 0.054 | 0.237 | 0.035 |

(*Means were calculated by changing below and above detection concentrations to the lower and upper limits, respectively.)

| Table 36: Nutrient Concentration Ranges and Means (mg/L) Subecoregion 75f | | | | |
|---|---|---|---|---|
| **Sea Island Flatwoods - 75f Subecoregion** | | | **Means*** | |
| Nutrient Parameter | Impaired Sites | Reference Sites | Impaired Sites | Reference Sites |
| Ammonia | <0.03 - 0.86 | <0.03 - 12.72 | 0.33 | 3.23 |
| Nitrite | <0.01 - 0.02 | <0.01 - 0.07 | 0.18 | 0.03 |
| Nitrite-Nitrate | <0.01 - 0.59 | <0.01 - 0.32 | 0.30 | 0.09 |
| Total Phosphorus | <0.01 - 0.67 | <0.01 - 0.11 | 0.17 | 0.04 |
| Total Phosphorus Sediment | 0.367 - 1.032 | 0.382 - 0.382 | 0.669 | 0.382 |

(*Means were calculated by changing below and above detection concentrations to the lower and upper limits, respectively.)

**<u>Subecoregion 75h (Bacon Terraces)</u>**

For the Bacon Terraces, five reference and six impaired sites were analyzed. The ranges and means for the nutrient concentrations are in table 37. (Refer to Appendix 41 for map, nutrient concentrations, and complete macroinvertebrate taxa list.)

| Table 37: Nutrient Concentration Ranges and Means (mg/L) Subecoregion 75h | | | | |
|---|---|---|---|---|
| **Bacon Terraces - 75h Subecoregion** | | | **Means*** | |
| Nutrient Parameter | Impaired Sites | Reference Sites | Impaired Sites | Reference Sites |
| Ammonia | <0.03 - 1.30 | <0.03 - 6.69 | 0.26 | 1.38 |
| Nitrite | <0.01 - 0.08 | <0.01 - 0.01 | 0.04 | 0.01 |
| Nitrite-Nitrate | <0.01 - 0.36 | <0.01 - >1.00 | 0.15 | 0.29 |
| Total Phosphorus | <0.01 - 0.29 | <0.01 - 0.17 | 0.11 | 0.04 |
| Total Phosphorus Sediment | 0.282 - 0.282 | 0.020 - 0.216 | 0.282 | 0.087 |

(*Means were calculated by changing below and above detection concentrations to the lower and upper limits, respectively.)

**Subecoregion 75j (Sea Islands/Coastal Marsh)**

For the Sea Islands/Coastal Marsh, eleven reference and ten impaired sites were analyzed. The ranges and means for the nutrient concentrations are in table 38. (Refer to Appendix 42 for map, nutrient concentrations, and complete macroinvertebrate taxa list.)

**Table 38: Nutrient Concentration Ranges and Means (mg/L) Subecoregion 75j**

| **Sea Islands/Coastal Marsh - 75j Subecoregion** | | | **Means*** | |
|---|---|---|---|---|
| Nutrient Parameter | Impaired Sites | Reference Sites | Impaired Sites | Reference Sites |
| Ammonia | 0.04 - 1.75 | <0.03 - 48.92 | 0.57 | 4.59 |
| Nitrite | <0.01 - 0.03 | <0.01 - 0.12 | 0.01 | 0.03 |
| Nitrite-Nitrate | <0.01 - 0.54 | <0.01 - >1.00 | 0.08 | 0.11 |
| Total Phosphorus | 0.01 - 0.83 | <0.01 - 0.32 | 0.26 | 0.06 |
| Total Phosphorus Sediment | 0.172 to 1.093 | 0.225 - 0.832 | 0.701 | 0.529 |

(*Means were calculated by changing below and above detection concentrations to the lower and upper limits, respectively.)

**Macroinvertebrates**

The Blue Ridge ecoregion had 348 macroinvertebrate taxa for reference sites and 298 macroinvertebrate taxa for impaired sites. For the Ridge and Valley ecoregion, reference sites had 309 macroinvertebrate taxa and 275 for impaired sites. There were 109 macroinvertebrate taxa for reference and 179 macroinvertebrate taxa for impaired sites in the Southwestern Appalachians ecoregion. The Piedmont ecoregion had 395 macroinvertebrate taxa for reference sites and 348 macroinvertebrate taxa for impaired sites. For the Southeastern Plains ecoregion, reference sites had 407 macroinvertebrate taxa and 443 for impaired sites. There were 189 macroinvertebrate taxa for reference and

239 macroinvertebrate taxa for impaired sites in the Coastal Plain. Table 39 includes the abundant macroinvertebrate taxa at ecoregion level for reference and impaired sites. Abundant taxa was calculated by number of individuals of a taxa in an ecoregion divided by total sites (*i.e.* reference or impaired) in the ecoregion (<=1 Rare; >1 & <=5 Common; >5 Abundant). (For a complete list of macroinvertebrate taxa refer to Appendices 15-42.)

**Table 39: Macroinvertebrate Abundant Taxa for Ecoregion Level**

| Ecoregion | Condition | Final Identification |
|---|---|---|
| 45 | Reference | *Polypedilum aviceps* |
| | | *Stenonema sp.* |
| | | Capniidae |
| | | *Strophopteryx limata* |
| | | *Cheumatopsyche sp.* |
| | | *Chimarra sp.* |
| 45 | Impaired | Oligochaeta |
| | | *Hyalella azteca* |
| | | *Chironomus sp.* |
| | | *Thienemannimyia group* |
| | | *Stenonema sp.* |
| | | Capniidae |
| | | *Cheumatopsyche sp.* |
| 66 | Reference | *Microtendipes pedellus group* |
| | | *Simulium sp.* |
| | | *Stenonema sp.* |
| | | Capniidae |
| | | *Tallaperla sp.* |
| | | *Cheumatopsyche sp.* |
| 66 | Impaired | *Optioservus sp.* |
| | | *Eukiefferiella brehmi group* |
| | | *Microtendipes pedellus group* |
| | | *Simulium sp.* |

| Table 39 (Continued): Macroinvertebrate Abundant Taxa for Ecoregion Level | | |
|---|---|---|
| **Ecoregion** | **Condition** | **Final Identification** |
| 66 | Impaired | Heptageniidae |
| | | *Stenonema sp.* |
| | | *Stenonema modestum* |
| | | *Isonychia sp.* |
| | | *Cheumatopsyche sp.* |
| 67 | Reference | *Hyalella azteca* |
| | | *Prosimulium sp.* |
| | | *Simulium sp.* |
| | | *Elimia sp.* |
| | | *Elimia caelatura georgiana* |
| | | *Elimia proxima* |
| | | *Chimarra sp.* |
| 67 | Impaired | Oligochaeta |
| | | *Stenelmis sp.* |
| | | *Caenis sp.* |
| | | *Lirceus sp.* |
| | | *Lirceus fontinalis* |
| | | *Elimia sp.* |
| 68 | Reference | *Diplocladius cultriger* |
| | | *Eukiefferiella claripennis group* |
| | | *Microtendipes pedellus group* |
| | | *Parametriocnemus F* |
| | | *Pseudorthocladius sp.* |
| | | *Prosimulium sp.* |
| | | *Lirceus sp.* |
| | | *Elimia proxima* |
| 68 | Impaired | *Phaenopsectra obediens group* |
| | | *Phaenopsectra/Tribelos complex* |
| | | *Tribelos jucundus* |
| | | *Baetis sp.* |
| | | *Isonychia sp.* |
| | | *Lirceus sp.* |
| | | *Cheumatopsyche sp.* |
| 65 | Reference | *Apedilum sp.* |
| | | *Phaenopsectra sp.* |

| Table 39 (Continued): Macroinvertebrate Abundant Taxa for Ecoregion Level | | |
|---|---|---|
| **Ecoregion** | **Condition** | **Final Identification** |
| 65 | Reference | *Polypedilum aviceps* |
| | | *Simulium sp.* |
| | | Leptophlebiidae |
| | | *Caecidotea sp.* |
| | | *Lirceus sp.* |
| 65 | Impaired | Oligochaeta |
| | | *Crangonyx sp.* |
| | | *Hydrobaenus sp.* |
| | | *Polypedilum tritum* |
| | | *Tanytarsus sp.* |
| | | *Thienemannimyia group* |
| | | *Simulium sp.* |
| | | *Caecidotea sp.* |
| 75 | Reference | *Crangonyx sp.* |
| | | *Gammarus sp.* |
| | | *Cladocera sp.* |
| | | *Chironomus sp.* |
| | | *Kiefferulus sp.* |
| | | *Polypedilum illinoense group* |
| | | *Polypedilum scalaenum group* |
| | | *Polypedilum tritum* |
| | | *Caecidotea sp.* |
| | | *Lirceus sp.* |
| 75 | Impaired | Oligochaeta |
| | | *Gammarus sp.* |
| | | *Hyalella azteca* |
| | | *Physella sp.* |
| | | *Palaemonetes sp.* |
| | | *Polypedilum illinoense group* |
| | | *Polypedilum tritum* |
| | | *Tanypus neopunctipennis* |
| | | *Caecidotea sp.* |
| | | *Lirceus sp.* |

**Analyses**

The Mann-Whitney-U-Test indicated which nutrient parameters were significantly correlated between reference and impaired sites. Since the cluster analysis determined if benthic populations were significantly different between reference and impaired sites, then the nutrient parameter that was different could be concluded to be one of the factors that affected the distribution of macroinvertebrates. The Spearman Rank Correlation Coefficient indicated if a correlation existed between nutrient concentrations and macroinvertebrate multi-metric index scores. If no nutrient parameters were significantly different, but there was good discrimination between reference and impaired sites, then the conclusion was that, other conditions (physical, chemical, or biological) were affecting the distribution. Nutrient parameters determined to be significantly correlated with the index score were plotted on a scatter plot, to determine if it was a linear correlation (less than values and greater than values were changed to the lower and upper limits, respectively to create the scatter plots).

**Ecoregion 66 (Blue Ridge)**

Nitrite in the water column and total phosphorous in the sediment were significantly different between reference and impaired sites (Table 40). Ammonia, nitrate-nitrite, and total phosphorus in the water column were not found to be significantly different. Cluster analysis did not distinguish between reference and impaired macroinvertebrate communities at the ecoregion level (Figure 2). Nitrite concentrations, positively correlated, and total phosphorus concentrations in sediment, negatively correlated, were significantly correlated with the macroinvertebrate multi-metric index scores (Table 41). Figures 3 and 4 are the scatter plots for nitrite vs. index score and total phosphorus in sediment vs. index score, respectively.

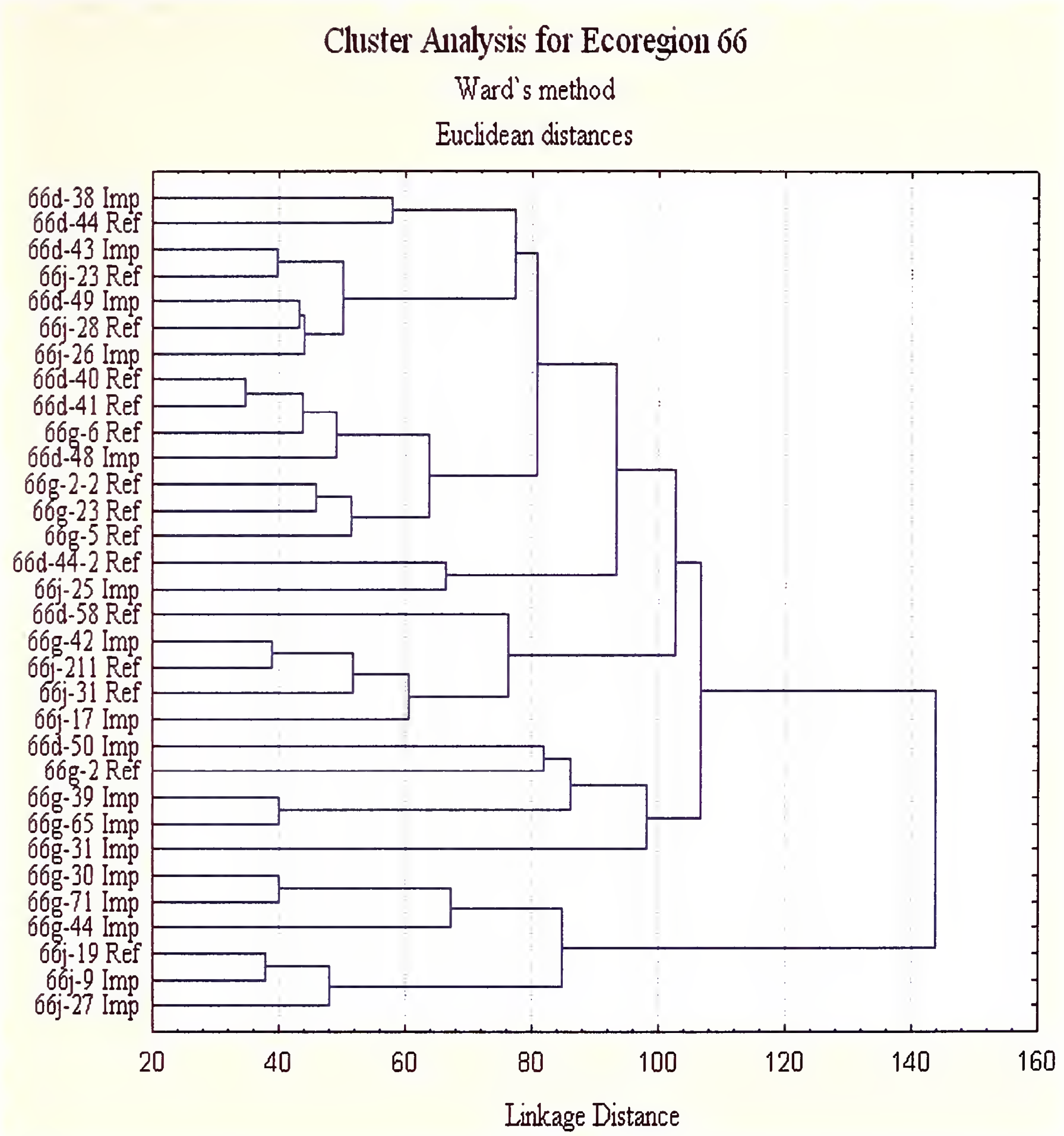


Figure 2: Cluster Analysis for Ecoregion 66

| Table 40: Mann - Whitney U Test for Ecoregion 66<br>Red marked tests are significant at p <.05000 | | | | | |
|---|---|---|---|---|---|
| | Z | p-level | Z adjusted | p-level | 2*1sided exact p |
| Ammonia | 0.58532 | 0.558331 | 0.60600 | 0.544514 | 0.576054 |
| Nitrite | -3.43641 | 0.000590 | -3.46446 | 0.000531 | 0.000290 |
| Nitrate-Nitrite | -0.32098 | 0.748223 | -0.32137 | 0.747933 | 0.765779 |
| Total Phosphorus | -1.43498 | 0.151293 | -1.61378 | 0.106576 | 0.153201 |
| TP Sediment | 2.073903 | 0.038089 | 2.074134 | 0.038065 | 0.036718 |

| Table 41: Spearman Rank Correlation Test - Nutrient Concentration vs. Index Score for Ecoregion 66<br>Red marked tests are significant at p<.05000 | | | | | |
|---|---|---|---|---|---|
| **Variable** | Ammonia | Nitrite | Nitrate-Nitrite | Total Phosphorus | TP Sediment |
| Index Score | 0.022012 | 0.543255 | 0.099275 | 0.101618 | -0.491767 |

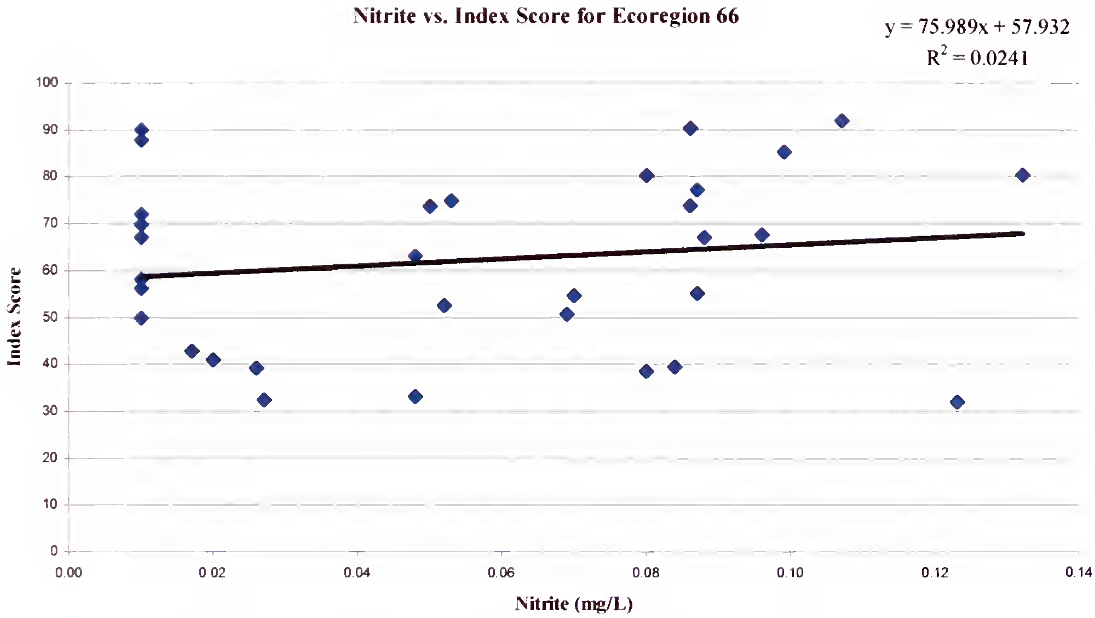


Figure 3: Nitrite vs. Index Score for Ecoregion 66

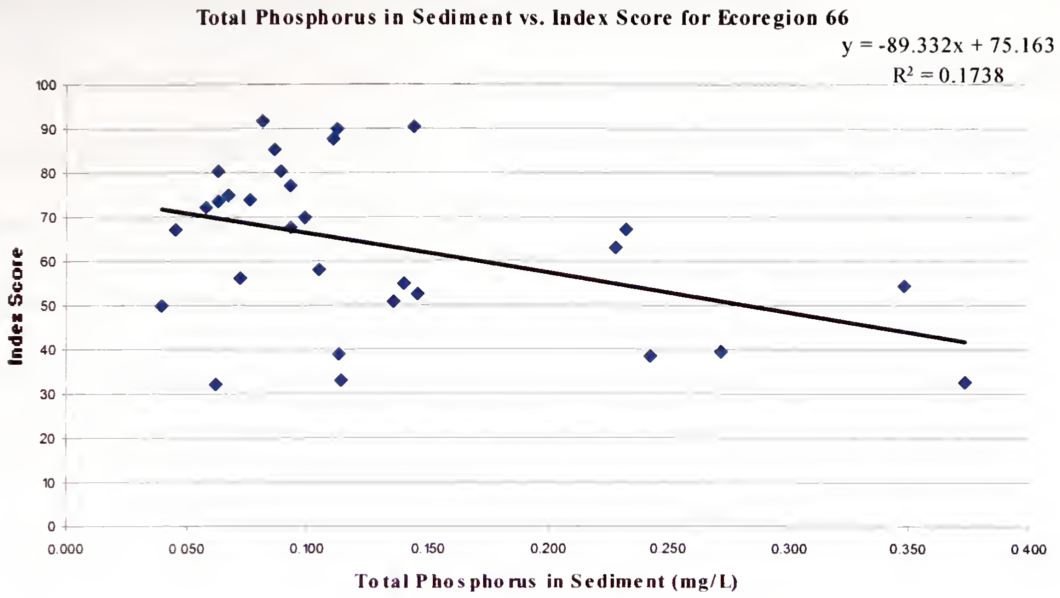


Figure 4: Total Phosphorus in Sediment vs. Index Score for Ecoregion 66

Discrimination efficiency between reference and impaired sites was 76%, with 13 of the 17 impaired sites being classified correctly. The box and whisker plot illustrated no overlap of interquartile range, but some overall overlap between reference and impaired sites (Figure 5).

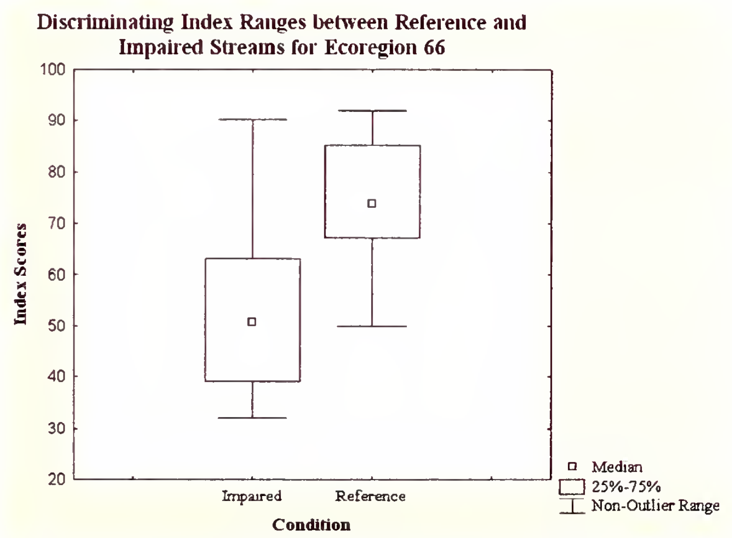


Figure 5: Discriminating Index Ranges between Reference and Impaired Streams for Ecoregion 66

**Subecoregion 66d (Southern Crystalline Ridges and Mountains)**

Total phosphorus in the water column was significantly different between reference and impaired sites (Table 42). Ammonia, nitrate-nitrite, and nitrite in the water column and total phosphorus in sediment were not found to be significantly different. Cluster analysis did not distinguish between reference and impaired macroinvertebrate communities (Figure 6). No nutrient parameters were significantly correlated with the macroinvertebrate multi-metric index scores (Table 43).

**Table 42: Mann - Whitney U Test for Subecoregion 66d**

Red marked tests are significant at p <.05000

| | Z | p-level | Z adjusted | p-level | 2*1sided exact p |
|---|---|---|---|---|---|
| Ammonia | 1.77559 | 0.075801 | 1.79751 | 0.072255 | 0.095238 |
| Nitrite | -0.73113 | 0.464703 | -0.74015 | 0.459208 | 0.547619 |
| Nitrate-Nitrite | 0.52223 | 0.601509 | 0.52382 | 0.600402 | 0.690476 |
| Total Phosphorus | -2.19338 | 0.028281 | -2.27036 | 0.023186 | 0.031746 |
| TP Sediment | 0.940019 | 0.347208 | 0.940019 | 0.347208 | 0.420635 |

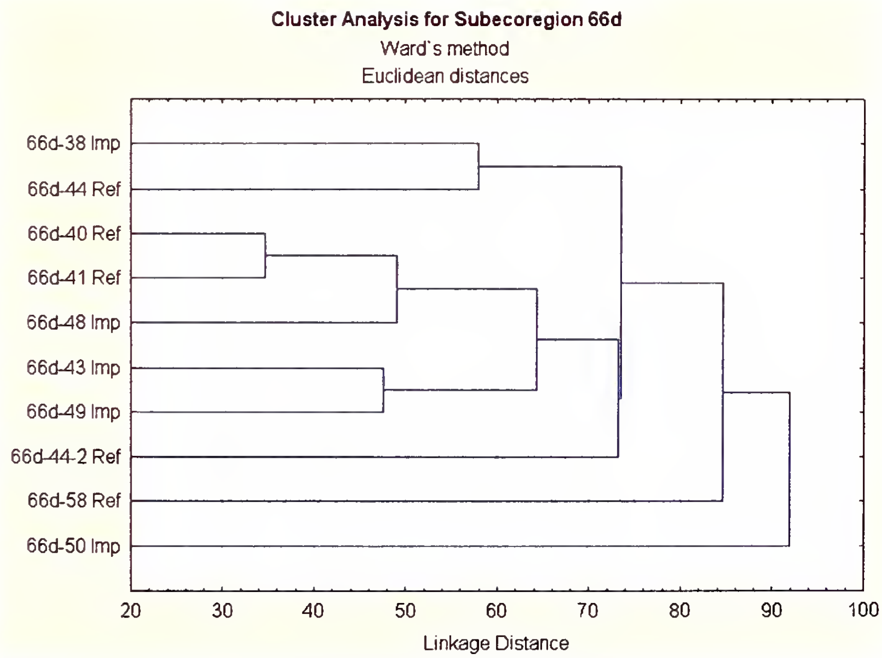


Figure 6: Cluster Analysis for Subecoregion 66d

**Table 43: Spearman Rank Correlation Test - Nutrient Concentration vs. Index Score for Subecoregion 66d**

Red marked tests are significant at p<.05000

| **Variable** | Ammonia | Nitrite | Nitrate-Nitrite | Total Phosphorus | TP Sediment |
|---|---|---|---|---|---|
| Index Score | -0.190198 | -0.018406 | 0.176293 | 0.219566 | -0.345455 |

Discrimination efficiency between reference and impaired sites was 80%, with four of the five impaired sites being classified correctly based on the reference condition. The box and whisker plot illustrated no overlap of interquartile range, but some overall overlap between reference and impaired sites (Figure 7).

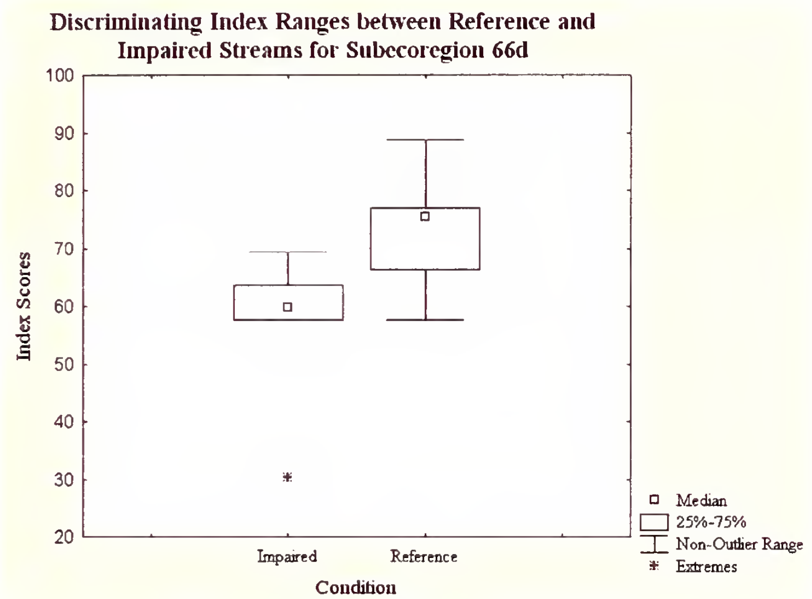


Figure 7: Discriminating Index Ranges between Reference and Impaired Streams for Subecoregion 66d

## <u>Subecoregion 66g (Southern Metasedimentary Mountains)</u>

Nitrate-nitrite in the water column was significantly different between reference and impaired sites (Table 44). Ammonia, nitrite, and total phosphorus in the water column and total phosphorus in sediment were not found to be significantly different.

Cluster analysis did distinguish between reference and impaired macroinvertebrate communities (Figure 8). Total phosphorus concentrations were negatively correlated with the macroinvertebrate multi-metric index scores (Table 45). Figure 9 is the scatter plot for total phosphorus vs. index score.

**Table 44: Mann - Whitney U Test for Subecoregion 66g**

Red marked tests are significant at $p < .05000$

| | Z | p-level | Z adjusted | p-level | 2*1sided exact p |
|---|---|---|---|---|---|
| Ammonia | 0.89320 | 0.371752 | 0.90111 | 0.367531 | 0.431818 |
| Nitrite | 1.86760 | 0.061819 | 1.88079 | 0.060001 | 0.073232 |
| Nitrate-Nitrite | 2.35479 | 0.018534 | 2.35892 | 0.018329 | 0.017677 |
| Total Phosphorus | -1.70520 | 0.088159 | -2.23822 | 0.025207 | 0.106061 |
| TP Sediment | 1.148913 | 0.250593 | 1.148913 | 0.250593 | 0.309528 |

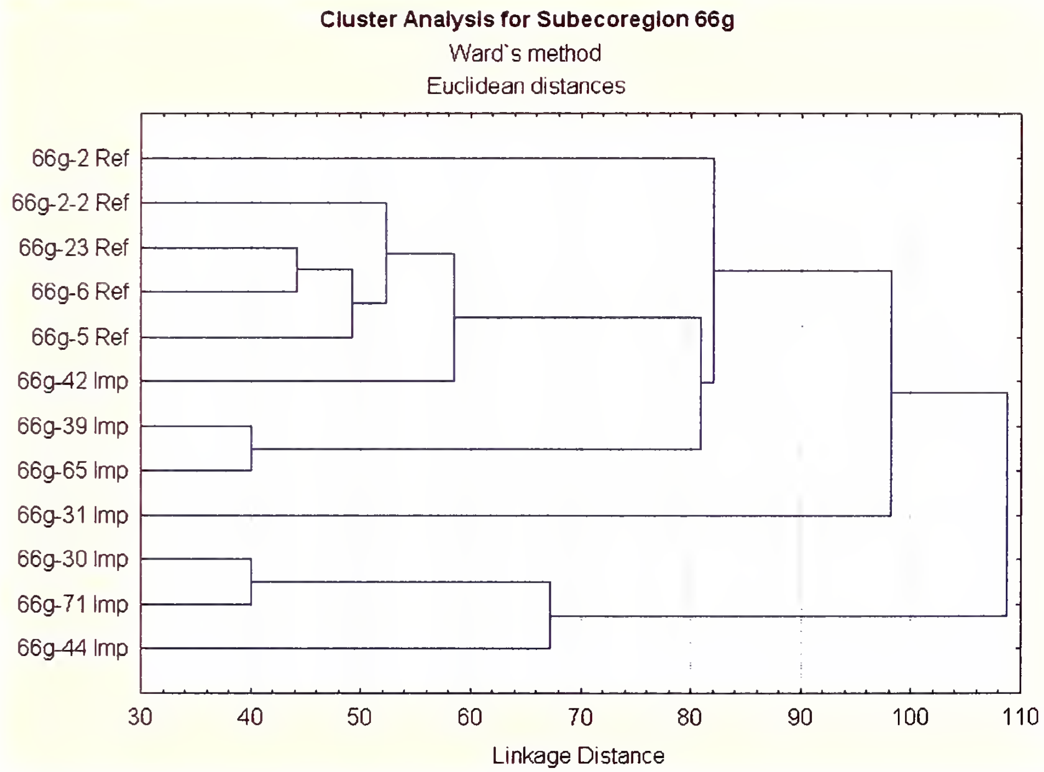


Figure 8: Cluster Analysis for Subecoregion 66g

| Table 45: Spearman Rank Correlation Test - Nutrient Concentration vs. Index Score for Subecoregion 66g | | | | | |
|---|---|---|---|---|---|
| Red marked tests are significant at p<.05000 | | | | | |
| **Variable** | Ammonia | Nitrite | Nitrate-Nitrite | Total Phosphorus | TP Sediment |
| Index Score | 0.402132 | 0.443673 | 0.521892 | -0.660884 | -0.328269 |

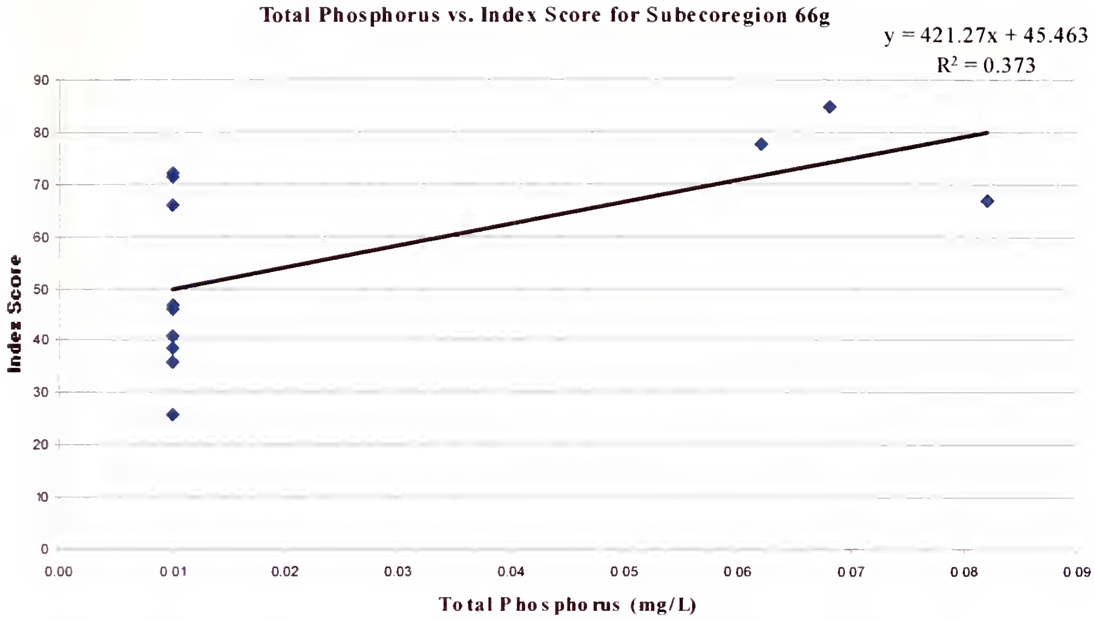


Figure 9: Total Phosphorus vs. Index Score for Subecoregion 66g

Discrimination efficiency between reference and impaired sites was 100%, with all the impaired sites being classified correctly based on the reference condition. The box and whisker plot illustrated no overlap between reference and impaired sites, thus indicating complete discrimination for the metrics (Figure 10).

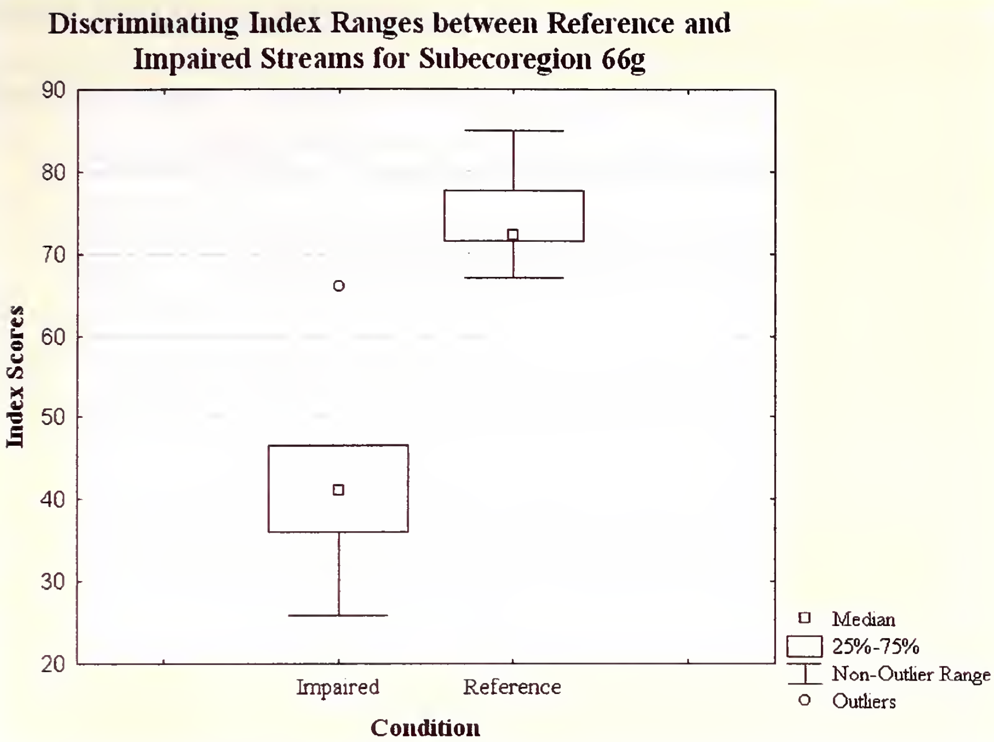


Figure 10: Discriminating Index Ranges between Reference and Impaired Streams for Subecoregion 66g

**Subecoregion 66j (Broad Basin)**

Total phosphorus in the water column was significantly different between reference and impaired sites (Table 46). Ammonia, nitrite, and nitrate-nitrite in the water column and total phosphorus in sediment were not found to be significantly different. Cluster analysis did not distinguish between reference and impaired macroinvertebrate communities (Figure 11). Nitrite concentrations, positive correlation; total phosphorus concentrations, positive correlation; and total phosphorus concentraions in sediment, negative correlation, were significantly correlated with the macroinvertebrate multi-metric index scores (Table 47). Figures 12, 13 and 14 are the scatter plots for nitrite vs. index score, total phosphorus in water column vs. index score and total phosphorus in sediment vs. index score, respectively.

**Table 46: Mann - Whitney U Test for Subecoregion 66j**

Red marked tests are significant at p <.05000

| | Z | p-level | Z adjusted | p-level | 2*1sided exact p |
|---|---|---|---|---|---|
| Ammonia | 0.31334 | 0.754023 | 0.38552 | 0.699854 | 0.841270 |
| Nitrite | -1.98449 | 0.047203 | -1.99662 | 0.045867 | 0.055556 |
| Nitrate-Nitrite | 0.94002 | 0.347208 | 0.94002 | 0.347208 | 0.420635 |
| Total Phosphorus | -2.08893 | 0.036715 | -2.35339 | 0.018604 | 0.031746 |
| TP Sediment | 1.462252 | 0.143676 | 1.466704 | 0.142458 | 0.150794 |

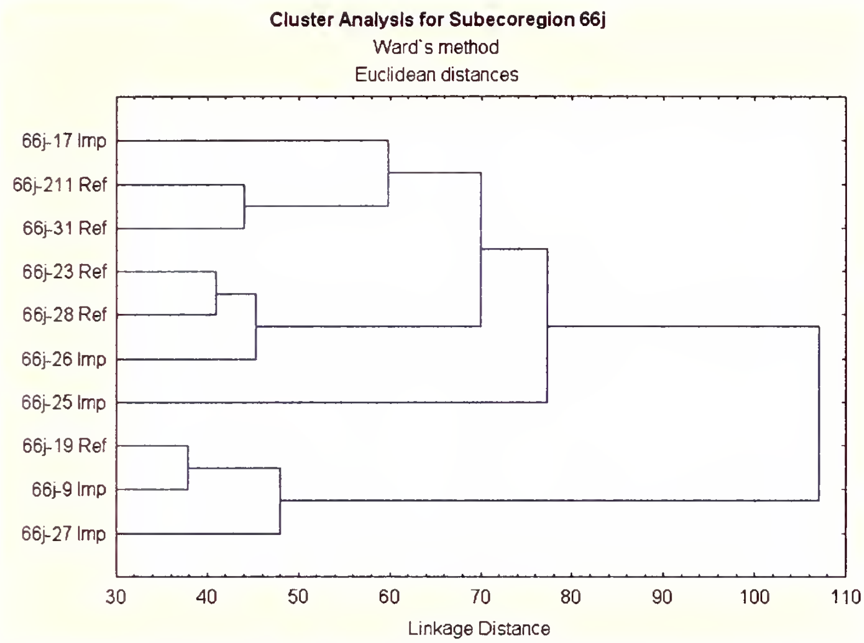


Figure 11: Cluster Analysis for Subecoregion 66j

**Table 47: Spearman Rank Correlation Test - Nutrient Concentration vs. Index Score for Subecoregion 66j**

Red marked tests are significant at p<.05000

| **Variable** | Ammonia | Nitrite | Nitrate-Nitrite | Total Phosphorus | TP Sediment |
|---|---|---|---|---|---|
| Index Score | -0.156590 | 0.664647 | -0.260606 | 0.792035 | -0.838910 |

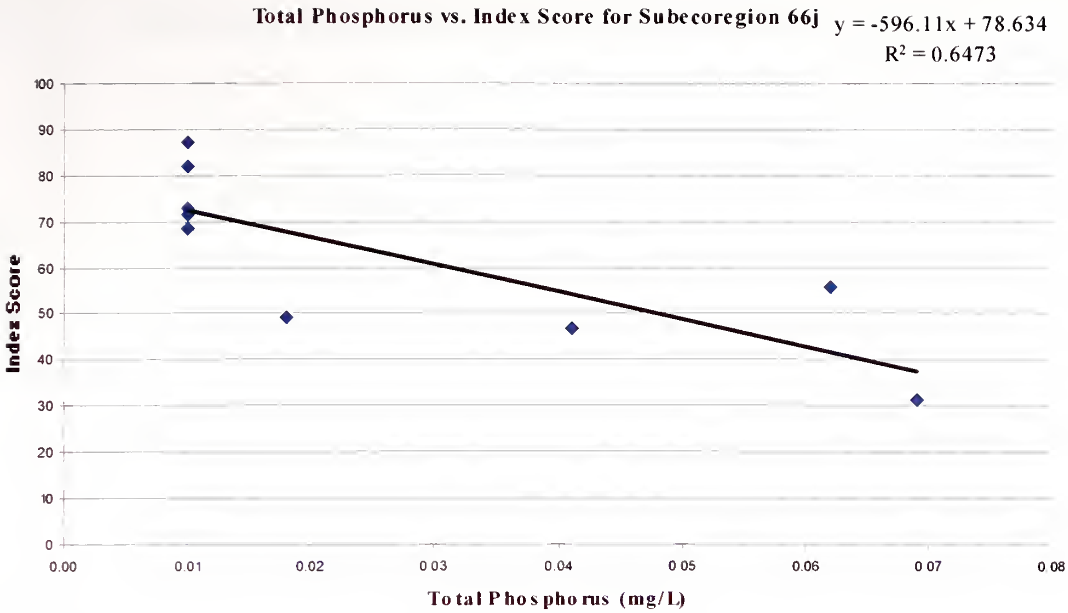


Figure 12: Total Phosphorus vs. Index Score for Subecoregion 66j

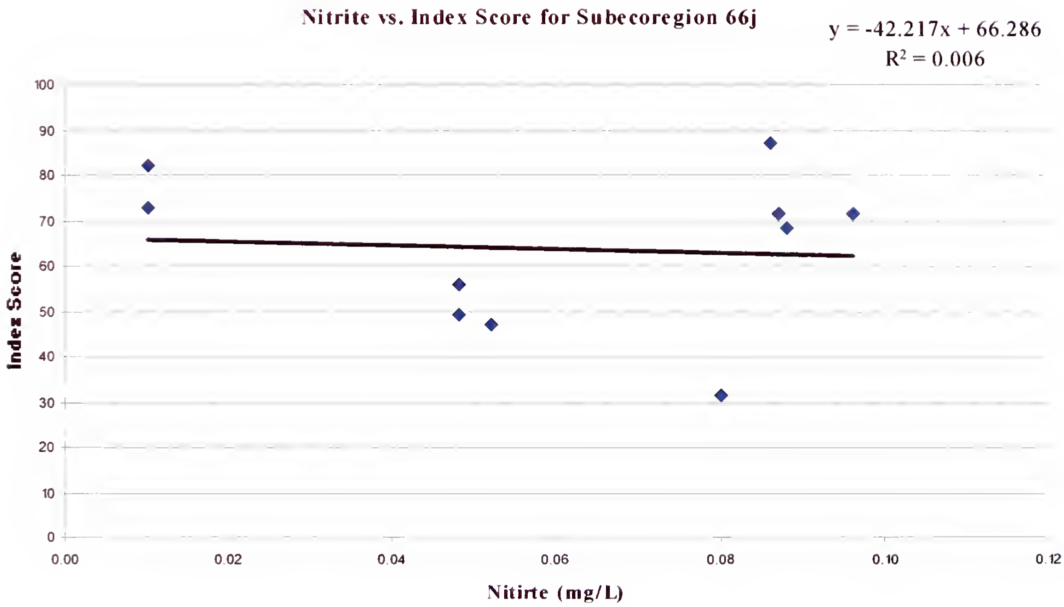


Figure 13: Nitrite vs. Index Score for Subecoregion 66j

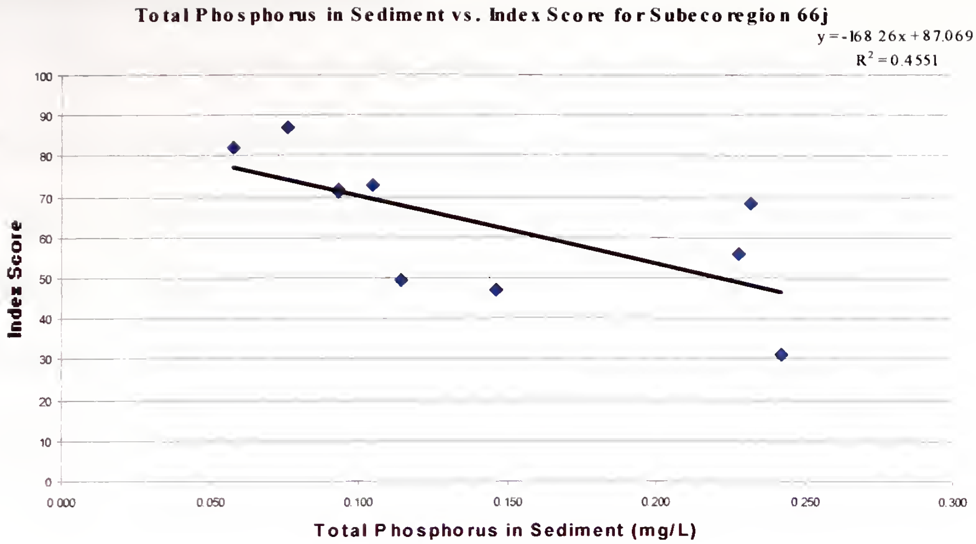


Figure 14: Total Phosphorus in Sediment vs. Index Score for Subecoregion 66j

Discrimination efficiency between reference and impaired sites was 100%, with all impaired sites being classified correctly based on the reference condition. The box and whisker plot illustrated no overlap between reference and impaired sites, thus indicating complete discrimination for the metrics (Figure 15).

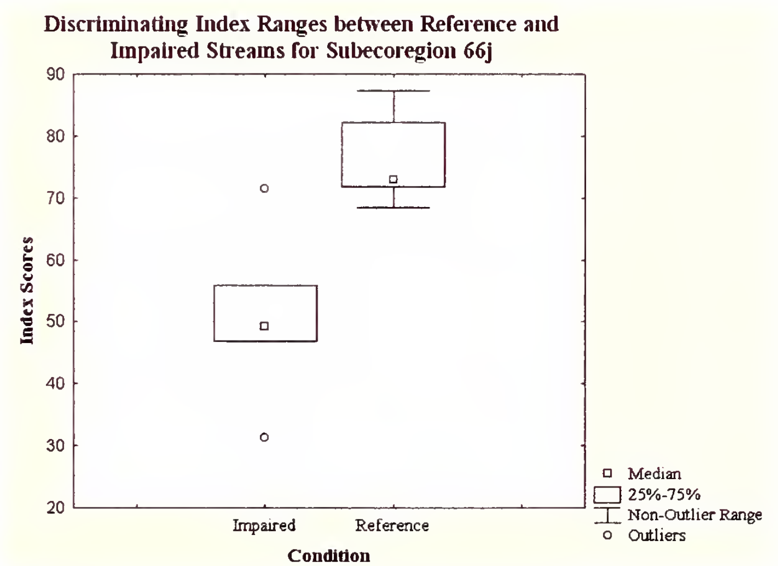


Figure 15: Discriminating Index Ranges between Reference and Impaired Streams for Subecoregion 66j

**Ecoregion 67 (Ridge and Valley)**

Total phosphorus in the water column was significantly different between reference and impaired sites (Table 48). Ammonia, nitrate-nitrite, and total phosphorus in the water column and total phosphorus in sediment were not found to be significantly different. Cluster analysis did not distinguish between reference and impaired macroinvertebrate communities at the ecoregion level (Figure 16). Total phosphorus concentrations in the water column were positively correlated with the macroinvertebrate multi-metric index scores (Table 49). Figure 17 is the scatter plot for total phosphorus in water column vs. index score.

**Table 48: Mann - Whitney U Test for Ecoregion 67**

Red marked tests are significant at p <.05000

| | Z | p-level | Z adjusted | p-level | 2*1sided exact p |
|---|---|---|---|---|---|
| Ammonia | 1.85164 | 0.064078 | 1.88014 | 0.060090 | 0.067266 |
| Nitrite | -1.72305 | 0.084880 | -1.77413 | 0.076042 | 0.084927 |
| Nitrate-Nitrite | 1.69734 | 0.089634 | 1.69763 | 0.089579 | 0.095012 |
| Total Phosphorus | -2.80318 | 0.005060 | -3.20038 | 0.001373 | 0.003708 |
| TP Sediment | 57.00000 | 0.553912 | 0.579640 | 0.579546 | 0.607524 |

**Table 49: Spearman Rank Correlation Test - Nutrient Concentration vs. Index Score for Ecoregion 67**

Red marked tests are significant at p<.05000

| **Variable** | Ammonia | Nitrite | Nitrate-Nitrite | Total Phosphorus | TP Sediment |
|---|---|---|---|---|---|
| Index Score | -0.270771 | 0.340751 | -0.072833 | 0.617493 | -0.136993 |

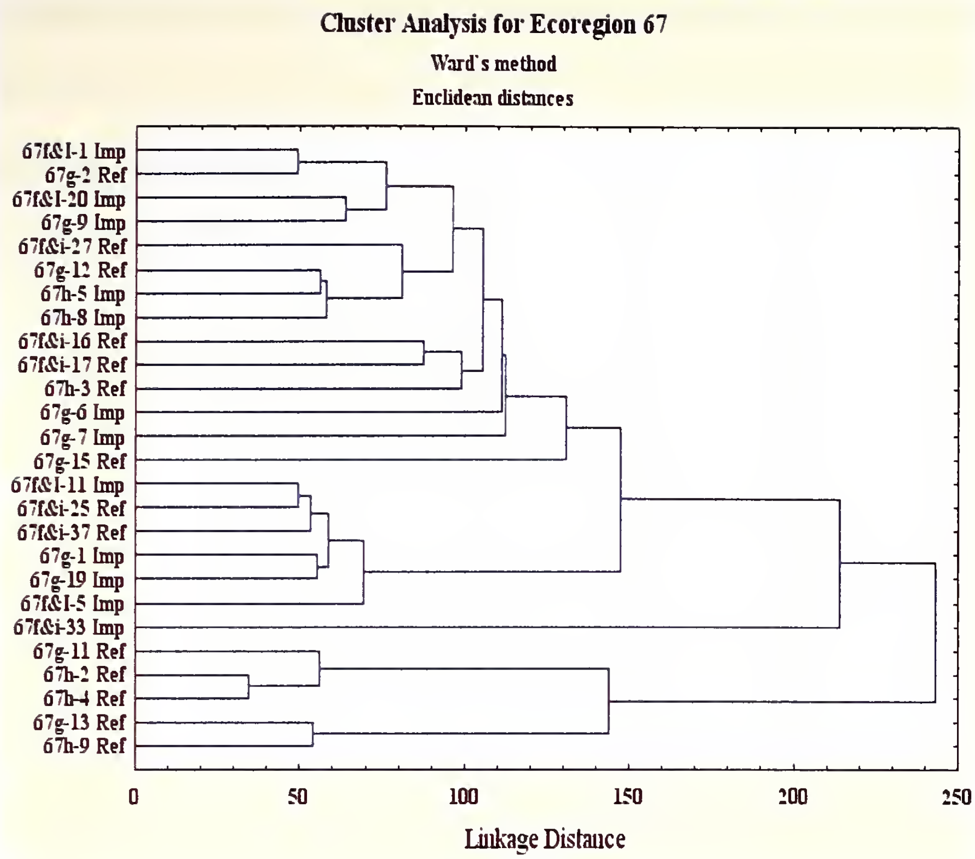


Figure 16: Cluster Analysis for Ecoregion 67

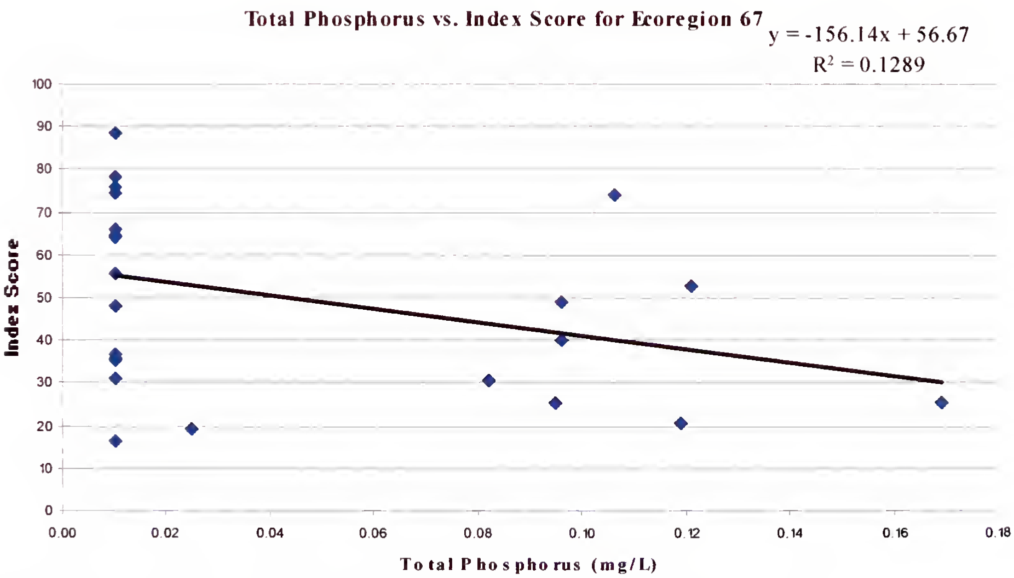


Figure 17: Total Phosphorus vs. Index Score for Ecoregion 67

Discrimination efficiency between reference and impaired sites was 92%, with 11 of the 12 impaired sites being classified correctly. The box and whisker plot illustrated no overlap of interquartile range, but some overall overlap between reference and impaired sites (Figure 18).

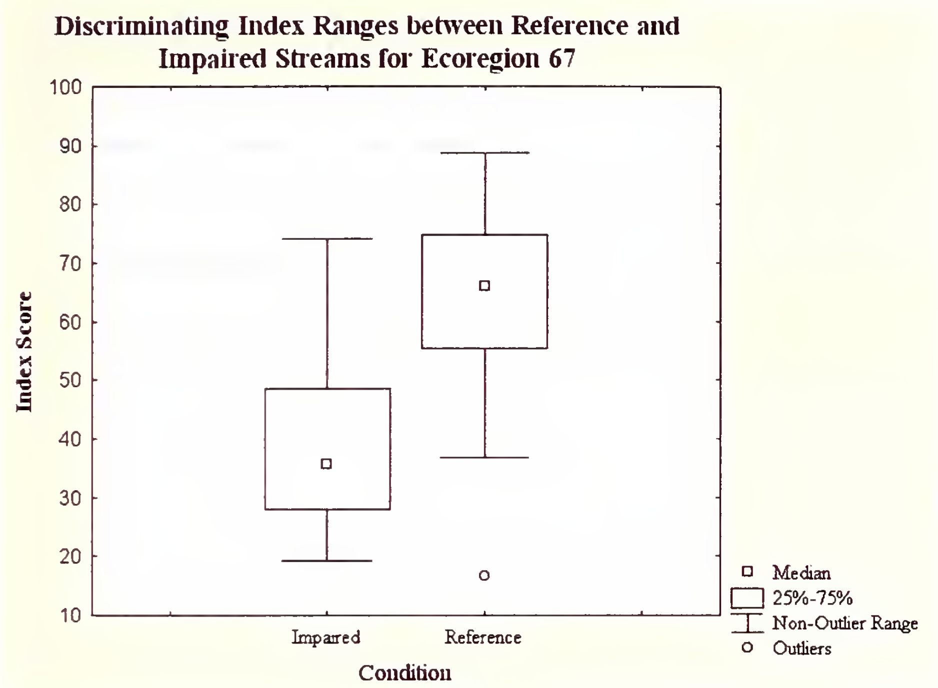


Figure 18: Discriminating Index Ranges between Reference and Impaired Streams for Ecoregion 67

## Subecoregion 67 f&i (Southern Limestone /Dolomite Valleys and Low Rolling Hills and Southern Dissected Ridges and Knobs)

Ammonia, nitrite, nitrate-nitrite, and total phosphorus in the water column and total phosphorus in sediment were not found to be significantly different between reference and impaired sites (Table 50). Cluster analysis did not distinguish between reference and impaired macroinvertebrate communities (Figure 19). Total phosphorus concentrations were positively correlated with the macroinvertebrate multi-metric index scores (Table 51). Figure 20 is the scatter plot for total phosphorus in water column vs. index score.

**Table 50: Mann - Whitney U Test for Subecoregion 67f&i**

Red marked tests are significant at p <.05000

| | Z | p-level | Z adjusted | p-level | 2*1sided exact p |
|---|---|---|---|---|---|
| Ammonia | 1.25336 | 0.210076 | 1.25717 | 0.208691 | 0.222222 |
| Nitrite | -1.25336 | 0.210076 | -1.29316 | 0.195957 | 0.222222 |
| Nitrate-Nitrite | 1.14891 | 0.250593 | 1.15241 | 0.249153 | 0.309524 |
| Total Phosphorus | -1.04447 | 0.296271 | -1.49071 | 0.136038 | 0.309524 |
| TP Sediment | 0.00 | 1.000000 | 0.00 | 1.000000 | 1.114286 |

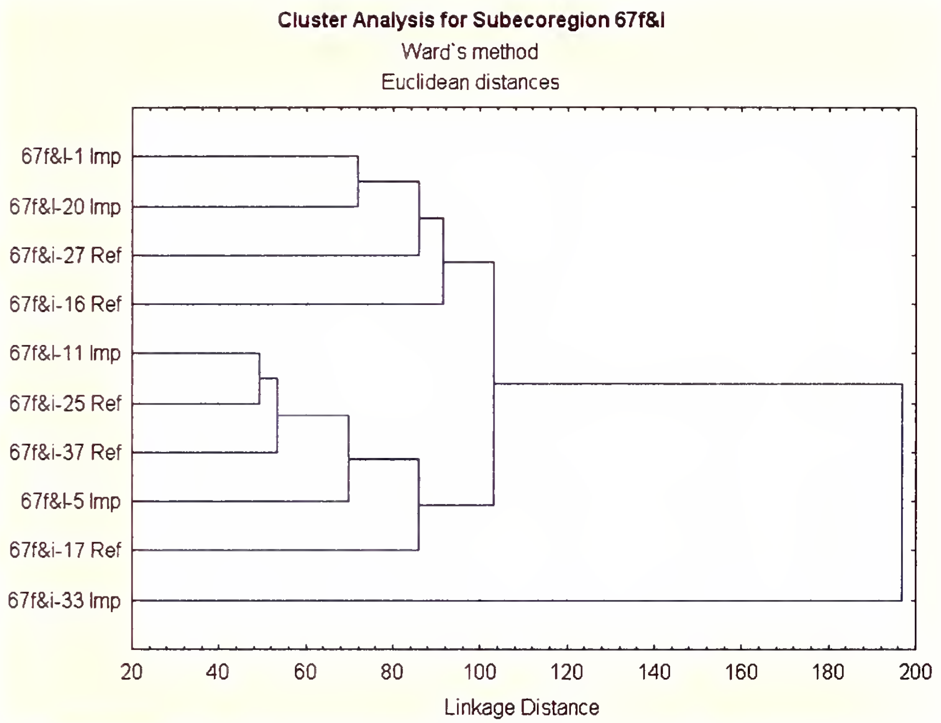


Figure 19: Cluster Analysis for Subecoregion 67f&i

**Table 51: Spearman Rank Correlation Test - Nutrient Concentration vs. Index Score for Subecoregion 67f&i**

Red marked tests are significant at p<.05000

| Variable | Ammonia | Nitrite | Nitrate-Nitrite | Total Phosphorus | TP Sediment |
|---|---|---|---|---|---|
| Index Score | -0.413376 | 0.118808 | -0.340427 | 0.700649 | -0.011976 |

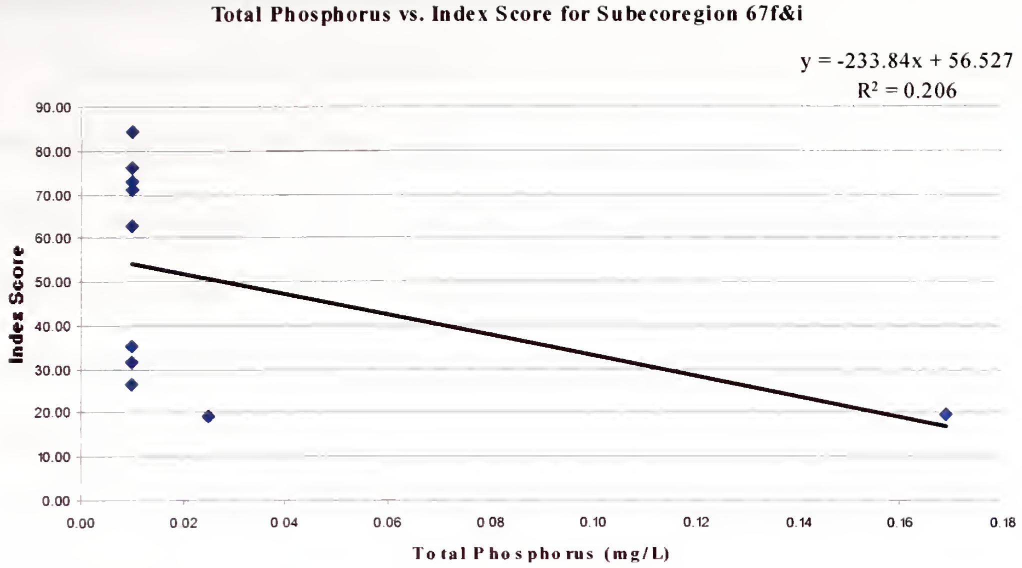


Figure 20: Total Phosphorus vs. Index Score for Subecoregion 67f&i

Discrimination efficiency between reference and impaired sites was 100%, with all impaired sites being classified correctly based on the reference condition. The box and whisker plot illustrated no overlap between reference and impaired sites, thus indicating complete discrimination for the metrics (Figure 21).

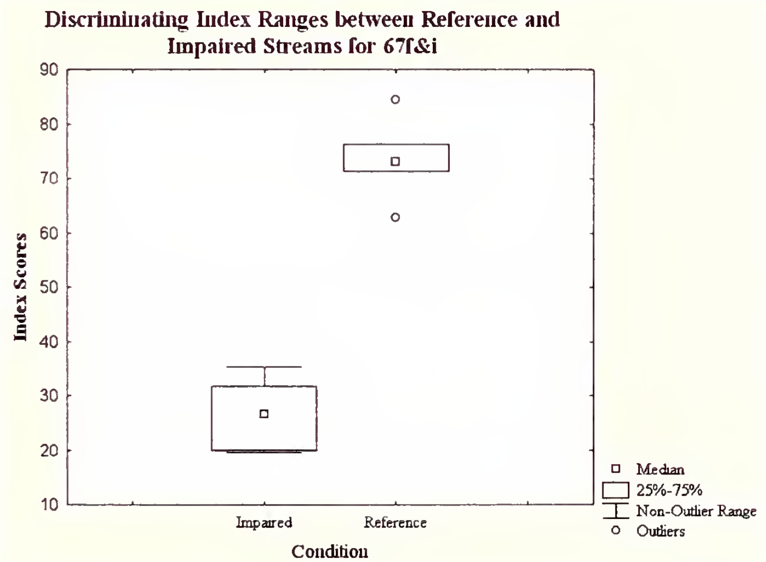


Figure 21: Discriminating Index Ranges between Reference and Impaired Streams for Subecoregion 67f&i

**Subecoregion 67g (Southern Shale Valleys)**

Ammonia, nitrite, nitrate-nitrite, and total phosphorus in the water column and total phosphorus in sediment were not found to be significantly different between reference and impaired sites (Table 52). Cluster analysis did not distinguish between reference and impaired macroinvertebrate communities (Figure 22). Total phosphorus concentrations in the water column were positively correlated and total phosphorus concentrations in the sediment were negatively correlated with the macroinvertebrate multi-metric index scores (Table 53). Figures 23 and 24 are the scatter plots for total phosphorus in water column vs. index score and total phosphorus in sediment vs. index score, respectively.

**Table 52: Mann - Whitney U Test for Subecoregion 67g**

Red marked tests are significant at p <.05000

| | Z | p-level | Z adjusted | p-level | 2*1sided exact p |
|---|---|---|---|---|---|
| Ammonia | 0.83557 | 0.403396 | 0.86211 | 0.388630 | 0.420635 |
| Nitrite | -0.94002 | 0.347208 | -0.95162 | 0.341288 | 0.420635 |
| Nitrate-Nitrite | 0.94002 | 0.347208 | 0.94002 | 0.347208 | 0.420635 |
| Total Phosphorus | -1.77559 | 0.075801 | -1.83791 | 0.066076 | 0.095238 |
| TP Sediment | 1.469694 | 0.141646 | 1.469694 | 0.141646 | 0.190476 |

**Table 53: Spearman Rank Correlation Test - Nutrient Concentration vs. Index Score for Subecoregion 67g**

Red marked tests are significant at p<.05000

| **Variable** | Ammonia | Nitrite | Nitrate-Nitrite | Total Phosphorus | TP Sediment |
|---|---|---|---|---|---|
| Index Score | -0.381436 | 0.546053 | 0.236364 | 0.696339 | -0.666667 |

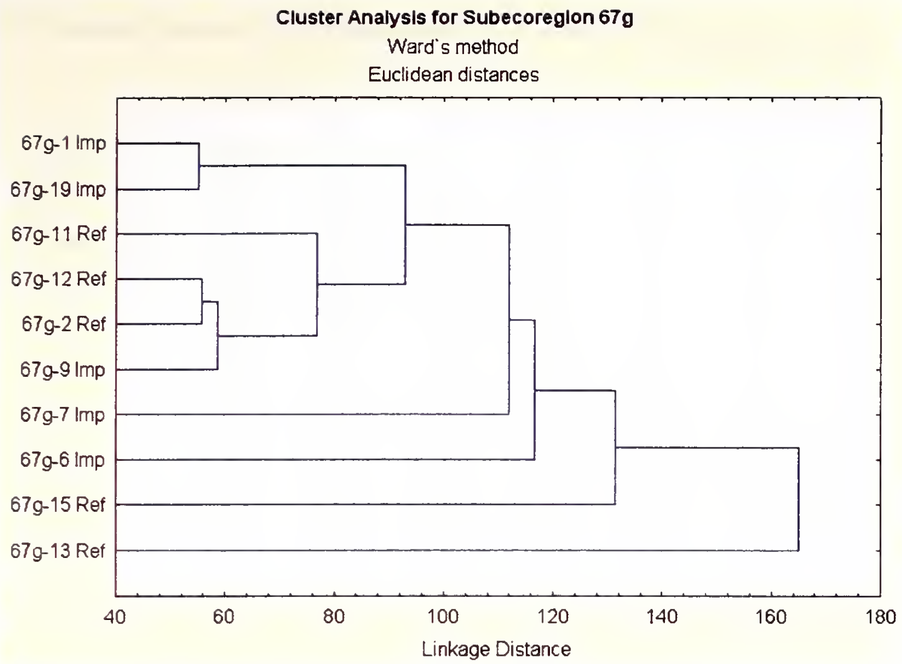


Figure 22: Cluster Analysis for Subecoregion 67g

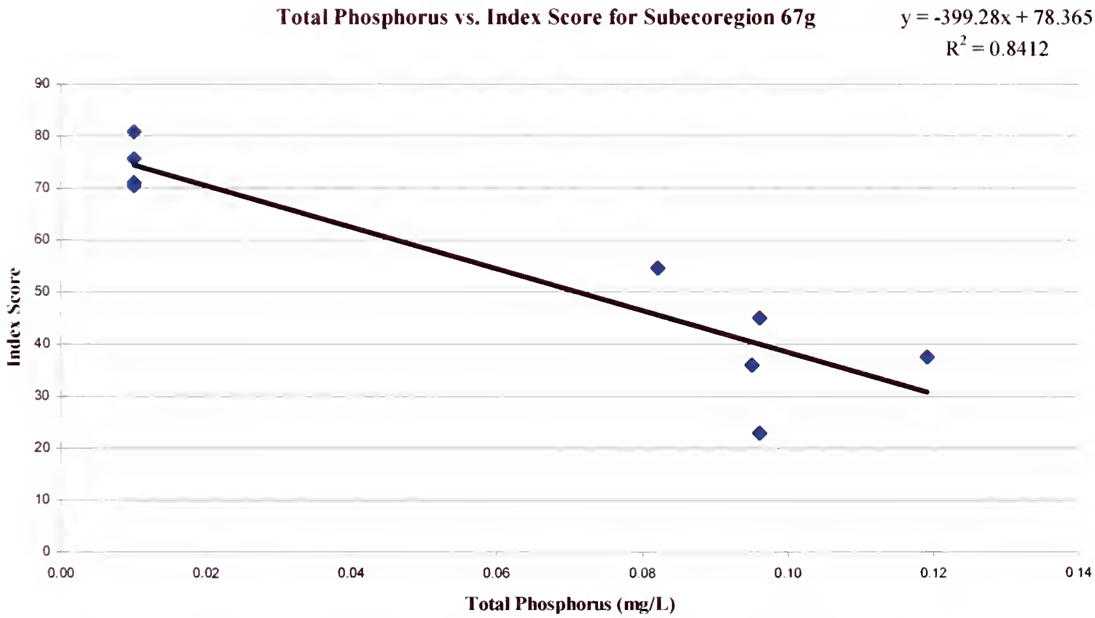


Figure 23: Total Phosphorus vs. Index Score for Subecoregion 67g

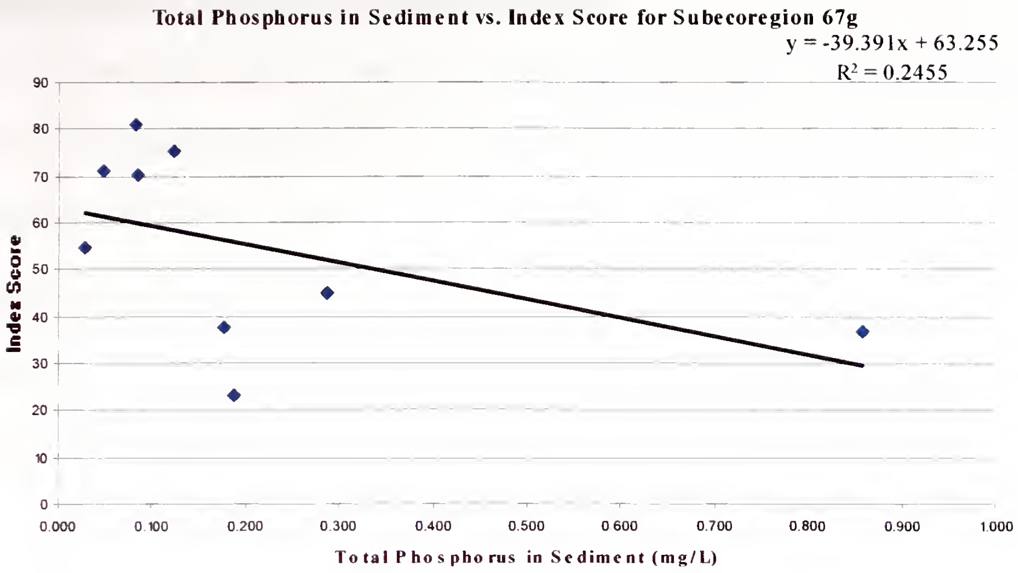


Figure 24: Total Phosphorus in Sediment vs. Index Score for Subecoregion 67g

Discrimination efficiency between reference and impaired sites was 100%, with all impaired sites being classified correctly based on the reference condition. The box and whisker plot illustrated no overlap between reference and impaired sites, thus indicating complete discrimination for the metrics (Figure 25).

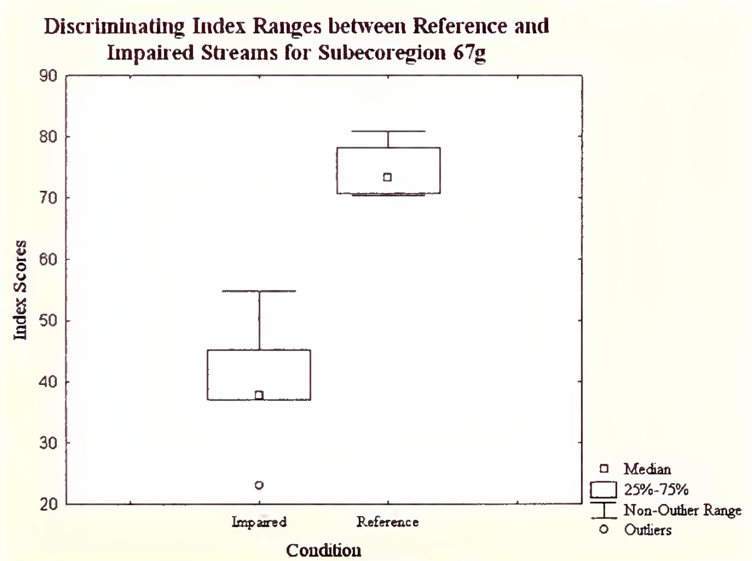


Figure 25: Discriminating Index Ranges between Reference and Impaired Streams for Subecoregion 67g

**Subecoregion 67h (Southern Sandstone Ridges)**

Ammonia, nitrite, nitrate-nitrite, and total phosphorus in the water column and total phosphorus in sediment were not found to be significantly different between reference and impaired sites (Table 54). Cluster analysis did distinguish between reference and impaired macroinvertebrate communities (Figure 26). No nutrient parameter was significantly correlated with the macroinvertebrate multi-metric index scores (Table 55).

**Table 54: Mann - Whitney U Test for Subecoregion 67h**

Red marked tests are significant at p <.05000

| | Z | p-level | Z adjusted | p-level | 2*1sided exact p |
|---|---|---|---|---|---|
| Ammonia | -1.15728 | 0.247161 | -1.17417 | 0.240328 | 0.266667 |
| Nitrite | 0.92582 | 0.354540 | 0.98374 | 0.325245 | 0.533333 |
| Nitrate-Nitrite | -0.92582 | 0.354540 | -0.92582 | 0.354540 | 0.533333 |
| Total Phosphorus | 1.85164 | 0.064078 | 2.19089 | 0.028460 | 0.133333 |
| TP Sediment | -0.925820 | 0.354540 | -0.939336 | 0.347559 | 0.533333 |

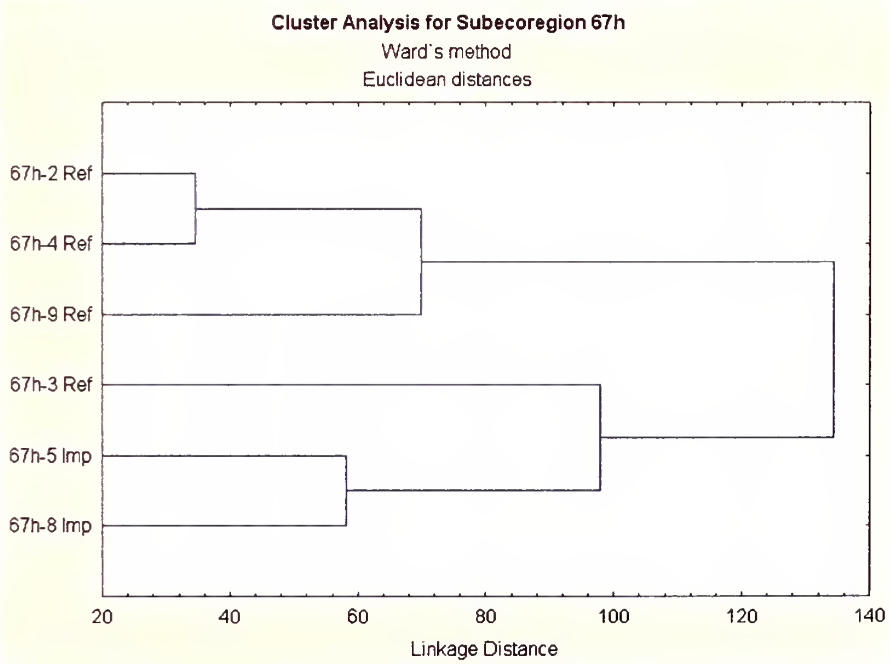


Figure 26: Cluster Analysis for Subecoregion 67h

**Table 55: Spearman Rank Correlation Test - Nutrient Concentration vs. Index Score for Subecoregion 67h**

Red marked tests are significant at p<.05000

| **Variable** | Ammonia | Nitrite | Nitrate-Nitrite | Total Phosphorus | TP Sediment |
|---|---|---|---|---|---|
| Index Score | -0.028989 | 0.455383 | 0.142857 | 0.777542 | 0.057977 |

Discrimination efficiency between reference and impaired sites was 100%, with all impaired sites being classified correctly based on the reference condition. The box and whisker plot illustrated no overlap between reference and impaired sites, thus indicating complete discrimination for the metrics (Figure 27).

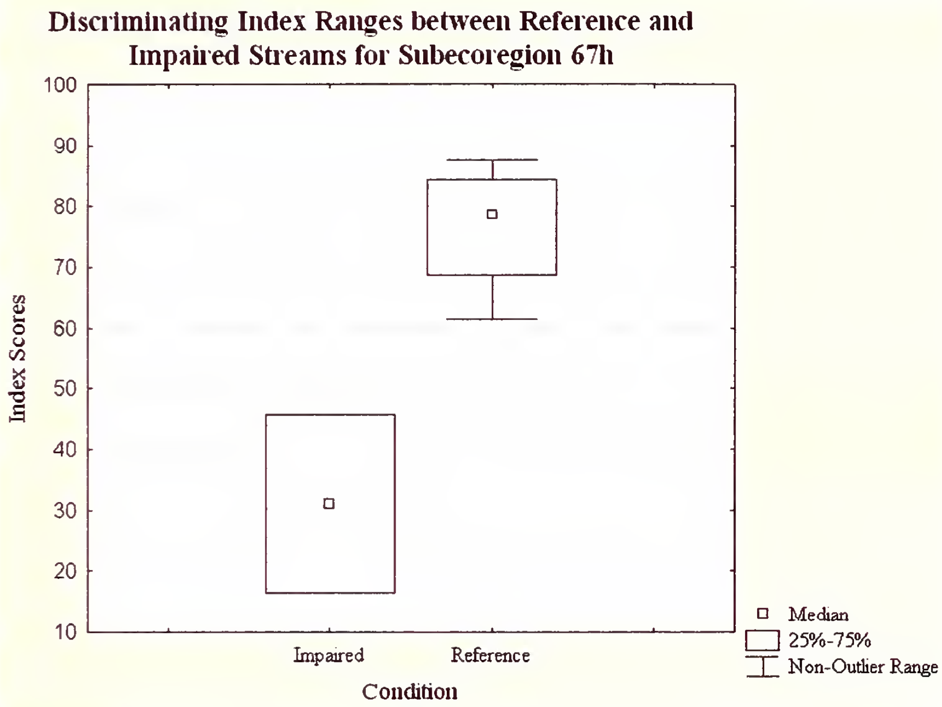


Figure 27: Discriminating Index Ranges between Reference and Impaired Streams for Subecoregion 67h

## Ecoregion 68 (Southwestern Appalachians) and Subecoregion 68c&d

In the State of Georgia, there is only one subecoregion in Southwestern

Appalachians ecoregion (Plateau Escarpment & Southern Table Plateaus subecoregion). Ammonia, nitrite, nitrate-nitrite, and total phosphorus in the water column and total phosphorus in sediment were not found to be significantly different between reference and impaired sites (Table 56). Cluster analysis did distinguish between reference and impaired macroinvertebrate communities, with the exception of one impaired site (Figure 28). Total phosphorus concentrations in the water column were positively correlated with the macroinvertebrate multi-metric index scores (Table 57). Figure 29 is the scatter plot for total phosphorus in the water column vs. index score.

**Table 56: Mann - Whitney U - Test for Ecoregion 68 and Subecoregion 68c&d**
Red marked tests are significant at p <.05000

| | Z | p-level | Z adjusted | p-level | 2*1sided exact p |
|---|---|---|---|---|---|
| Ammonia | 1.10227 | 0.270345 | 1.15128 | 0.249616 | 0.285714 |
| Nitrite | 0.97980 | 0.327188 | 1.16417 | 0.244356 | 0.412698 |
| Nitrate-Nitrite | -0.48990 | 0.624206 | -0.48990 | 0.624206 | 0.730159 |
| Total Phosphorus | -1.46969 | 0.141646 | -1.74626 | 0.080767 | 0.190476 |
| TP Sediment | 0.00 | 1.000000 | 0.00 | 1.000000 | 1.095328 |

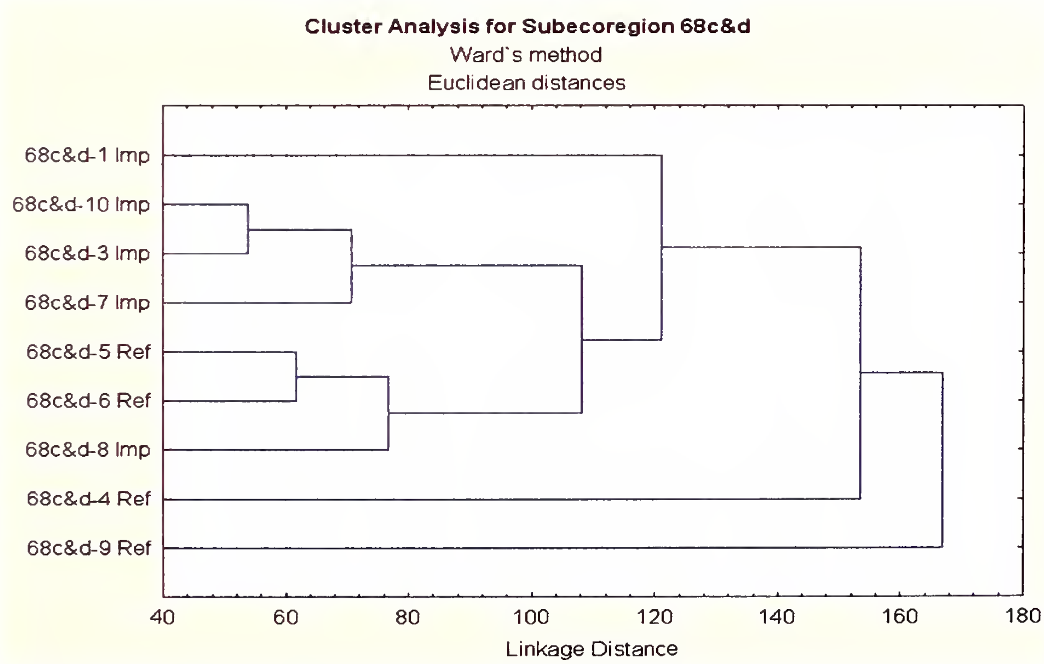


Figure 28: Cluster Analysis for Subecoregion 68c&d

| Table 57: Spearman Rank Correlation Test - Nutrient Concentration vs. Index Score for Subecoregion 68c&d | | | | | |
|---|---|---|---|---|---|
| Red marked tests are significant at p<.05000 | | | | | |
| **Variable** | Ammonia | Nitrite | Nitrate-Nitrite | Total Phosphorus | TP Sediment |
| Index Score | -0.130558 | -0.118818 | 0.433333 | 0.732709 | -0.033473 |

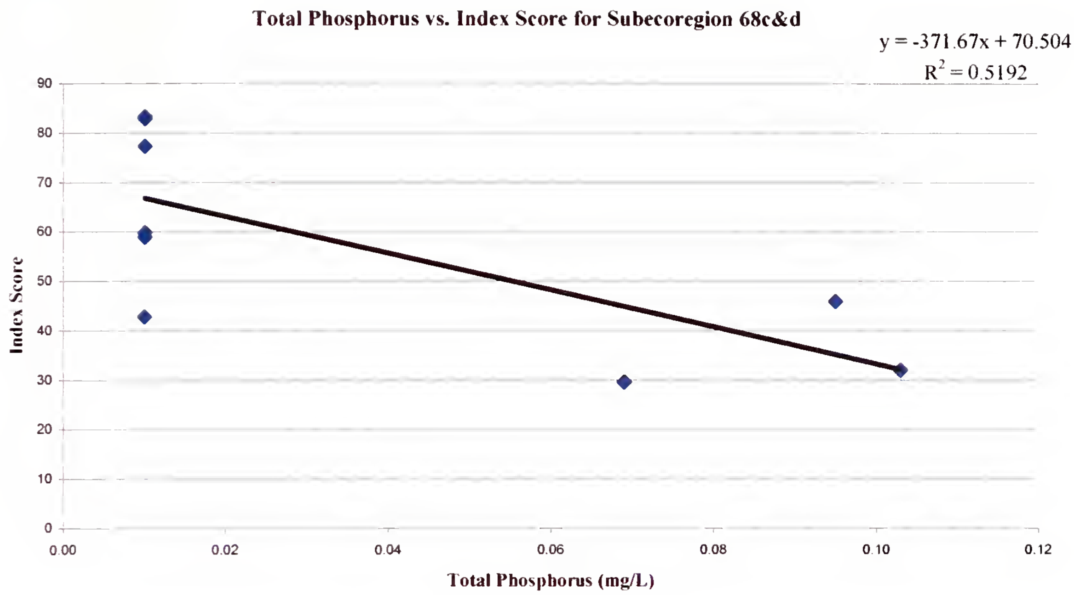


Figure 29: Total Phosphorus vs. Index Score for Subecoregion 68c&d

Discrimination efficiency between reference and impaired sites was 100%, with all impaired sites being classified correctly based on the reference condition. The box and whisker plot illustrated no overlap between reference and impaired sites, thus indicating complete discrimination for the metrics (Figure 30).

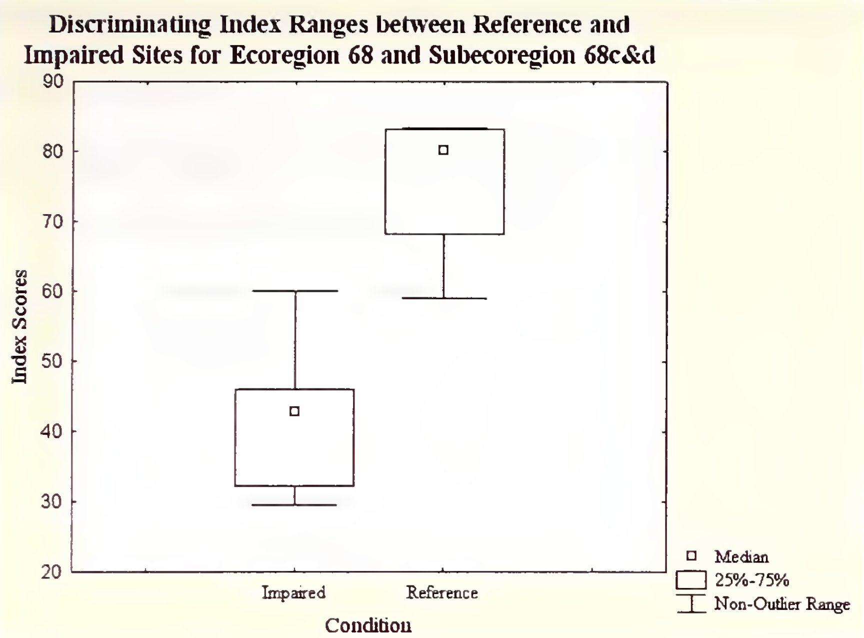


Figure 30: Discriminating Index Ranges between Reference and Impaired Sites for Ecoregion 68 and Subecoregion 68c&d

**Ecoregion 45 (Piedmont)**

Total phosphorus in the water column was significantly different between reference and impaired sites (Table 58). Ammonia, nitrate-nitrite, and nitrite in the water column and total phosphorous in the sediment were not found to be significantly different. Cluster analysis did not distinguish between reference and impaired macroinvertebrate communities at the ecoregion level (Figure 31). Total phosphorus concentrations in the water column were positively correlated with the macroinvertebrate multi-metric index scores (Table 59). Figure 32 is the scatter plot for total phosphorus in the water column vs. index score.

Discrimination efficiency between reference and impaired sites was 69%, with 18 of the 26 impaired sites being classified correctly based on the reference condition. The box and whisker plot illustrated some overlap of interquartile range between reference and impaired sites (Figure 33).

**Table 58: Mann - Whitney U - Test for Ecoregion 45**

Red marked tests are significant at p <.05000

| | Z | p-level | Z adjusted | p-level | 2*1sided exact p |
|---|---|---|---|---|---|
| Ammonia | -0.508740 | 0.610935 | -0.514155 | 0.607144 | 0.620438 |
| Nitrite | 1.874802 | 0.060821 | 1.981051 | 0.047586 | 0.060410 |
| Nitrate-Nitrite | -0.857321 | 0.391268 | -0.860540 | 0.389492 | 0.394487 |
| Total Phosphorus | 2.892282 | 0.003825 | 3.165816 | 0.001547 | 0.003248 |
| TP Sediment | 0.350839 | 0.725710 | 0.350886 | 0.725674 | 0.728109 |

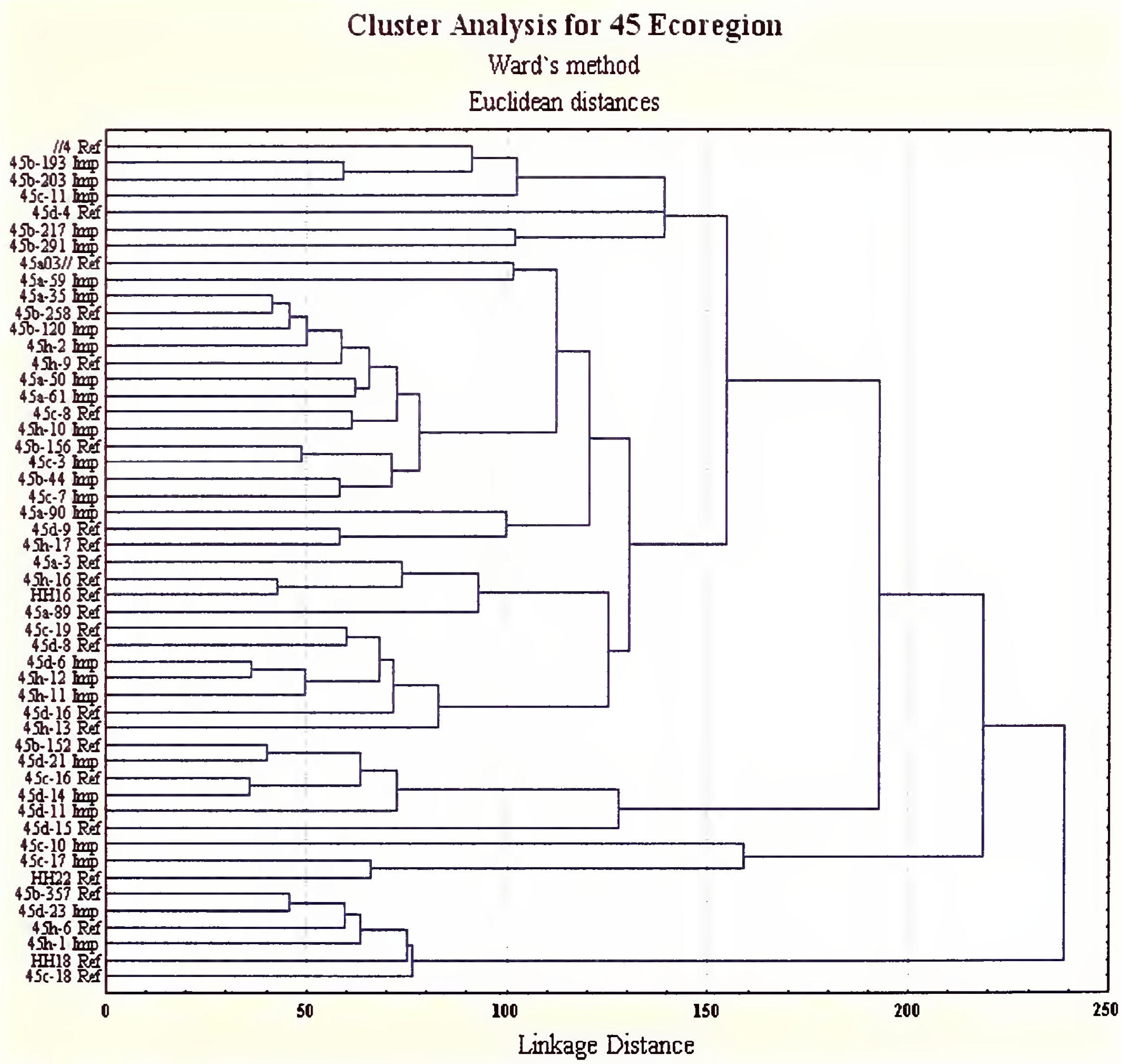


Figure 31: Cluster Analysis for 45 Ecoregion

| Table 59: Spearman Rank Correlation Test - Nutrient Concentration vs. Index Score for Ecoregion 45 | | | | | |
|---|---|---|---|---|---|
| Red marked tests are significant at p<.05000 | | | | | |
| **Variable** | Ammonia | Nitrite | Nitrate-Nitrite | Total Phosphorus | TP Sediment |
| Index Score | -0.066355 | 0.101890 | -0.134530 | 0.451351 | 0.000123 |

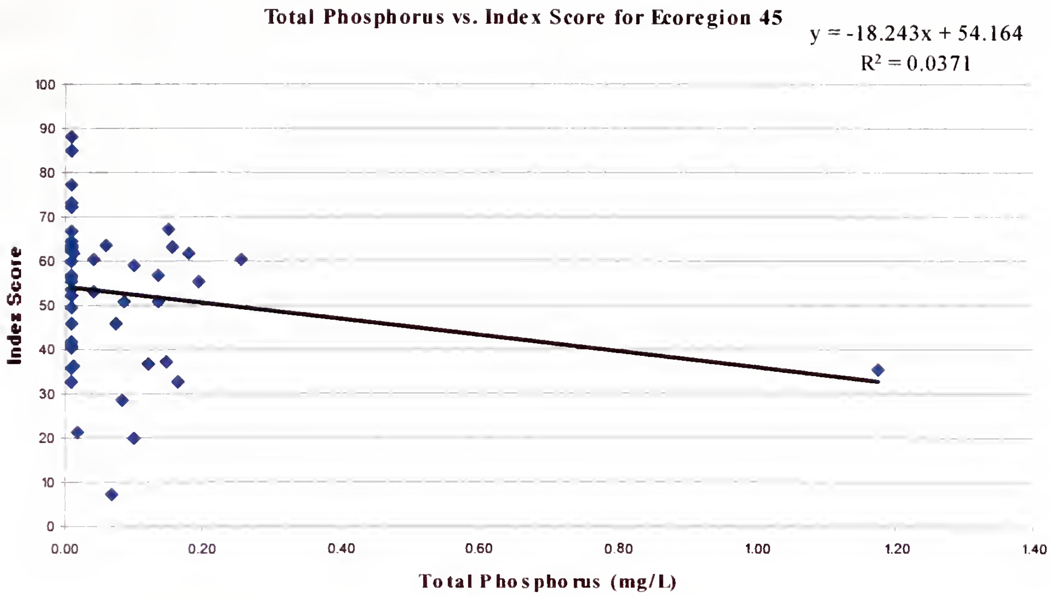


Figure 32: Total Phosphorus vs. Index Score for Ecoregion 45

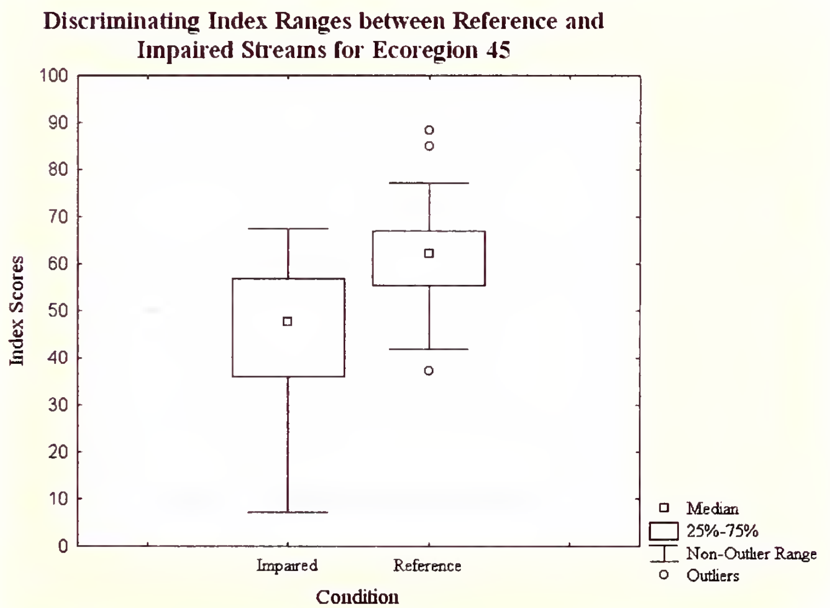


Figure 33: Discriminating Index Ranges between Reference and Impaired Streams for Ecoregion 45

**Subecoregion 45a (Southern Inner Piedmont)**

Total phosphorus in the water column was significantly different between reference and impaired sites (Table 60). Ammonia, nitrate-nitrite, and nitrite in the water column and total phosphorous in the sediment were not found to be significantly different. Cluster analysis did distinguish between reference and impaired macroinvertebrate communities, with the exception of one reference site (Figure 34). Total phosphorus concentrations in the water column were positively correlated with the macroinvertebrate multi-metric index scores (Table 61). Figure 35 is the scatter plot for total phosphorus in the water column vs. index score.

**Table 60: Mann - Whitney U - Test for Subecoregion 45a**
Red marked tests are significant at p <.05000

| | Z | p-level | Z adjusted | p-level | 2*1sided exact p |
|---|---|---|---|---|---|
| Ammonia | 1.044466 | 0.296271 | 1.047645 | 0.294803 | 0.309524 |
| Nitrite | 1.566699 | 0.117186 | 1.616448 | 0.105999 | 0.150794 |
| Nitrate-Nitrite | 0.522233 | 0.601509 | 0.528681 | 0.597027 | 0.690476 |
| Total Phosphorus | 2.088932 | 0.036715 | 2.353394 | 0.018604 | 0.031746 |
| TP Sediment | 0.522233 | 0.601509 | 0.522233 | 0.601509 | 0.690476 |

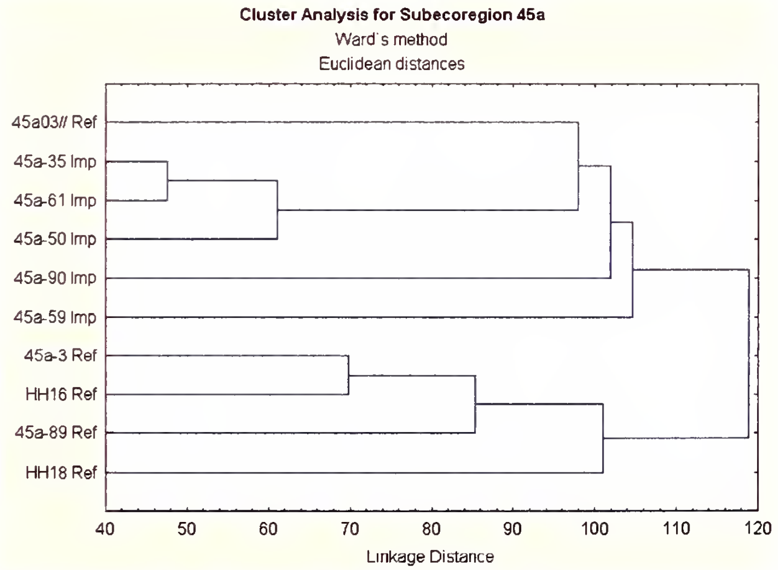


Figure 34: Cluster Analysis for Subecoregion 45a

**Table 61: Spearman Rank Correlation Test - Nutrient Concentration vs. Index Score for Subecoregion 45a**

Red marked tests are significant at p<.05000

| Variable | Ammonia | Nitrite | Nitrate-Nitrite | Total Phosphorus | TP Sediment |
|---|---|---|---|---|---|
| Index Score | 0.121581 | 0.256375 | -0.227011 | 0.873970 | -0.127273 |

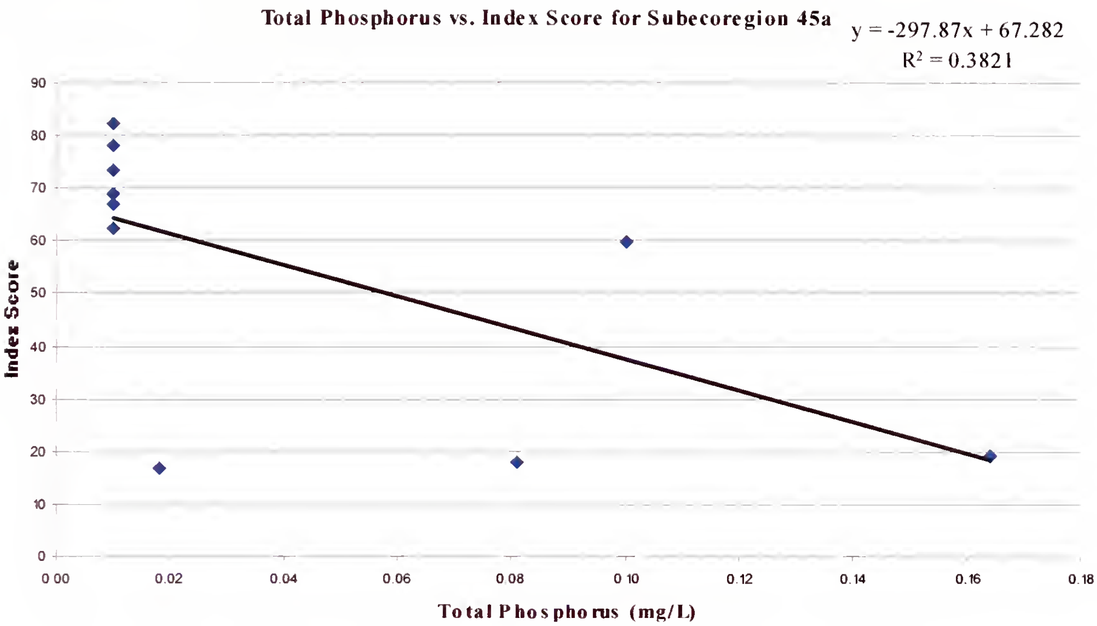


Figure 35: Total Phosphorus vs. Index Score for Subecoregion 45a

Discrimination efficiency between reference and impaired sites was 100%, with all impaired sites being classified correctly based on the reference condition. The box and whisker plot illustrated no overlap between reference and impaired sites, thus indicating complete discrimination for the metrics (Figure 36).

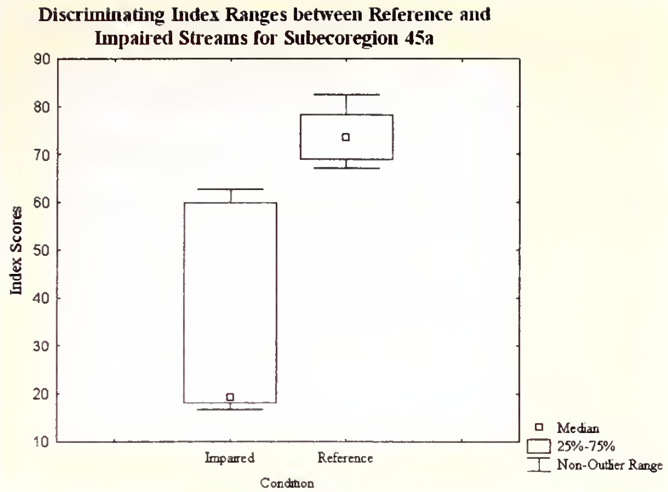


Figure 36: Discriminating Index Ranges between Reference and Impaired Streams for Subecoregion 45a

## Subecoregion 45b (Southern Outer Piedmont)

Nitrate-nitrite in the water column was significantly different between reference and impaired sites (Table 62). Ammonia, nitrite, and total phosphorus in the water column and total phosphorous in the sediment were not found to be significantly different. Cluster analysis did not distinguish between reference and impaired macroinvertebrate communities (Figure 37). Total phosphorus concentrations in the water column were positively correlated with the macroinvertebrate multi-metric index scores (Table 63). Figure 38 is the scatter plot for total phosphorus in the water column vs. index score.

**Table 62: Mann - Whitney U - Test for Subecoregion 45b**

Red marked tests are significant at p <.05000

| | Z | p-level | Z adjusted | p-level | 2*1sided exact p |
|---|---|---|---|---|---|
| Ammonia | 0.730297 | 0.465209 | 0.765942 | 0.443712 | 0.536797 |
| Nitrite | -0.365148 | 0.715001 | -0.382971 | 0.701742 | 0.792208 |
| Nitrate-Nitrite | 2.738613 | 0.006170 | 2.738613 | 0.006170 | 0.004329 |
| Total Phosphorus | -0.730297 | 0.465209 | -0.845841 | 0.397642 | 0.536797 |
| TP Sediment | 1.357806 | 0.174526 | 1.357806 | 0.174526 | 0.222222 |

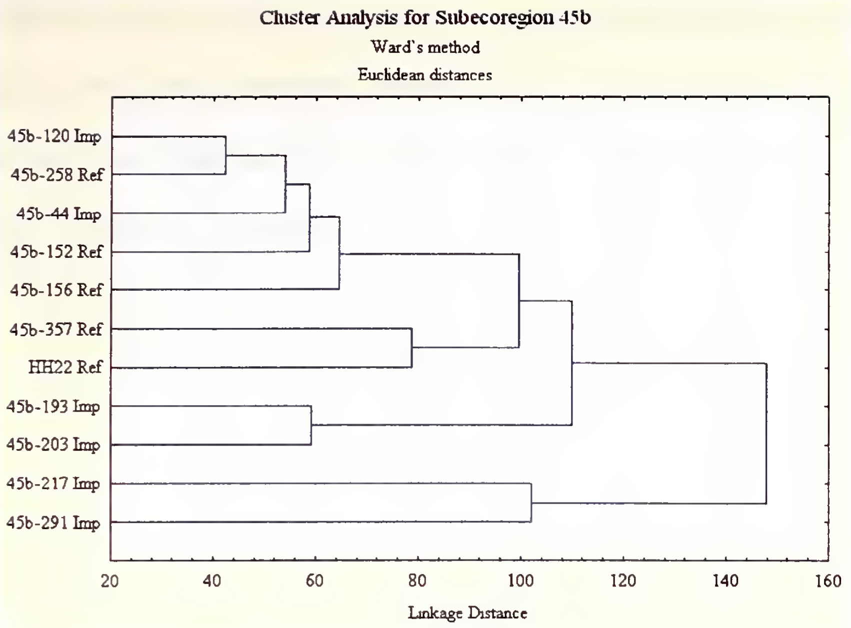


Figure 37: Cluster Analysis for Subecoregion 45b

| **Table 63: Spearman Rank Correlation Test - Nutrient Concentration vs. Index Score for Subecoregion 45b** Red marked tests are significant at p<.05000 | | | | | |
|---|---|---|---|---|---|
| **Variable** | Ammonia | Nitrite | Nitrate-Nitrite | Total Phosphorus | TP Sediment |
| Index Score | -0.2193 | 0.019069 | -0.718182 | 0.231643 | -0.418182 |

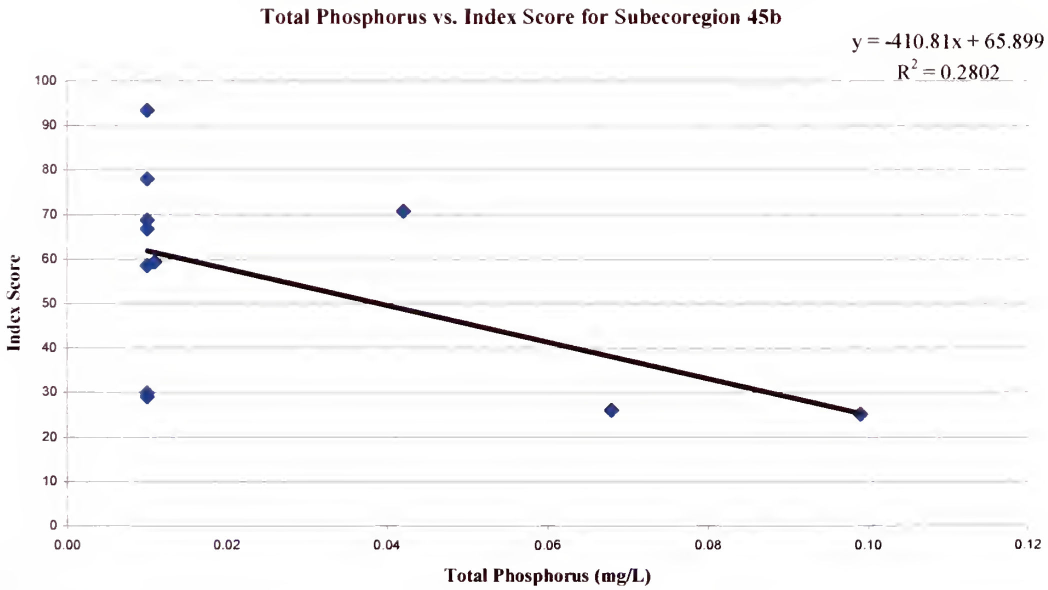


Figure 38: Total Phosphorus vs. Index Score for Subecoregion 45b

Discrimination efficiency between reference and impaired sites was 100%, with all impaired sites being classified correctly based on the reference condition. The box and whisker plot illustrated no overlap between reference and impaired sites, thus indicating complete discrimination for the metrics (Figure 39).

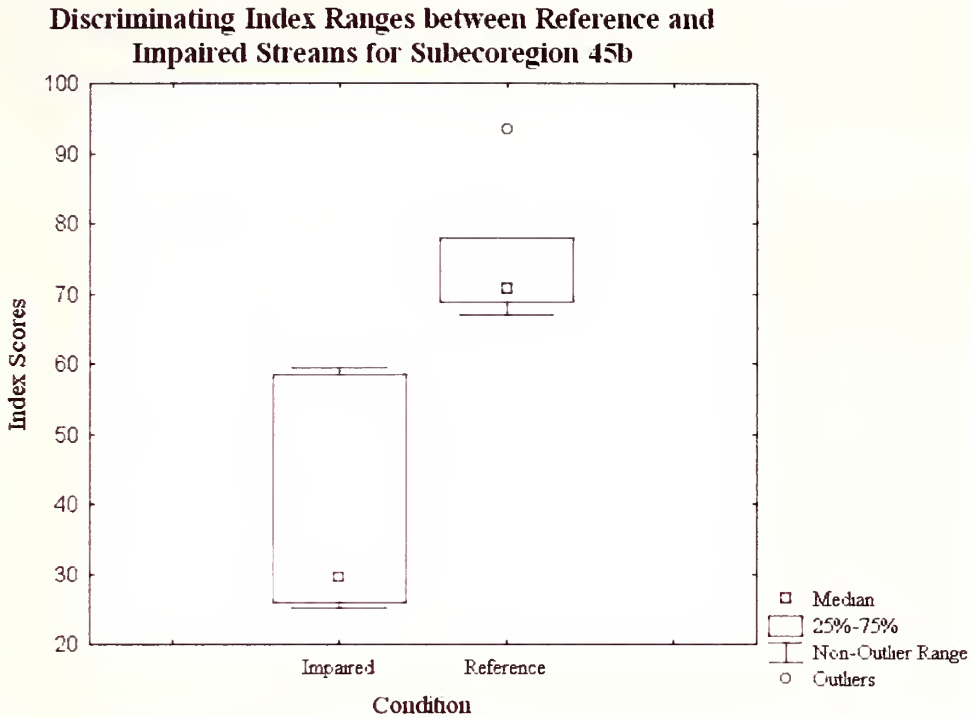


Figure 39: Discriminating Index Ranges between Reference and Impaired Streams for Subecoregion 45b

## Subecoregion 45c (Carolina Slate Belt)

Ammonia, nitrite, nitrate-nitrite, and total phosphorus in the water column and total phosphorus in sediment were not found to be significantly different between reference and impaired sites (Table 64). Cluster analysis did not distinguish between reference and impaired macroinvertebrate communities (Figure 40). No nutrient parameter was significantly correlated with the macroinvertebrate multi-metric index scores (Table 65).

| Table 64: Mann - Whitney U - Test for Subecoregion 45c<br>Red marked tests are significant at p <.05000 | | | | | |
|---|---|---|---|---|---|
| | Z | p-level | Z adjusted | p-level | 2*1sided exact p |
| Ammonia | -0.731126 | 0.464703 | -0.731126 | 0.464703 | 0.547619 |
| Nitrite | 0.104447 | 0.916815 | 0.108465 | 0.913627 | 1.000000 |
| Nitrate-Nitrite | 1.984485 | 0.047203 | 2.116927 | 0.034267 | 0.055556 |
| Total Phosphorus | -0.417786 | 0.676104 | -0.431053 | 0.666430 | 0.690476 |
| TP Sediment | -0.979796 | 0.327188 | -0.979796 | 0.327188 | 0.412698 |

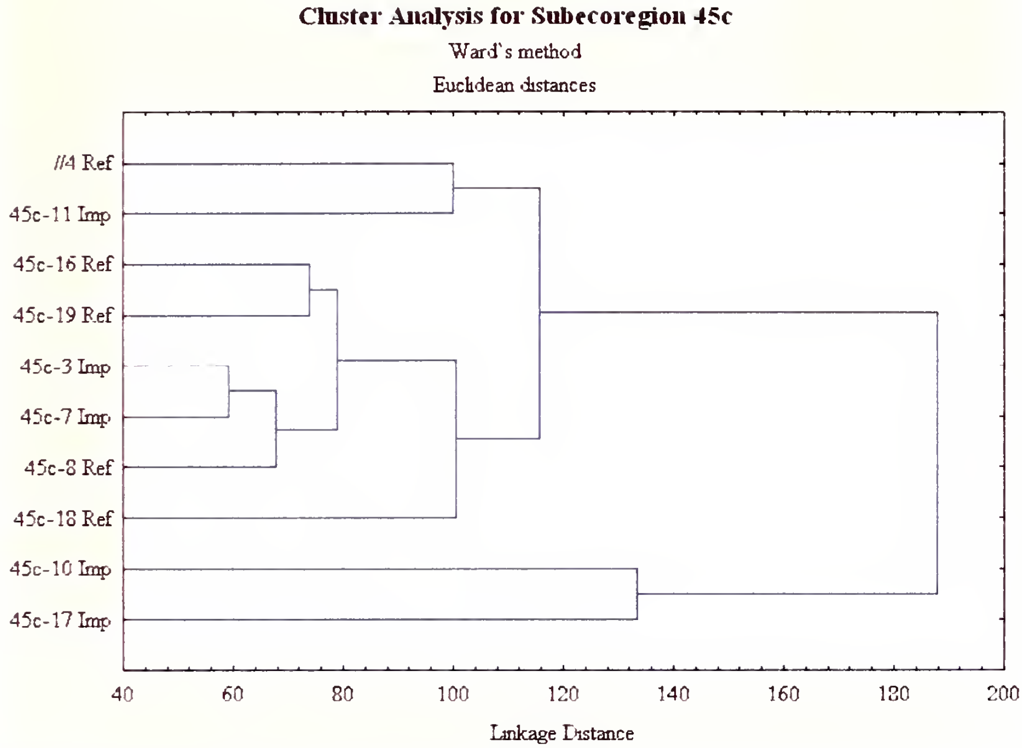


Figure 40: Cluster Analysis for Subecoregion 45c

| Table 65: Spearman Rank Correlation Test - Nutrient Concentration vs. Index Score for Subecoregion 45c<br>Red marked tests are significant at p<.05000 | | | | | |
|---|---|---|---|---|---|
| **Variable** | Ammonia | Nitrite | Nitrate-Nitrite | Total Phosphorus | TP Sediment |
| Index Score | -0.200000 | 0.132170 | 0.588322 | 0.212604 | 0.416667 |

Discrimination efficiency between reference and impaired sites was 80%, with four of the five impaired sites being classified correctly based on the reference condition. The box and whisker plot illustrated no overlap of interquartile range, but some overall overlap between reference and impaired sites (Figure 41).

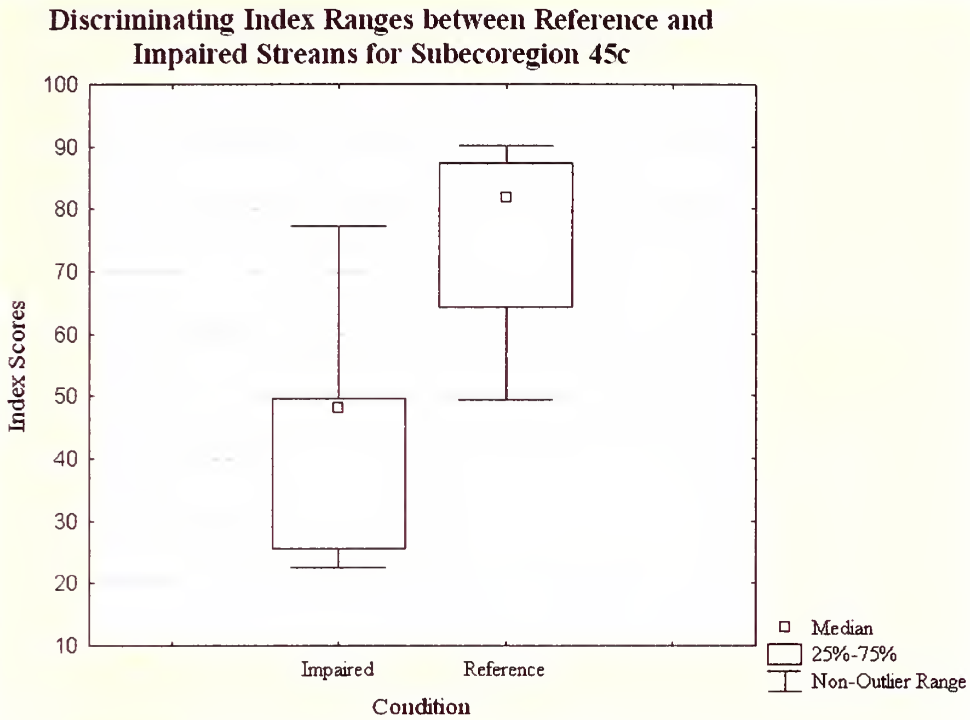


Figure 41: Discriminating Index Ranges between Reference and Impaired Streams for Subecoregion 45c

## Subecoregion 45d (Talladega Upland)

Total phosphorus in the water column was significantly different between reference and impaired sites (Table 66). Ammonia, nitrite, and nitrate-nitrite in the water column and total phosphorous in the sediment were not found to be significantly different. Cluster analysis did distinguish between reference and impaired macroinvertebrate communities, with the exception of one site (Figure 42). Total phosphorus concentrations in the water column were positively correlated with the macroinvertebrate multi-metric index scores (Table 67). Figure 43 is the scatter plot for total phosphorus in the water column vs. index score.

**Table 66: Mann - Whitney U - Test for Subecoregion 45d**

Red marked tests are significant at p <.05000

| | Z | p-level | Z adjusted | p-level | 2*1sided exact p |
|---|---|---|---|---|---|
| Ammonia | -0.31334 | 0.754023 | -0.31429 | 0.753298 | 0.841270 |
| Nitrite | -0.73113 | 0.464703 | -0.74015 | 0.459208 | 0.547619 |
| Nitrate-Nitrite | 0.52223 | 0.601509 | 0.52382 | 0.600402 | 0.690476 |
| Total Phosphorus | -2.61116 | 0.009024 | -2.69408 | 0.007059 | 0.007937 |
| TP Sediment | 0.00 | 1.000000 | 0.00 | 1.000000 | 1.095238 |

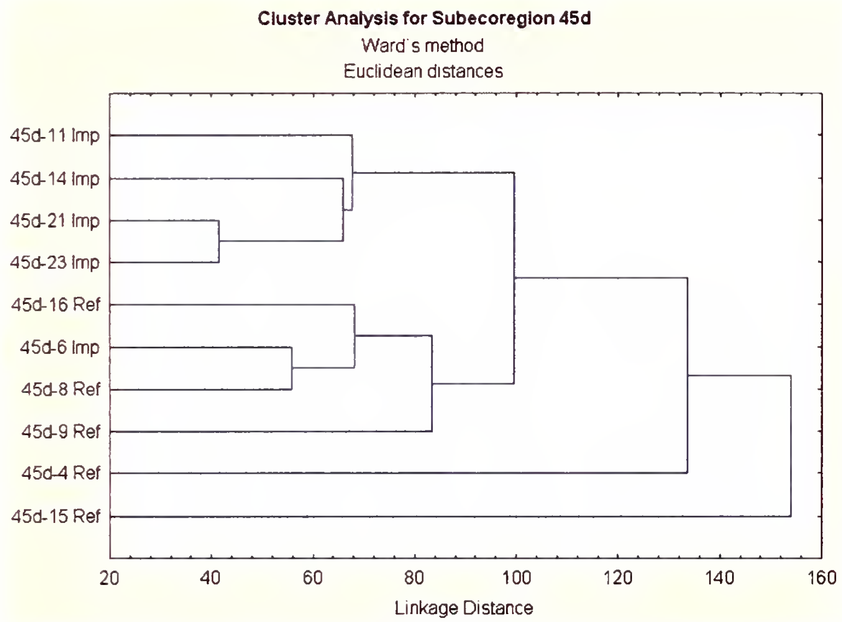


Figure 42: Cluster Analysis for Subecoregion 45d

**Table 67: Spearman Rank Correlation Test - Nutrient Concentration vs. Index Score for Subecoregion 45d**

Red marked tests are significant at p<.05000

| Variable | Ammonia | Nitrite | Nitrate-Nitrite | Total Phosphorus | TP Sediment |
|---|---|---|---|---|---|
| Index Score | -0.097265 | -0.042948 | -0.158055 | 0.775379 | -0.300000 |

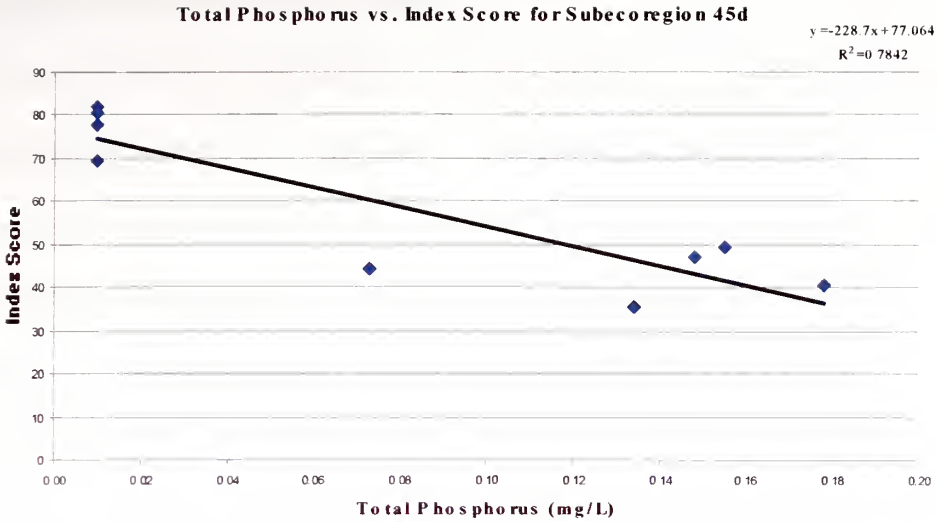


Figure 43: Total Phosphorus vs. Index Score for Subecoregion 45d

Discrimination efficiency between reference and impaired sites was 100%, with all impaired sites being classified correctly based on the reference condition. The box and whisker plot illustrated no overlap between reference and impaired sites, thus indicating complete discrimination for the metrics (Figure 44).

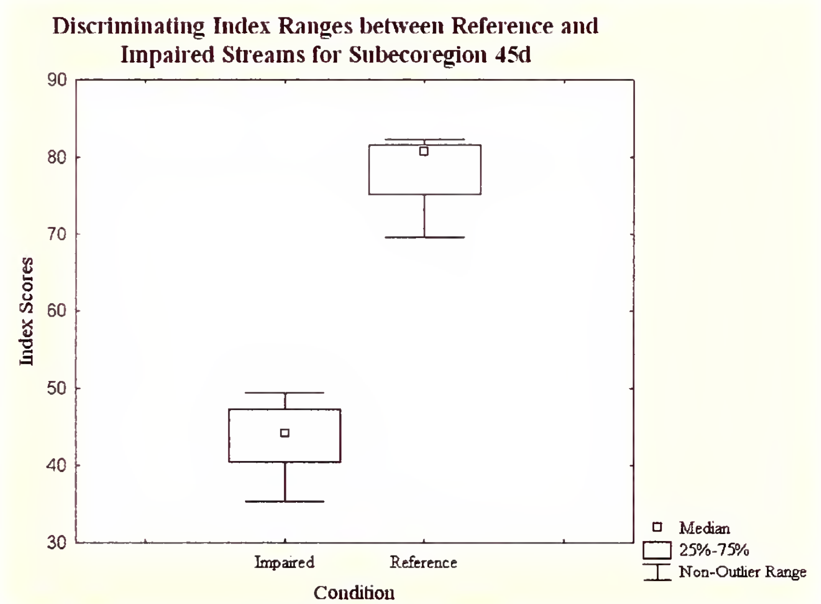


Figure 44: Discriminating Index Ranges between Reference and Impaired Streams for Subecoregion 45d

**Subecoregion 45h (Pine Mountain Ridge)**

Ammonia, nitrite, nitrate-nitrite, and total phosphorus in the water column and total phosphorus in sediment were not found to be significantly different between reference and impaired sites (Table 68). Cluster analysis did not distinguish between reference and impaired macroinvertebrate communities (Figure 45). Total phosphorus concentrations in the water column were positively correlated with the macroinvertebrate multi-metric index scores (Table 69).

**Table 68: Mann - Whitney U - Test for Subecoregion 45h**
Red marked tests are significant at p <.05000

| | Z | p-level | Z adjusted | p-level | 2*1sided exact p |
|---|---|---|---|---|---|
| Ammonia | 0.20889 | 0.834532 | 0.21553 | 0.829357 | 0.841270 |
| Nitrite | -1.04447 | 0.296271 | -1.49071 | 0.136038 | 0.309524 |
| Nitrate-Nitrite | 1.35781 | 0.174526 | 1.35781 | 0.174526 | 0.222222 |
| Total Phosphorus | -1.56670 | 0.117186 | -1.92759 | 0.053907 | 0.150794 |
| TP Sediment | -0.626680 | 0.530870 | -0.62859 | 0.529620 | 0.547619 |

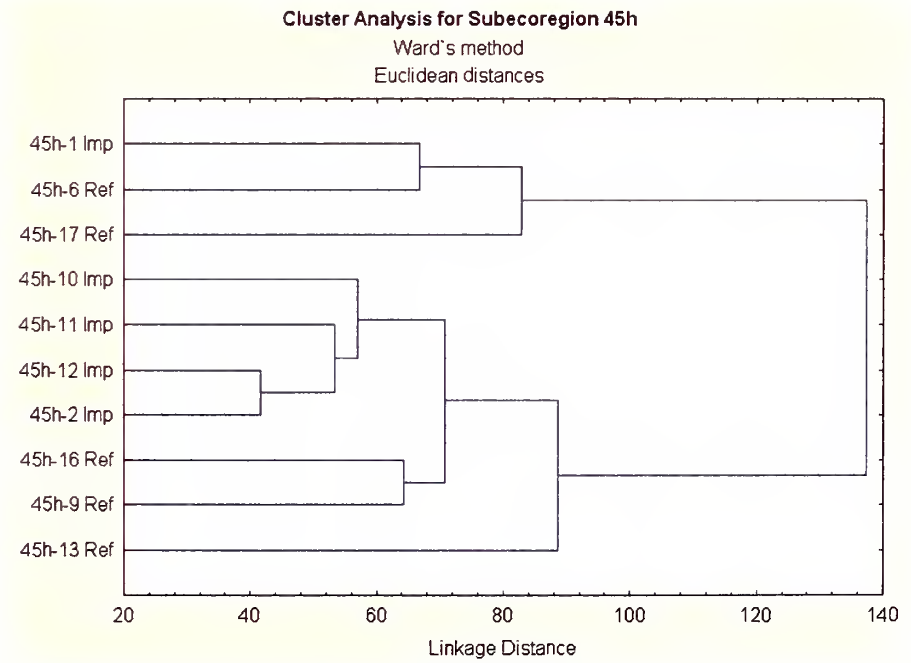


Figure 45: Cluster Analysis for Subecoregion 45h

**Table 69: Spearman Rank Correlation Test - Nutrient Concentration vs. Index Score for Subecoregion 45h**

Red marked tests are significant at p<.05000

| **Variable** | Ammonia | Nitrite | Nitrate-Nitrite | Total Phosphorus | TP Sediment |
|---|---|---|---|---|---|
| Index Score | -0.500240 | 0.311401 | -0.551515 | 0.320637 | 0.214286 |

Discrimination efficiency between reference and impaired sites was 100%, with all impaired sites being classified correctly based on the reference condition. The box and whisker plot illustrated no overlap of interquartile range, but some overall overlap between reference and impaired sites (Figure 46).

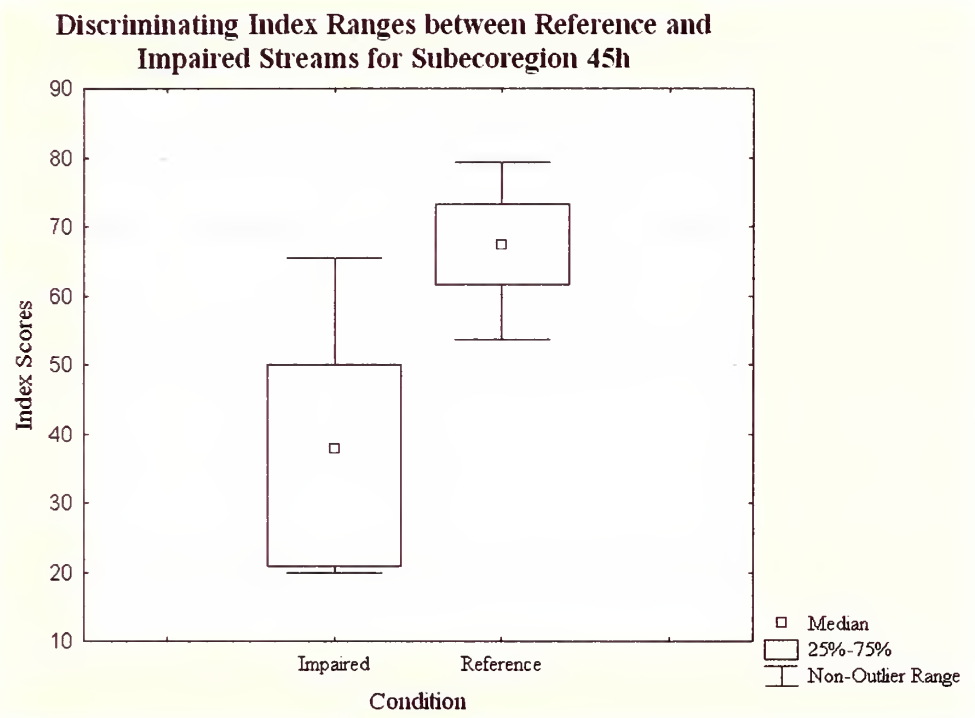


Figure 46: Discriminating Index Ranges between Reference and Impaired Streams for Subecoregion 45h

**Ecoregion 65 (Southeastern Plains)**

Nitrite in the water column was significantly different between reference and impaired sites (Table 70). Ammonia, nitrate-nitrite, and total phosphorus in the water column and total phosphorous in the sediment were not found to be significantly different. Cluster analysis did not distinguish between reference and impaired macroinvertebrate communities at the ecoregion level (Figure 47). Nitrite concentrations in the water column were positively correlated with the macroinvertebrate multi-metric index scores (Table 71). Figure 48 is the scatter plot for nitrite vs. index score.

**Table 70: Mann - Whitney U - Test for Ecoregion 65**

Red marked tests are significant at p <.05000

| | Z | p-level | Z adjusted | p-level | 2*1sided exact p |
|---|---|---|---|---|---|
| Ammonia | 0.85663 | 0.391648 | 0.86879 | 0.384965 | 0.396726 |
| Nitrite | -3.44743 | 0.000566 | -3.64762 | 0.000265 | 0.000448 |
| Nitrate-Nitrite | 0.69993 | 0.483970 | 0.70112 | 0.483228 | 0.489459 |
| Total Phosphorus | -0.86708 | 0.385899 | -1.10028 | 0.271209 | 0.390941 |
| TP Sediment | -1.13915 | 0.254643 | -1.14409 | 0.252587 | 0.261616 |

**Table 71: Spearman Rank Correlation Test - Nutrient Concentration vs. Index Score for Ecoregion 65**

Red marked tests are significant at p<.05000

| **Variable** | Ammonia | Nitrite | Nitrate-Nitrite | Total Phosphorus | TP Sediment |
|---|---|---|---|---|---|
| Index Score | 0.029656 | 0.378649 | -0.038096 | -0.024945 | 0.140031 |

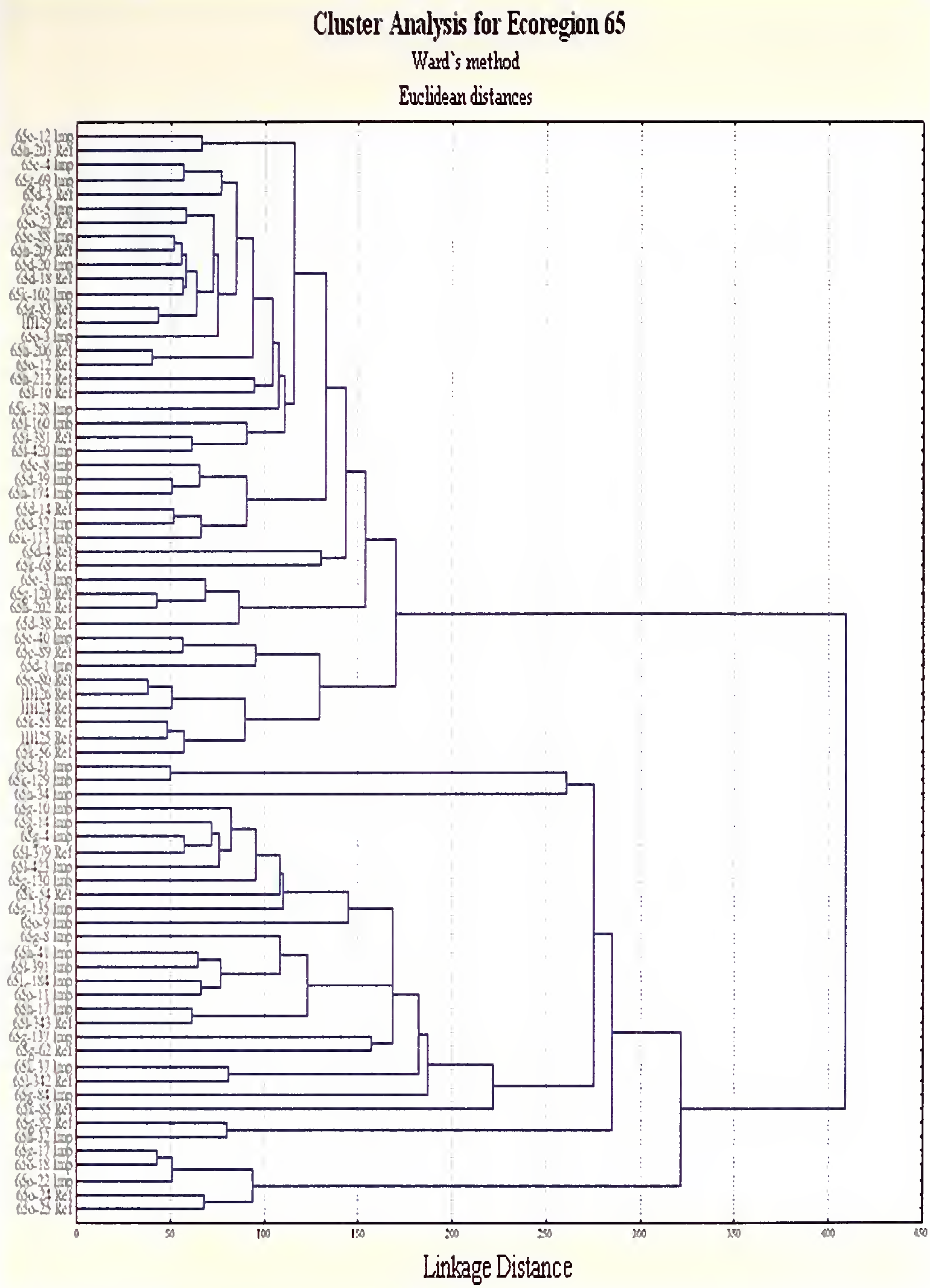


Figure 47: Cluster Analysis for Ecoregion 65

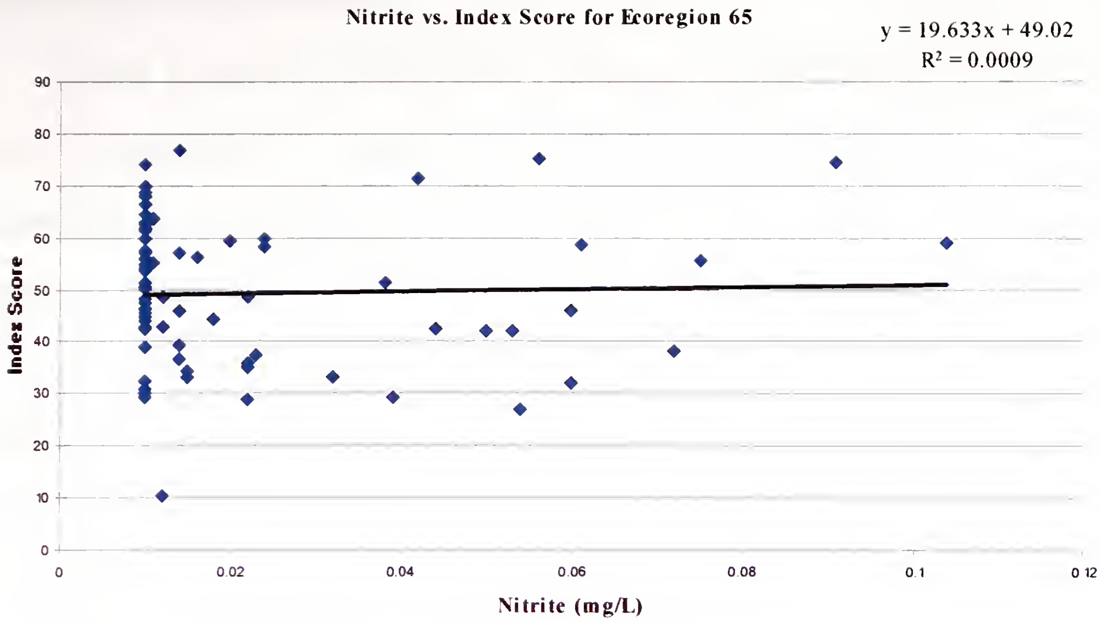


Figure 48: Nitrite vs. Index Score for Ecoregion 65

Discrimination efficiency between reference and impaired sites was 62%, with 26 of the 42 impaired sites being classified correctly. The box and whisker plot illustrated overlap of interquartile range between reference and impaired sites (Figure 49).

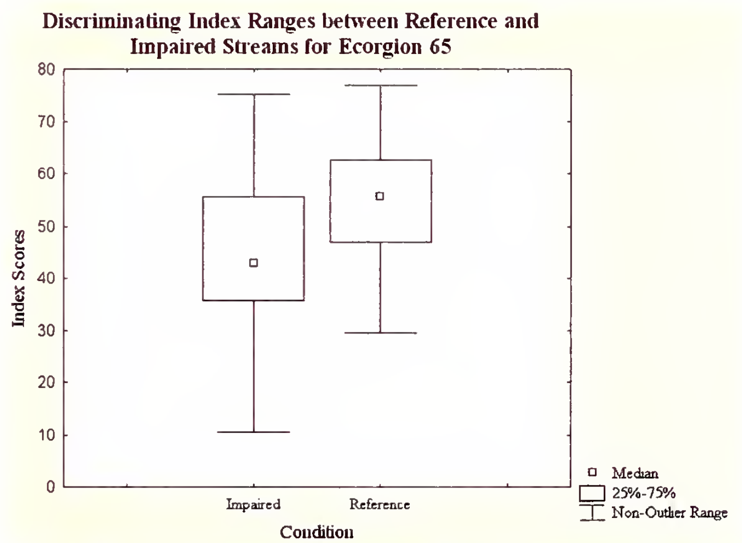


Figure 49: Discriminating Index Ranges between Reference and Impaired Streams for Ecoregion 65

**Subecoregion 65c (Sand Hills)**

Nitrite in the water column was significantly different between reference and impaired sites (Table 72). Ammonia, nitrate-nitrite, and total phosphorus in the water column and total phosphorous in the sediment were not found to be significantly different. Cluster analysis did distinguish between reference and impaired macroinvertebrate communities (Figure 50). Total phosphorus concentrations in sediment were positively correlated with the macroinvertebrate multi-metric index scores (Table 73). Figure 51 is the scatter plot for total phosphorus in sediment vs. index score.

**Table 72: Mann - Whitney U - Test for Subecoregion 65c**

Red marked tests are significant at p <.05000

| | Z | p-level | Z adjusted | p-level | 2*1sided exact p |
|---|---|---|---|---|---|
| Ammonia | 1.29920 | 0.193877 | 1.32252 | 0.185995 | 0.202020 |
| Nitrite | -2.02999 | 0.042358 | -2.26367 | 0.023595 | 0.047980 |
| Nitrate-Nitrite | 1.05560 | 0.291153 | 1.05560 | 0.291153 | 0.343434 |
| Total Phosphorus | -1.21800 | 0.223226 | -1.59873 | 0.109882 | 0.267677 |
| TP Sediment | -1.64317 | 0.100349 | -1.72337 | 0.084823 | 0.125541 |

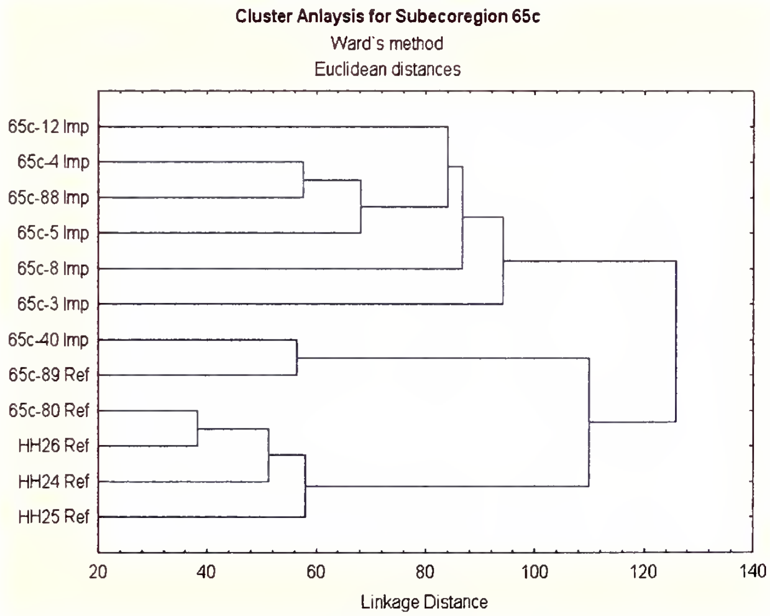


Figure 50: Cluster Analysis for Subecoregion 65c

| Table 73: Spearman Rank Correlation Test - Nutrient Concentration vs. Index Score for Subecoregion 65c<br>Red marked tests are significant at p<.05000 | | | | | |
|---|---|---|---|---|---|
| **Variable** | Ammonia | Nitrite | Nitrate-Nitrite | Total Phosphorus | TP Sediment |
| Index Score | 0.067626 | 0.405496 | -0.195804 | -0.064253 | 0.769345 |

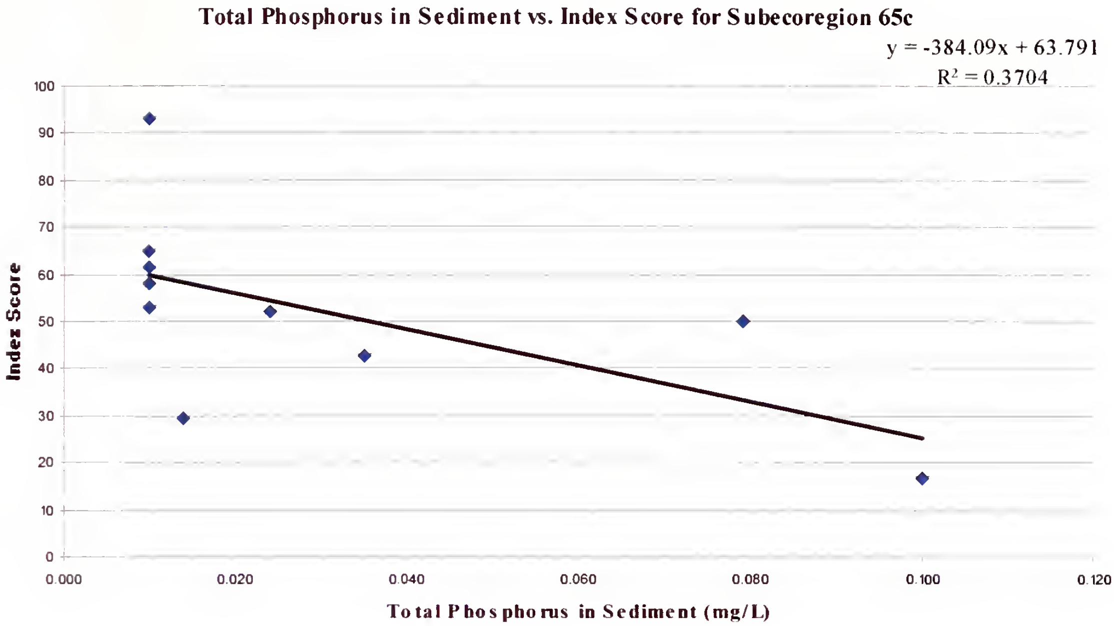


Figure 51: Total Phosphorus in Sediment vs. Index Score for Subecoregion 65c

Discrimination efficiency between reference and impaired sites was 86%, with six of seven impaired sites being classified correctly based on the reference condition. The box and whisker plot illustrated no overlap of interquartile range, but some overall overlap between reference and impaired sites (Figure 52).

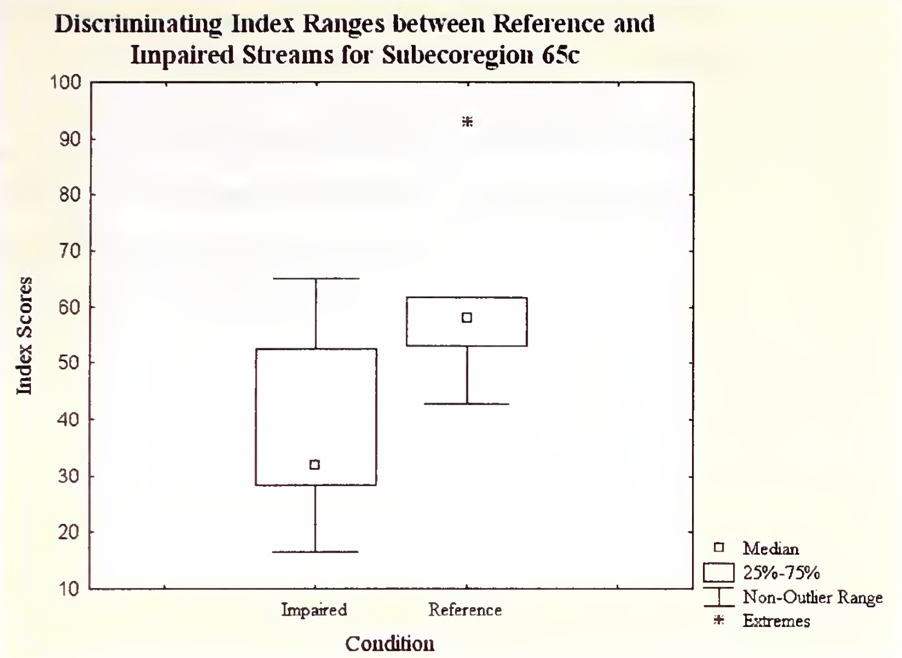


Figure 52: Discriminating Index Ranges between Reference and Impaired Streams for Subecoregion 65c

**Subecoregion 65d (Southern Hilly Gulf Coastal Plain)**

Total phosphorus in the water column was significantly different between reference and impaired sites (Table 74). Ammonia, nitrate-nitrite, and nitrite in the water column and total phosphorous in the sediment were not found to be significantly different. Cluster analysis did not distinguish between reference and impaired macroinvertebrate communities (Figure 53). Total phosphorus concentrations in the water column and nitrate-nitrite concentrations were positively correlated with the macroinvertebrate multi-metric index scores (Table 75). Figures 54 and 55 are the scatter plots for nitrate-nitrite vs. index score and total phosphorus in sediment vs. index score, respectively.

**Table 74: Mann - Whitney U - Test for Subecoregion 65d**

Red marked tests are significant at p <.05000

| | Z | p-level | Z adjusted | p-level | 2*1sided exact p |
|---|---|---|---|---|---|
| Ammonia | -0.31334 | 0.754023 | -0.33425 | 0.738190 | 0.841270 |
| Nitrite | 0.10445 | 0.916815 | 0.11180 | 0.910979 | 1.000000 |
| Nitrate-Nitrite | -1.14891 | 0.250593 | -1.15241 | 0.249153 | 0.309524 |
| Total Phosphorus | -2.08893 | 0.036715 | -2.36250 | 0.018153 | 0.031746 |
| TP Sediment | -0.313340 | 0.754023 | -0.323290 | 0.746476 | 0.841270 |

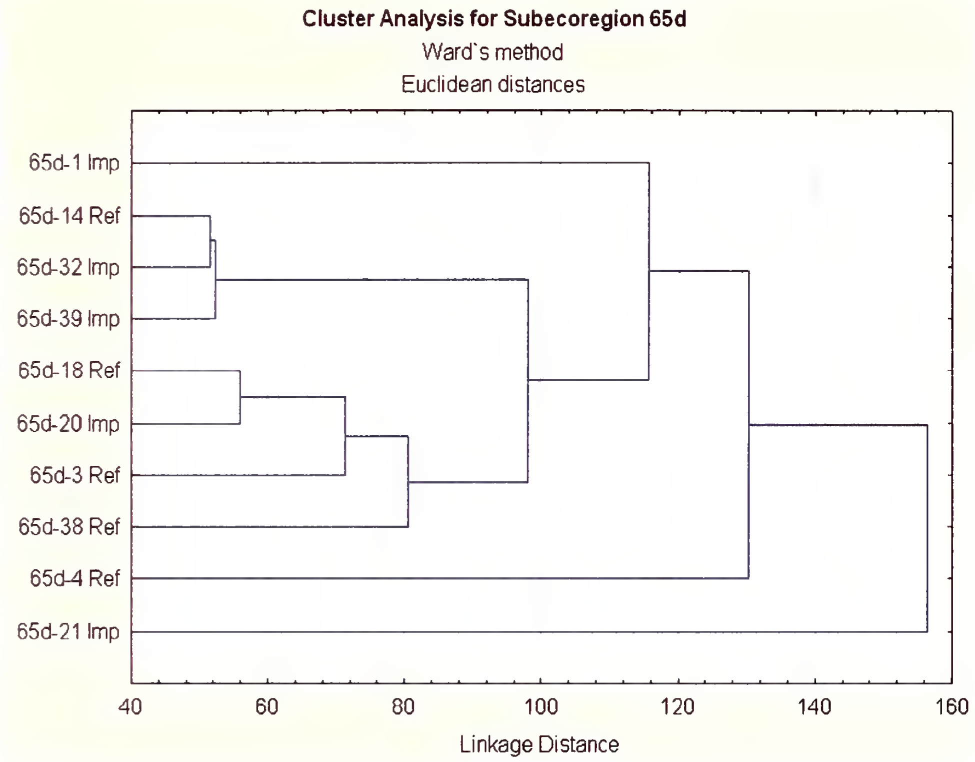


Figure 53: Cluster Analysis for subecoregion 65d

**Table 75: Spearman Rank Correlation Test - Nutrient Concentration vs. Index Score for Subecoregion 65d**

Red marked tests are significant at p<.05000

| **Variable** | Ammonia | Nitrite | Nitrate-Nitrite | Total Phosphorus | TP Sediment |
|---|---|---|---|---|---|
| Index Score | -0.096976 | -0.019462 | 0.644380 | 0.801953 | 0.187592 |

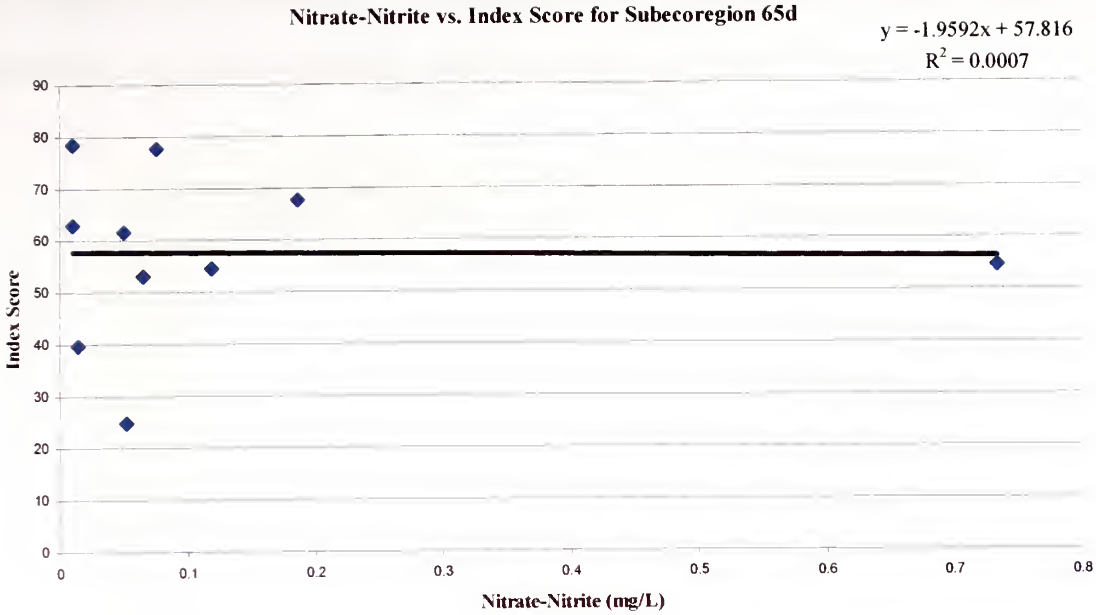


Figure 54: Nitrate-Nitrite vs. Index Score for Subecoregion 65d

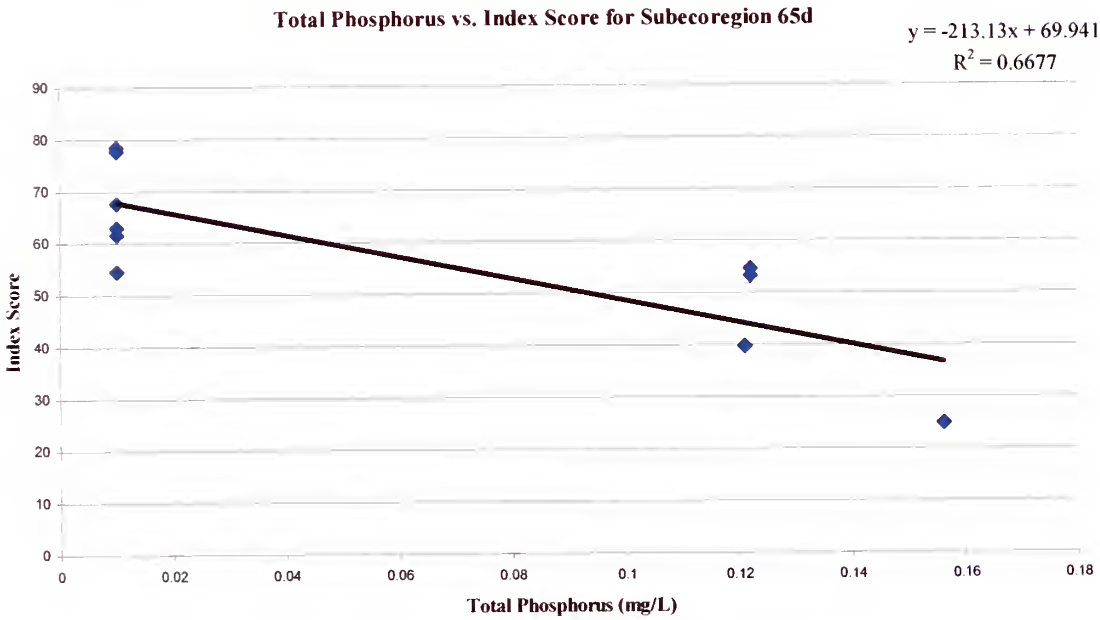


Figure 55: Total Phosphorus vs. Index Score for Subecoregion 65d

Discrimination efficiency between reference and impaired sites was 100%, with all impaired sites being classified correctly based on the reference condition. The box and whisker plot illustrated no overlap between reference and impaired sites, thus indicating complete discrimination for the metrics (Figure 56).

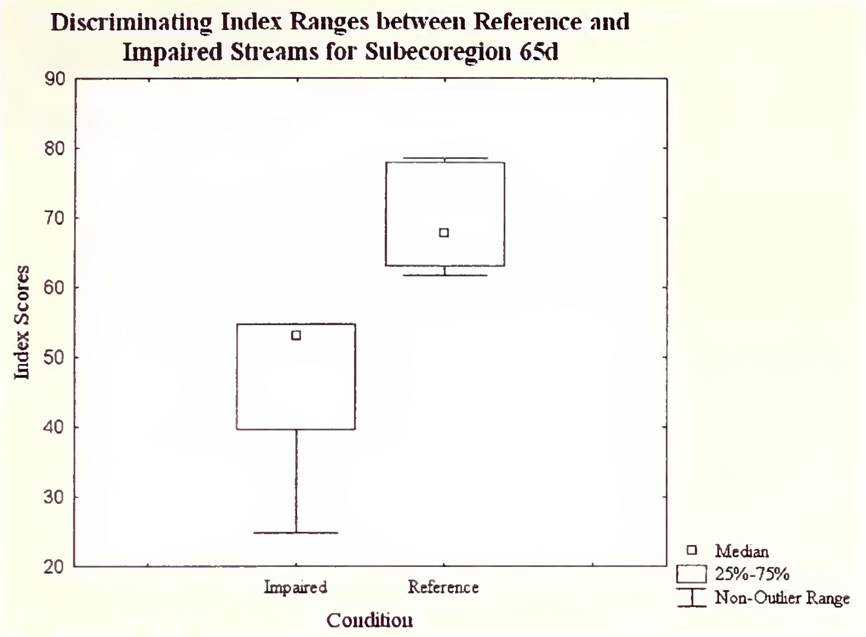


Figure 56: Discriminating Index Ranges between Reference and Impaired Streams for Subecoregion 65d

**Subecoregion 65g (Dougherty Plain)**

Ammonia, nitrite, nitrate-nitrite, and total phosphorus in the water column were not found to be significantly different between reference and impaired sites (Table 76). Due to the limited number of sediment samples for this subecoregion, analysis was not performed. However, the sediment data was used in analysis at the ecoregion level. Cluster analysis did not distinguish between reference and impaired macroinvertebrate communities (Figure 57). No nutrient parameter was significantly correlated with the macroinvertebrate multi-metric index scores (Table 77).

**Table 76: Mann - Whitney U - Test for Subecoregion 65g**

Red marked tests are significant at p <.05000

| | Z | p-level | Z adjusted | p-level | 2*1sided exact p |
|---|---|---|---|---|---|
| Ammonia | 1.71464 | 0.086412 | 1.71464 | 0.086412 | 0.099234 |
| Nitrite | -1.34722 | 0.177911 | -1.35450 | 0.175579 | 0.206460 |
| Nitrate-Nitrite | 0.85732 | 0.391268 | 0.86040 | 0.389569 | 0.439560 |
| Total Phosphorus | -0.61237 | 0.540292 | -1.03510 | 0.300624 | 0.594073 |

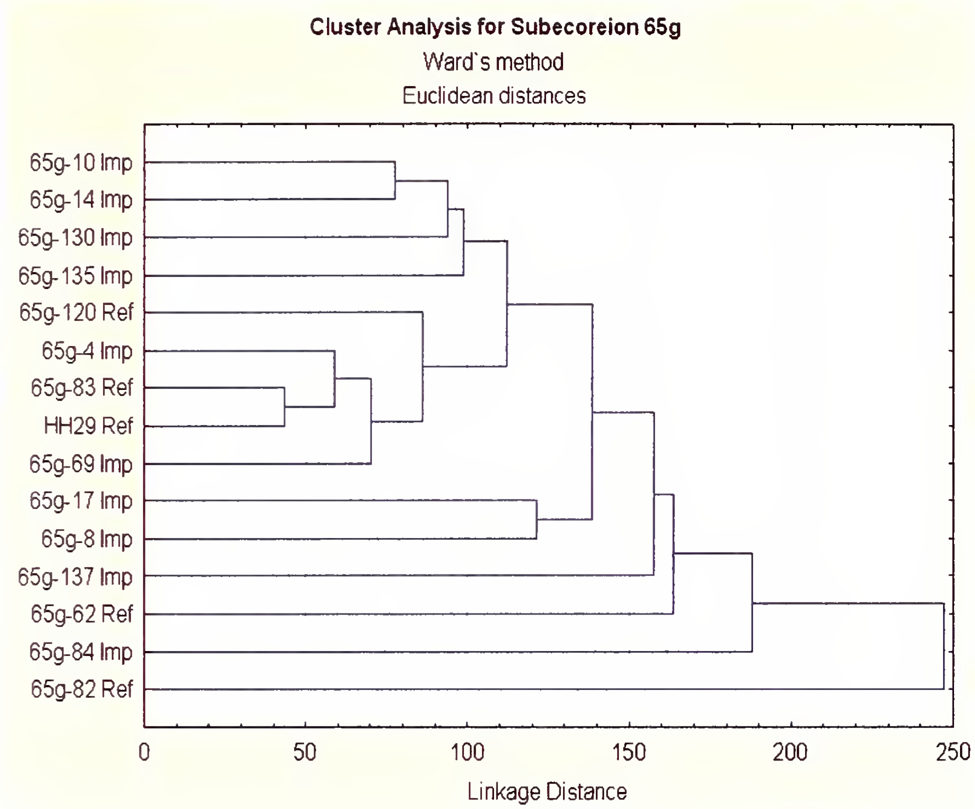


Figure 57: Cluster Analysis for Subecoregion 65g

**Table 77: Spearman Rank Correlation Test - Nutrient Concentration vs. Index Score for Subecoregion 65g**

Red marked tests are significant at p<.05000

| **Variable** | Ammonia | Nitrite | Nitrate-Nitrite | Total Phosphorus | TP Sediment |
|---|---|---|---|---|---|
| Index Score | -0.389286 | 0.380616 | -0.304661 | 0.187141 | -0.800000 |

Discrimination efficiency between reference and impaired sites was 100%, with all impaired sites being classified correctly based on the reference condition. The box and whisker plot illustrated no overlap between reference and impaired sites, thus indicating complete discrimination for the metrics (Figure 58).

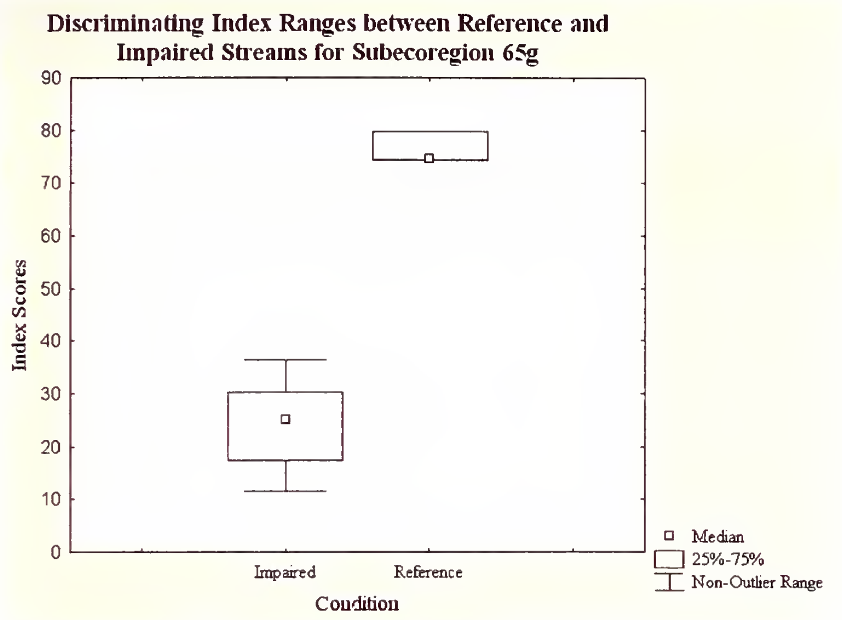


Figure 58: Discriminating Index Ranges between Reference and Impaired Streams for Subecoregion 65g

**Subecoregion 65h (Tifton Upland)**

Ammonia, nitrite, nitrate-nitrite, and total phosphorus in the water column and total phosphorous in the sediment were not found to be significantly different between reference and impaired sites (Table 78). Cluster analysis did distinguish between reference and impaired macroinvertebrate communities, with the exception of one impaired site (Figure 59). Ammonia concentrations in the water column were negatively correlated with the macroinvertebrate multi-metric index scores (Table 79). Figure 60 is the scatter plot for ammonia vs. index score.

| Table 78: Mann - Whitney U - Test for Subecoregion 65h<br>Red marked tests are significant at p <.05000 | | | | | |
|---|---|---|---|---|---|
| | Z | p-level | Z adjusted | p-level | 2*1sided exact p |
| Ammonia | 1.671145 | 0.094694 | 1.67623 | 0.093694 | 0.095238 |
| Nitrite | -0.940019 | 0.347208 | -1.05903 | 0.289588 | 0.420635 |
| Nitrate-Nitrite | 1.148913 | 0.250593 | 1.14891 | 0.250593 | 0.309524 |
| Total Phosphorus | -0.522233 | 0.601509 | -1.000000 | 0.317311 | 0.690476 |
| TP Sediment | -0.36742 | 0.713303 | -0.368964 | 0.712155 | 0.730159 |

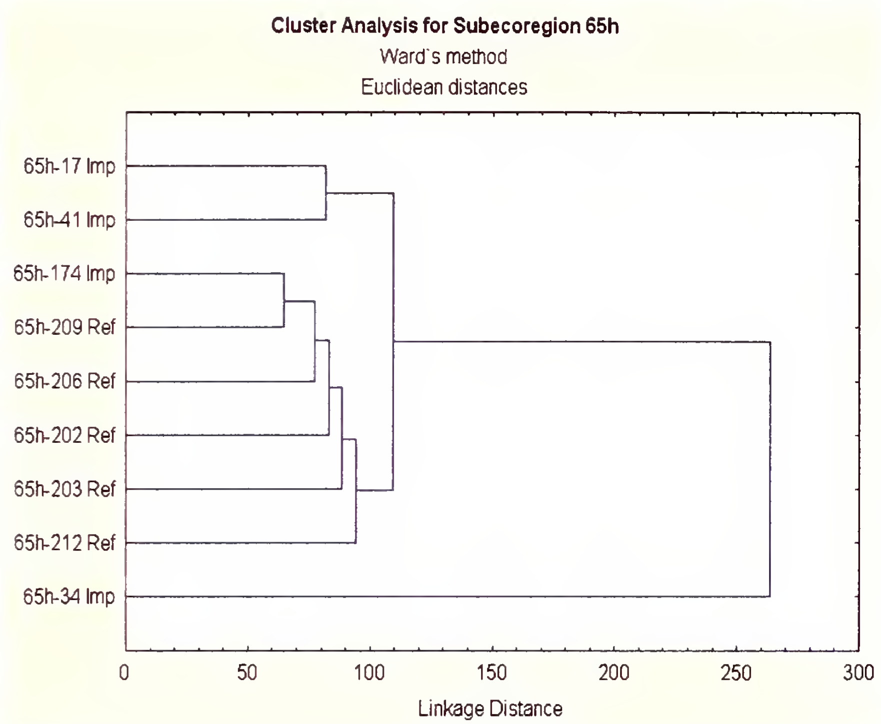


Figure 59: Cluster Analysis for Subecoregion 65h

| Table 79: Spearman Rank Correlation Test - Nutrient Concentration vs. Index Score for Subecoregion 65h<br>Red marked tests are significant at p<.05000 | | | | | |
|---|---|---|---|---|---|
| **Variable** | Ammonia | Nitrite | Nitrate-Nitrite | Total Phosphorus | TP Sediment |
| Index Score | -0.820672 | -0.013656 | -0.503030 | 0.174078 | 0.071429 |

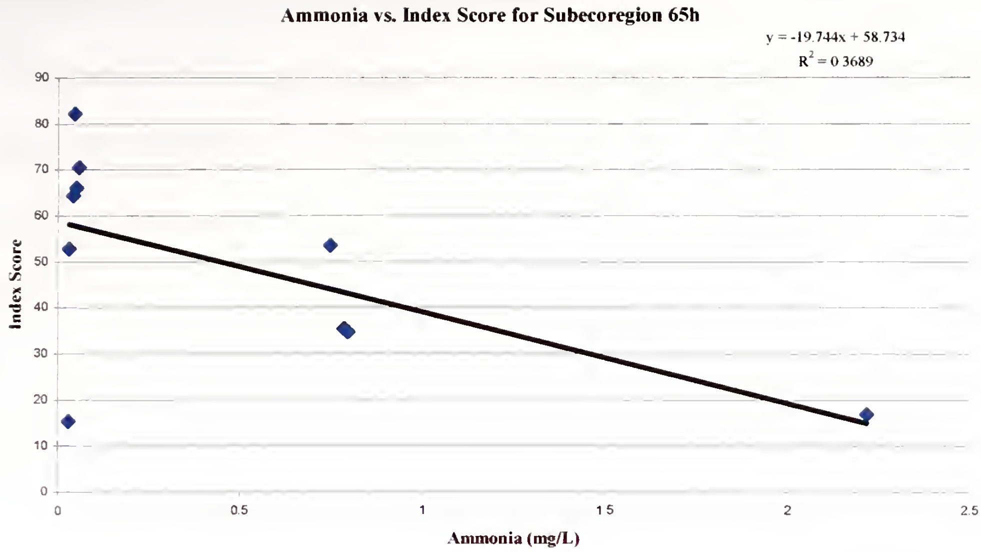


Figure 60: Ammonia vs. Index Score for Subecoregion 65h

Discrimination efficiency between reference and impaired sites was 100%, with all impaired sites being classified correctly based on the reference condition. The box and whisker plot illustrated no overlap between reference and impaired sites, thus indicating complete discrimination for the metrics (Figure 61).

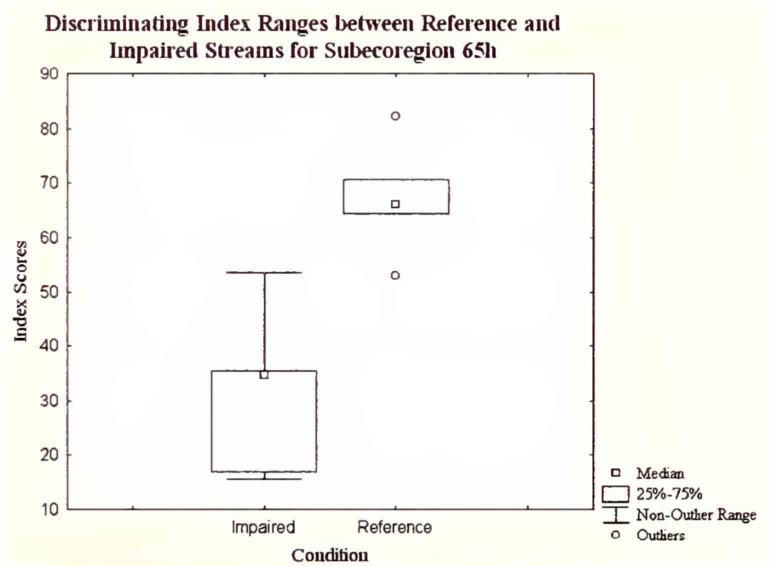


Figure 61: Discriminating Index Ranges between Reference and Impaired Streams for Subecoregion 65h

**Subecoregion 65k (Coastal Plain Red Uplands)**

Ammonia, nitrite, nitrate-nitrite, and total phosphorus in the water column were not found to be significantly different between reference and impaired sites (Table 80). Due to the limited number of sediment samples for this subecoregion, analysis was not performed. However, the sediment data was used in analysis at the ecoregion level. Cluster analysis did not distinguish between reference and impaired macroinvertebrate communities (Figure 62). No nutrient parameter was correlated with the macroinvertebrate multi-metric index scores (Table 81).

**Table 80: Mann - Whitney U - Test for Subecoregion 65k**
Red marked tests are significant at p <.05000

| | Z | p-level | Z adjusted | p-level | 2*1sided exact p |
|---|---|---|---|---|---|
| Ammonia | 1.04447 | 0.296271 | 1.07763 | 0.281199 | 0.309524 |
| Nitrite | -1.56670 | 0.117186 | -1.92759 | 0.053907 | 0.150794 |
| Nitrate-Nitrite | 0.94002 | 0.347208 | 0.94002 | 0.347208 | 0.420635 |
| Total Phosphorus | -1.56670 | 0.117186 | -1.92759 | 0.053907 | 0.150794 |

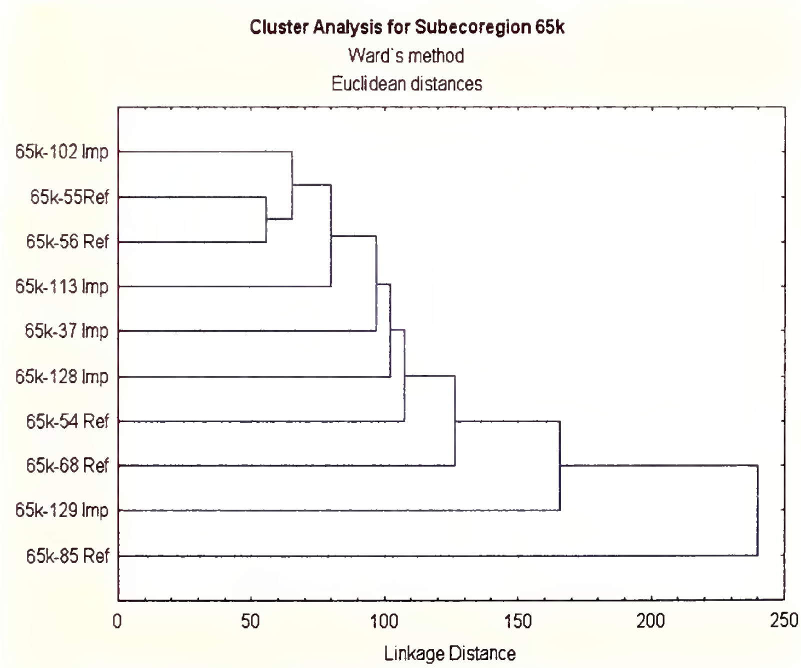


Figure 62: Cluster Analysis for Subecoregion 65k

| Table 81: Spearman Rank Correlation Test - Nutrient Concentration vs. Index Score for Subecoregion 65k<br>Red marked tests are significant at p<.05000 | | | | | |
|---|---|---|---|---|---|
| **Variable** | Ammonia | Nitrite | Nitrate-Nitrite | Total Phosphorus | TP Sediment |
| Index Score | -0.225110 | 0.246070 | -0.272727 | 0.275897 | -0.035714 |

Discrimination efficiency between reference and impaired sites was 100%, with all impaired sites being classified correctly based on the reference condition. The box and whisker plot illustrated no overlap of interquartile range, but some overall overlap between reference and impaired sites (Figure 63).

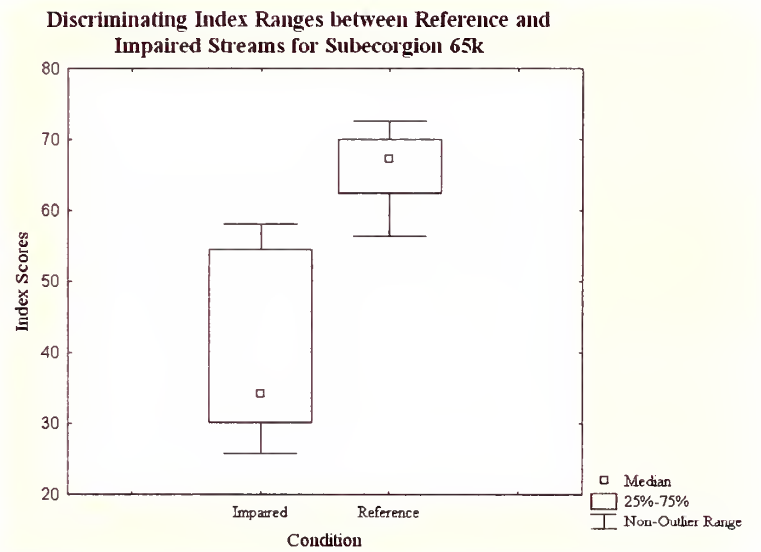


Figure 63: Discriminating Index Ranges between Reference and Impaired Streams for Subecoregion 65k

## Subecoregion 65l (Atlantic Southern Loam Plains)

Ammonia, nitrite, nitrate-nitrite, and total phosphorus in the water column were not found to be significantly different between reference and impaired sites (Table 82). Due to the limited number of sediment samples for this subecoregion, analysis was not performed. However, the sediment data were used in analysis at the ecoregion level.

Cluster analysis did not distinguish between reference and impaired macroinvertebrate communities (Figure 64). Ammonia and nitrite concentrations in the water column were positively correlated with the macroinvertebrate multi-metric index scores (Table 83). Figures 65 and 66 are the scatter plots for ammonia vs. index score and nitrite vs. index score, respectively.

**Table 82: Mann - Whitney U - Test for Subecoregion 65l**
Red marked tests are significant at p <.05000

| | Z | p-level | Z adjusted | p-level | 2*1sided exact p |
|---|---|---|---|---|---|
| Ammonia | 0.52223 | 0.601509 | 0.52868 | 0.597027 | 0.690476 |
| Nitrite | 1.14891 | 0.250593 | 1.22559 | 0.220354 | 0.309524 |
| Nitrate-Nitrite | 0.52223 | 0.601509 | 0.52868 | 0.597027 | 0.690476 |
| Total Phosphorus | -1.56670 | 0.117186 | -1.92759 | 0.053907 | 0.150794 |

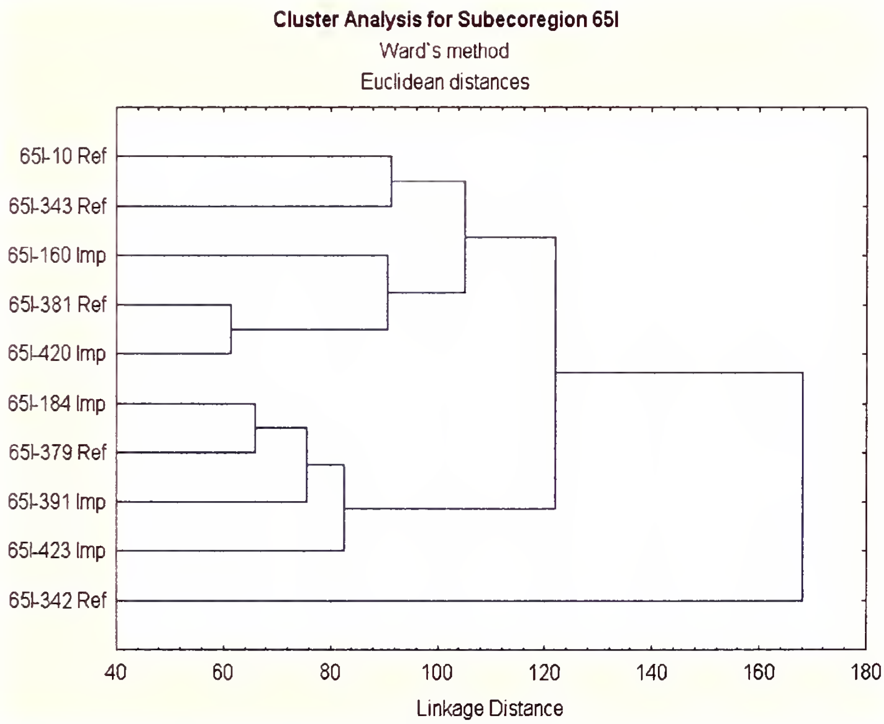


Figure 64: Cluster Analysis for Subecoregion 65l

| Table 83: Spearman Rank Correlation Test - Nutrient Concentration vs. Index Score for Subecoregion 65l | | | | | |
|---|---|---|---|---|---|
| Red marked tests are significant at p<.05000 | | | | | |
| **Variable** | Ammonia | Nitrite | Nitrate-Nitrite | Total Phosphorus | TP Sediment |
| Index Score | 0.681033 | 0.821065 | -0.006135 | -0.544337 | -0.200000 |

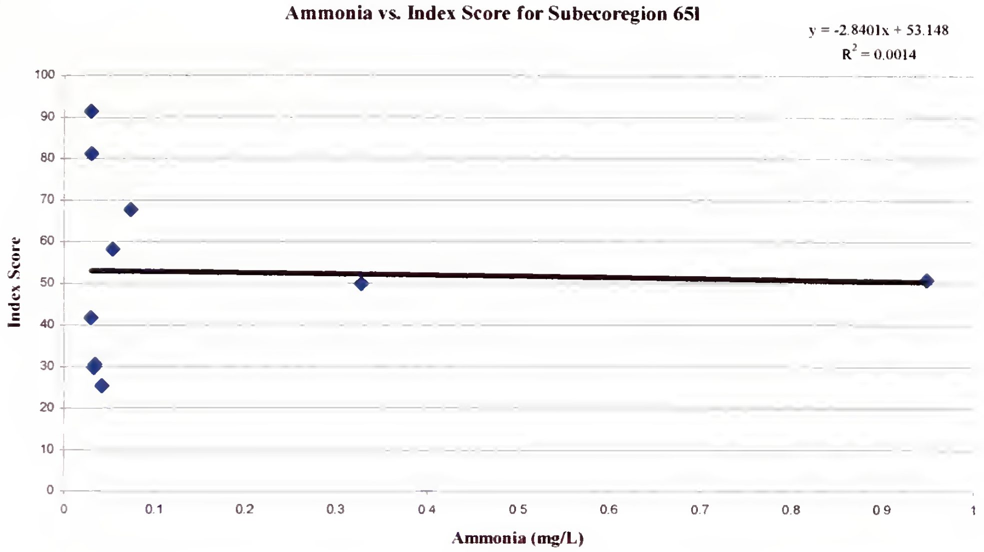


Figure 65: Ammonia vs. Index Score for Subecoregion 65l

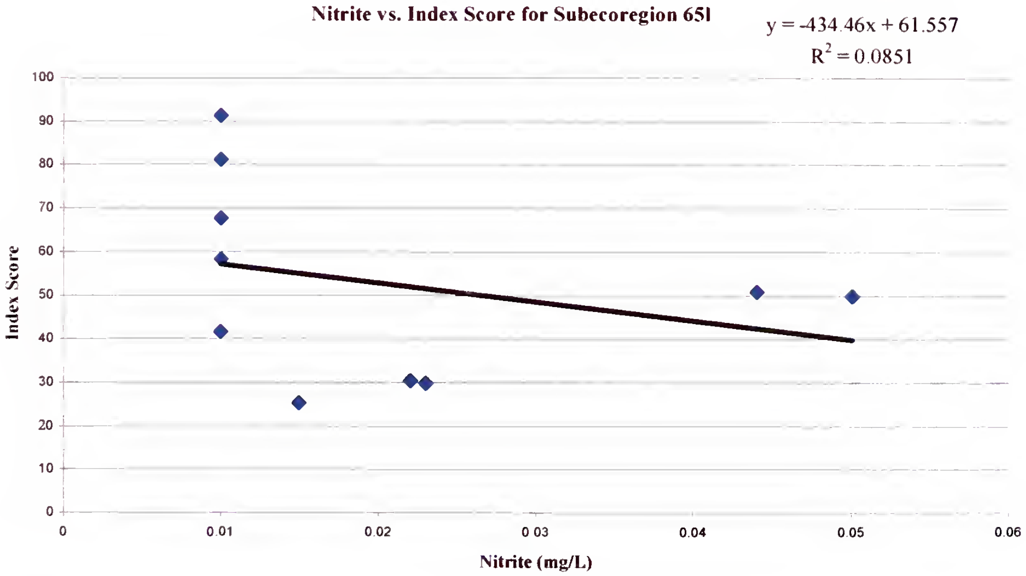


Figure 66: Nitrite vs. Index Score for Subecoregion 65l

Discrimination efficiency between reference and impaired sites was 100%, with all impaired sites being classified correctly based on the reference condition. The box and whisker plot illustrated no overlap of interquartile range, but overall overlap between reference and impaired sites (Figure 67).

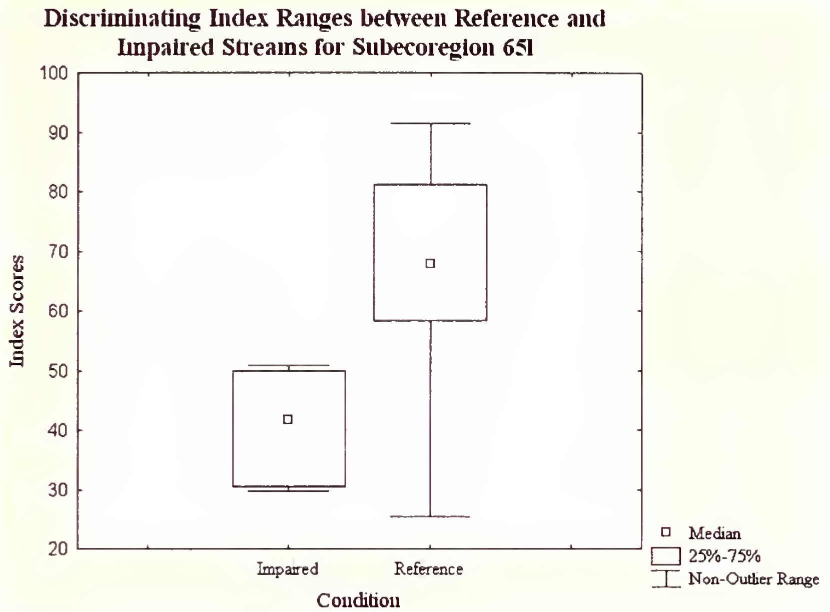


Figure 67: Discriminating Index Ranges between Reference and Impaired Streams for Subecoregion 65l

**Subecoregion 65o (Tallahassee Hill/Valdosta Limesink)**

Total phosphorus in the water column was significantly different between reference and impaired sites (Table 84). Ammonia, nitrite, and nitrate-nitrite in the water column were not found to be significantly different between reference and impaired sites. Due to the limited number of sediment samples for this subecoregion, analysis was not performed. However, the sediment data were used in analysis at the ecoregion level. Cluster analysis did not distinguish between reference and impaired macroinvertebrate communities (Figure 68). Nitrite concentrations in the water column were positively correlated with the macroinvertebrate multi-metric index scores (Table 85). Figure 69 is the scatter plot for nitrite vs. index score.

**Table 84: Mann - Whitney U - Test for Subecoregion 65o**

Red marked tests are significant at p <.05000

| | Z | p-level | Z adjusted | p-level | 2*1sided exact p |
|---|---|---|---|---|---|
| Ammonia | -1.59217 | 0.111348 | -1.66297 | 0.096320 | 0.111111 |
| Nitrite | -1.83712 | 0.066193 | -1.92759 | 0.053907 | 0.063492 |
| Nitrate-Nitrite | -0.61237 | 0.540292 | -0.62554 | 0.531615 | 0.555556 |
| Total Phosphorus | 2.20454 | 0.027487 | 2.30257 | 0.021304 | 0.031746 |

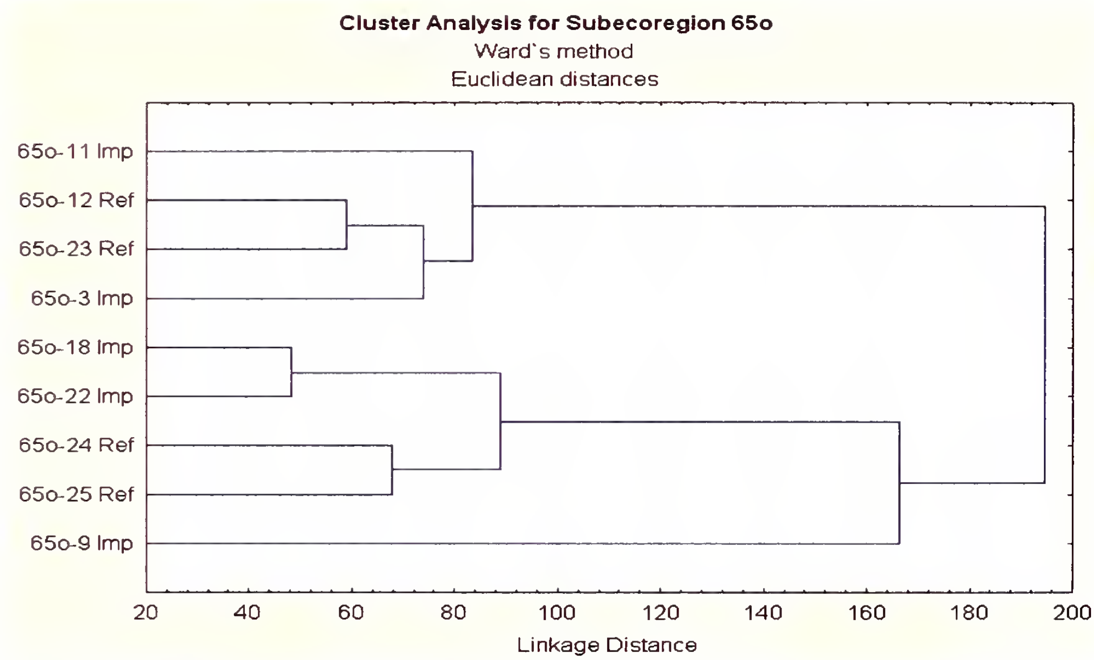


Figure 68: Cluster Analysis for Subecoregion 65o

**Table 85: Spearman Rank Correlation Test - Nutrient Concentration vs. Index Score for Subecoregion 65o**

Red marked tests are significant at p<.05000

| **Variable** | Ammonia | Nitrite | Nitrate-Nitrite | Total Phosphorus | TP Sediment |
|---|---|---|---|---|---|
| Index Score | 0.382971 | 0.708241 | 0.442653 | -0.409082 | 0.800000 |

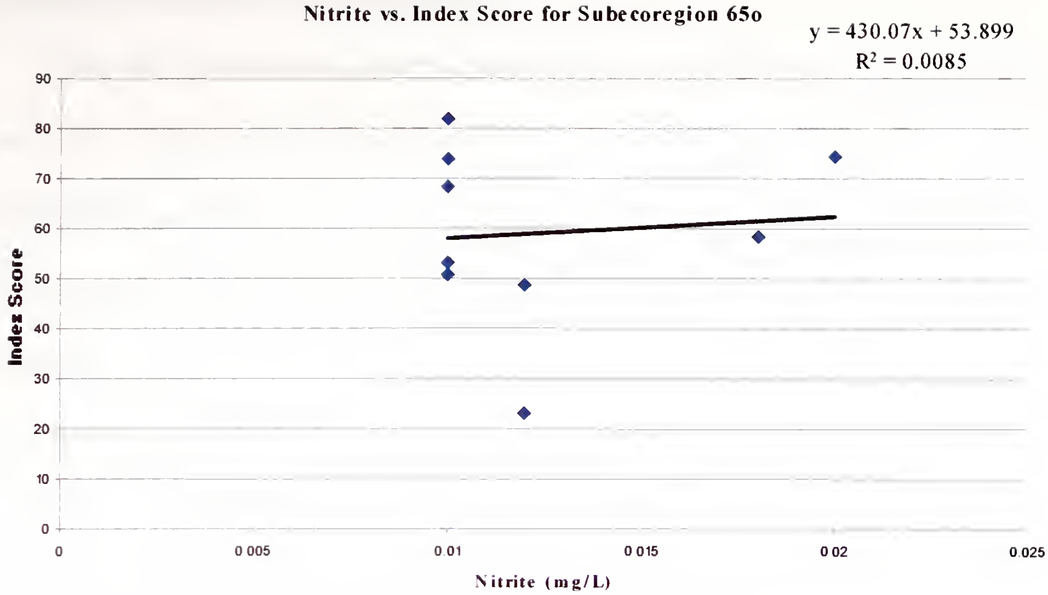


Figure 69: Nitrite vs. Index Score for Subecoregion 65o

Discrimination efficiency between reference and impaired sites was 100%, with all impaired sites being classified correctly based on the reference condition. The box and whisker plot illustrated no overlap of interquartile range, but some overall overlap between reference and impaired sites (Figure 70).

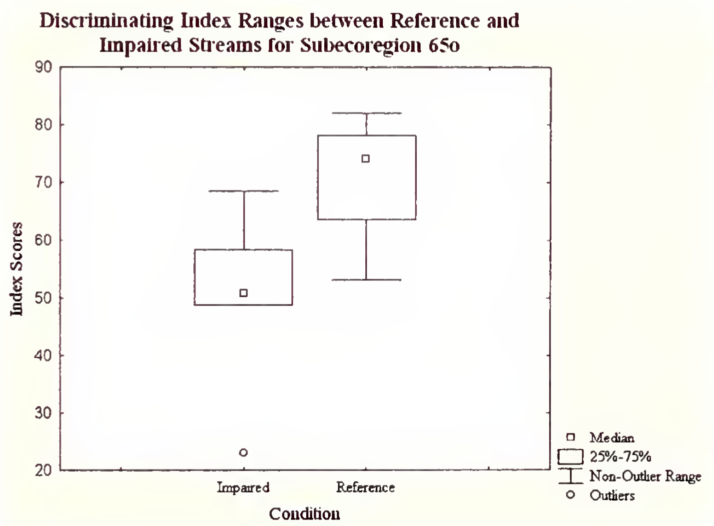


Figure 70: Discriminating Index Ranges between Reference and Impaired Streams for Subecoregion 65o

**Ecoregion 75 (Southern Coastal Plains)**

Total phosphorus in the water column and total phosphorous in the sediment were significantly different between reference and impaired sites (Table 86). Ammonia, nitrate-nitrite, and nitrite in the water column were not found to be significantly different. Cluster analysis did not distinguish between reference and impaired macroinvertebrate communities at the ecoregion level (Figure 71). Total phosphorus concentrations in the water column and total phosphorus concentrations in sediment were positively correlated with the macroinvertebrate multi-metric index scores (Table 87). Figures 72 and 73 are the scatter plots for total phosphorus in water column vs. index score and total phosphorus in sediment vs. index score, respectively.

**Table 86: Mann - Whitney U - Test for Ecoregion 75**
Red marked tests are significant at p <.05000

| | Z | p-level | Z adjusted | p-level | 2*1sided exact p |
|---|---|---|---|---|---|
| Ammonia | -1.76175 | 0.078113 | -1.77330 | 0.076180 | 0.078016 |
| Nitrite | -0.28263 | 0.777458 | -0.28640 | 0.774569 | 0.786517 |
| Nitrate-Nitrite | -0.22611 | 0.821119 | -0.23444 | 0.814640 | 0.829935 |
| Total Phosphorus | -2.27991 | 0.022614 | -2.42727 | 0.015213 | 0.022259 |
| TP Sediment | 2.480695 | 0.013113 | 2.480695 | 0.013113 | 0.012095 |

**Table 87: Spearman Rank Correlation Test - Nutrient Concentration vs. Index Score for Ecoregion 75**
Red marked tests are significant at p<.05000

| **Variable** | Ammonia | Nitrite | Nitrate-Nitrite | Total Phosphorus | TP Sediment |
|---|---|---|---|---|---|
| Index Score | 0.264727 | 0.149133 | 0.077338 | 0.381679 | -0.586275 |

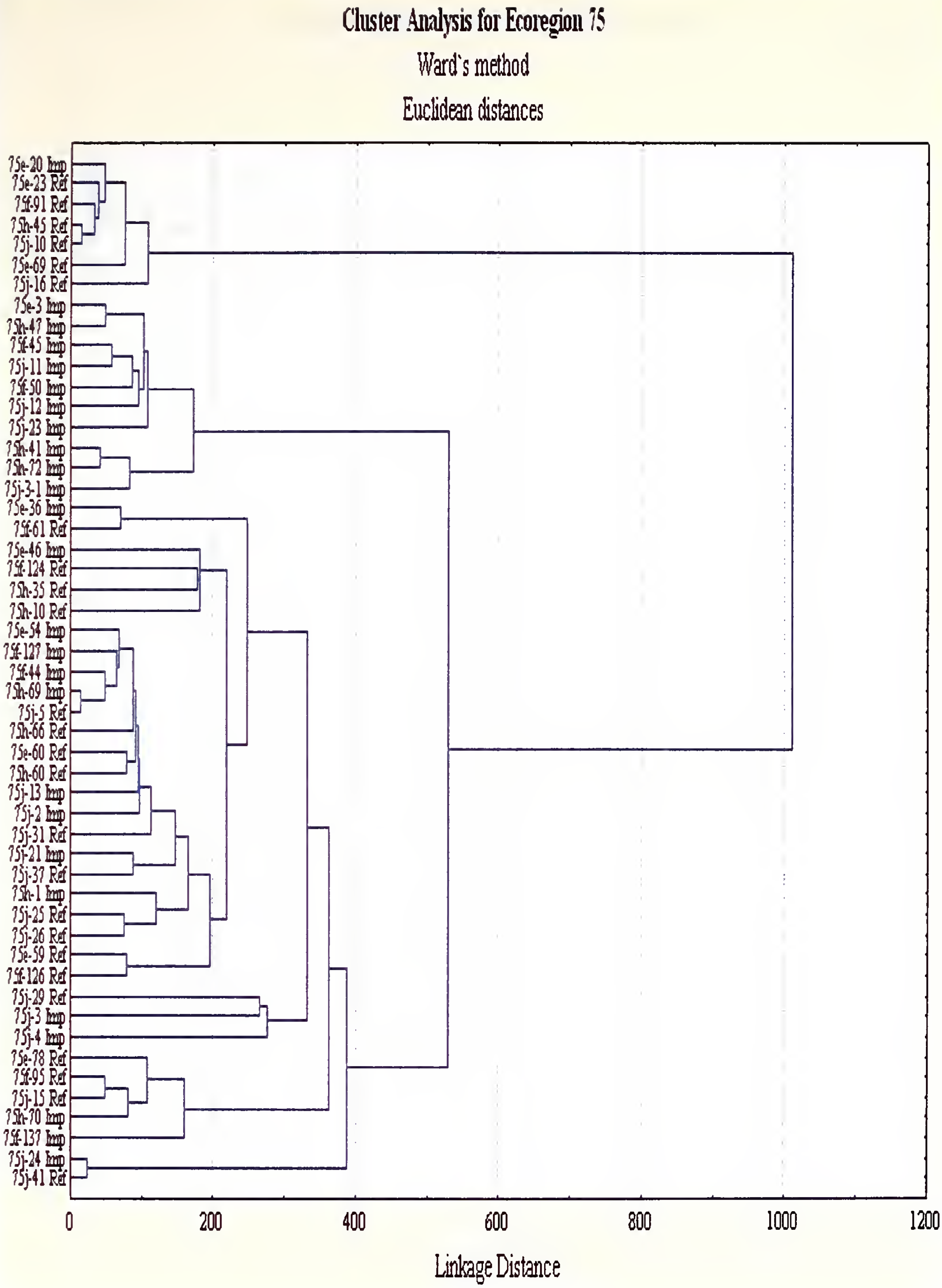


Figure 71: Cluster Analysis for Ecoregion 75

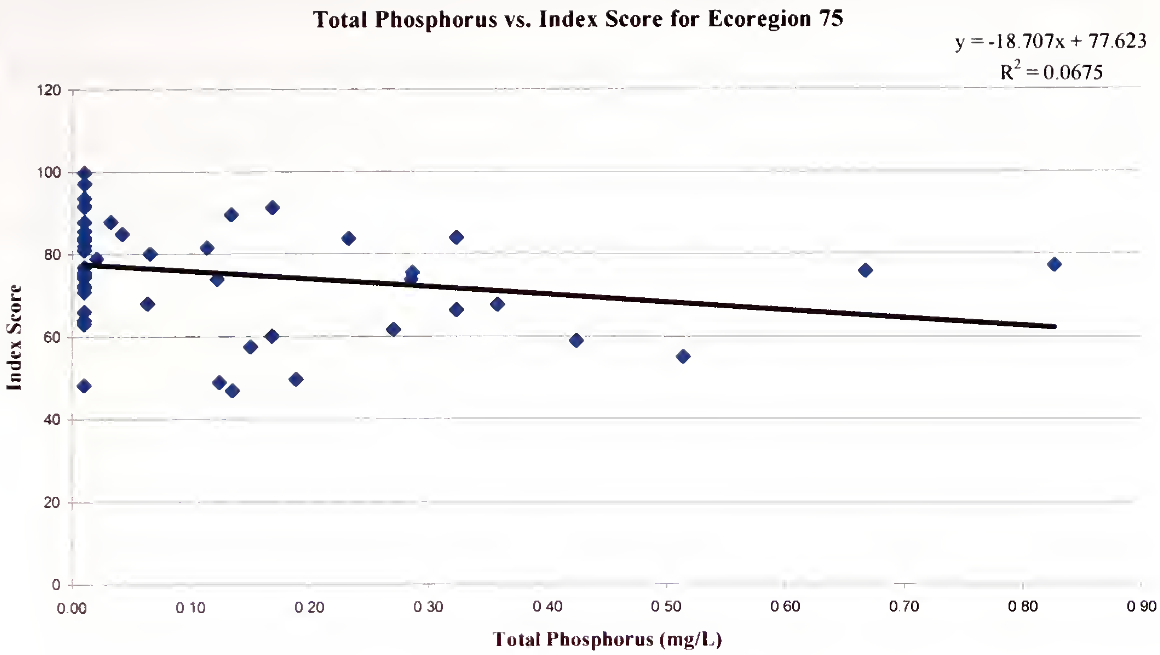


Figure 72: Total Phosphorus vs. Index Score for Ecoregion 75

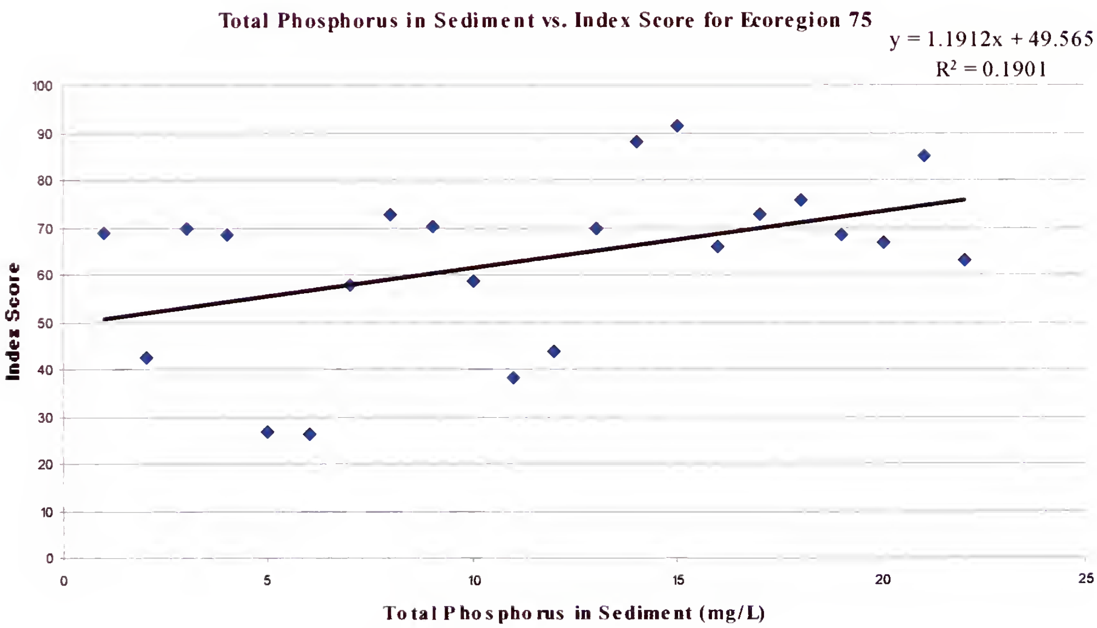


Figure 73: Total Phosphorus in Sediment vs. Index Score for Ecoregion 75

Discrimination efficiency between reference and impaired sites was 77%, with 20 of the 26 impaired sites being classified correctly. The box and whisker plot illustrated no overlap of interquartile range, but some overall overlap between reference and impaired sites (Figure 74).

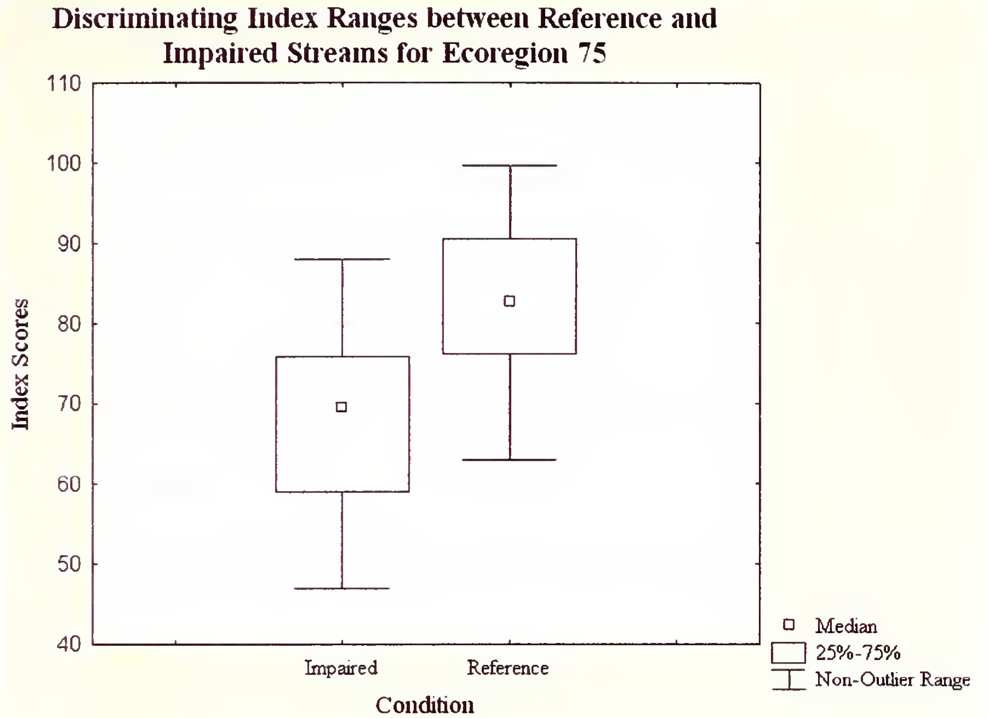


Figure 74: Discriminating Index Ranges between Reference and Impaired Streams for Ecoregion 75

**Subecoregion 75e (Okefenokee Plains)**

Ammonia, nitrite, nitrate-nitrite, and total phosphorus in the water column and total phosphorous in the sediment were not found to be significantly different between reference and impaired sites (Table 88). Cluster analysis did not distinguish between reference and impaired macroinvertebrate communities (Figure 75). No nutrient parameters were significantly correlated with the macroinvertebrate multi-metric index scores (Table 89).

| Table 88: Mann - Whitney U - Test for Subecoregion 75e Red marked tests are significant at p <.05000 | | | | | |
|---|---|---|---|---|---|
| | Z | p-level | Z adjusted | p-level | 2*1sided exact p |
| Ammonia | 0.313340 | 0.754023 | 0.31429 | 0.753298 | 0.841270 |
| Nitrite | -0.940019 | 0.347208 | -0.94002 | 0.347208 | 0.420635 |
| Nitrate-Nitrite | -0.522233 | 0.601509 | -0.55709 | 0.577469 | 0.690476 |
| Total Phosphorus | -0.940019 | 0.347208 | -1.05903 | 0.289588 | 0.420635 |
| TP Sediment | 0.654654 | 0.512691 | 0.654654 | 0.512691 | 0.700000 |

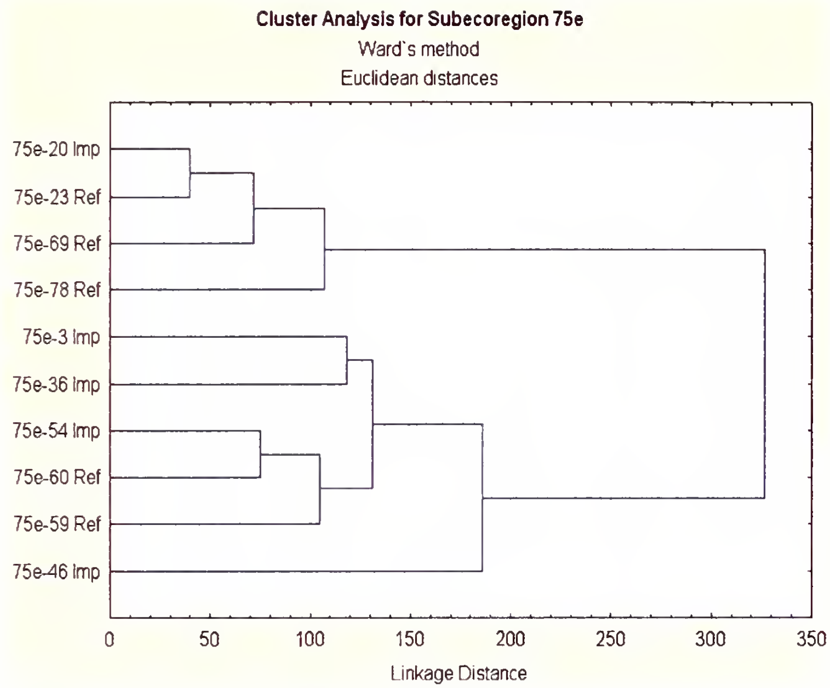


Figure 75: Cluster Analysis for Subecoregion 75e

| Table 89: Spearman Rank Correlation Test - Nutrient Concentration vs. Index Score for Subecoregion75e Red marked tests are significant at p<.05000 | | | | | |
|---|---|---|---|---|---|
| **Variable** | Ammonia | Nitrite | Nitrate-Nitrite | Total Phosphorus | TP Sediment |
| Index Score | 0.085107 | 0.321212 | 0.084046 | 0.375534 | -0.542857 |

Discrimination efficiency between reference and impaired sites was 100%, with all impaired sites being classified correctly based on the reference condition. The box and whisker plot illustrated no overlap between reference and impaired sites, thus

indicating complete discrimination for the metrics (Figure 76).

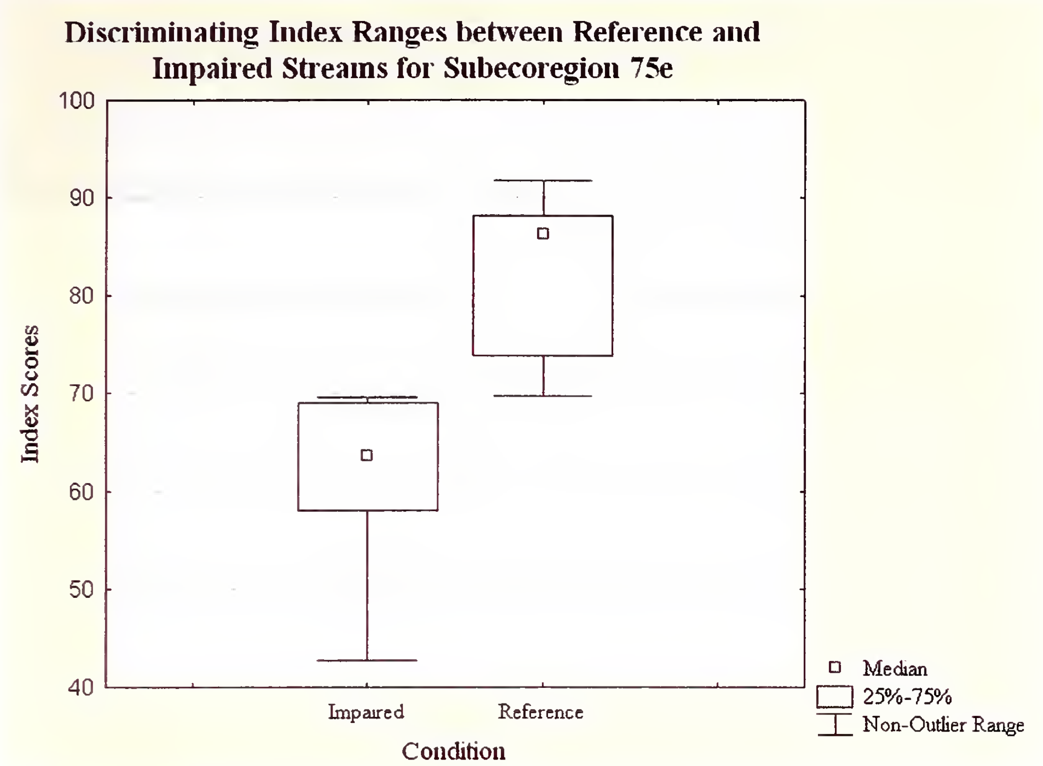


Figure 76: Discriminating Index Ranges between Reference and Impaired Streams for Subecoregion 75e

**Subecoregion 75f (Sea Island Flatwoods)**

Ammonia, nitrite, nitrate-nitrite, and total phosphorus in the water column were not found to be significantly different between reference and impaired sites (Table 90). Due to the limited number of sediment samples for this subecoregion, analysis was not performed. However, the sediment data were used in analysis at the ecoregion level. Cluster analysis did distinguish between reference and impaired macroinvertebrate communities, with the exception of one reference site (Figure 77). No nutrient parameters were significantly correlated with the macroinvertebrate multi-metric index scores (Table 91).

| Table 90: Mann - Whitney U - Test for Subecoregion 75f<br>Red marked tests are significant at p <.05000 | | | | | |
|---|---|---|---|---|---|
| | Z | p-level | Z adjusted | p-level | 2*1sided exact p |
| Ammonia | 0.734847 | 0.462433 | 0.737928 | 0.460559 | 0.555556 |
| Nitrite | -0.244949 | 0.806496 | -0.249136 | 0.803255 | 0.904762 |
| Nitrate-Nitrite | -0.244949 | 0.806496 | -0.249136 | 0.803255 | 0.904762 |
| Total Phosphorus | 0.489898 | 0.624206 | 0.536656 | 0.591505 | 0.730159 |

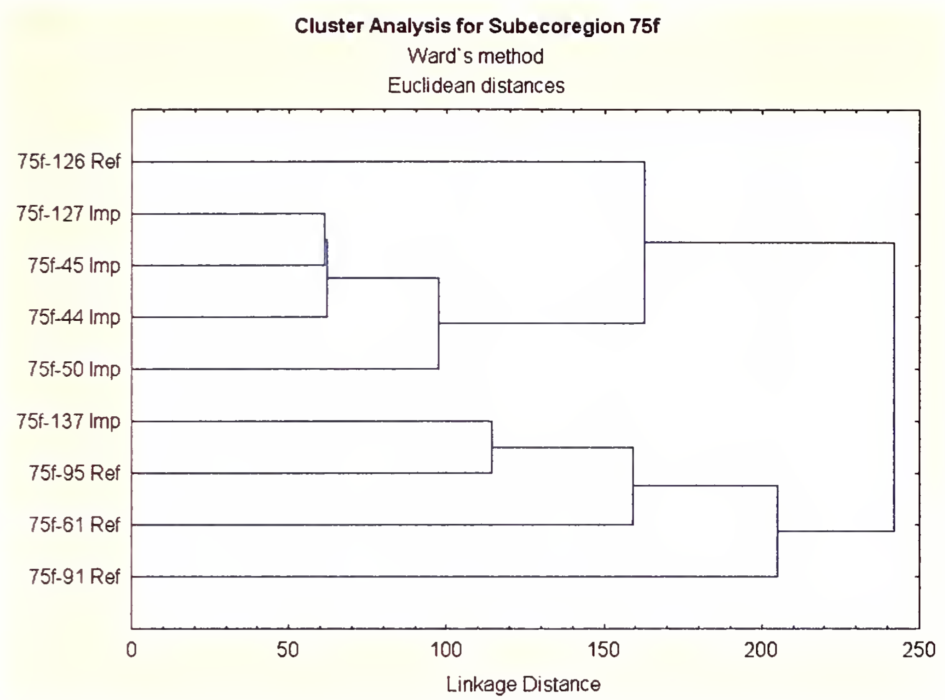


Figure 77: Cluster Analysis for Subecoregion 75f

| Table 91: Spearman Rank Correlation Test - Nutrient Concentration vs. Index Score for Subecoregion 75f<br>Red marked tests are significant at p<.05000 | | | | | |
|---|---|---|---|---|---|
| **Variable** | Ammonia | Nitrite | Nitrate-Nitrite | Total Phosphorus | TP Sediment |
| Index Score | 0.325178 | -0.153386 | -0.423345 | 0.341394 | -0.600000 |

Discrimination efficiency between reference and impaired sites was 100%, with all impaired sites being classified correctly based on the reference condition. The box and whisker plot illustrated no overlap between reference and impaired sites, thus

indicating complete discrimination for the metrics (Figure 78).

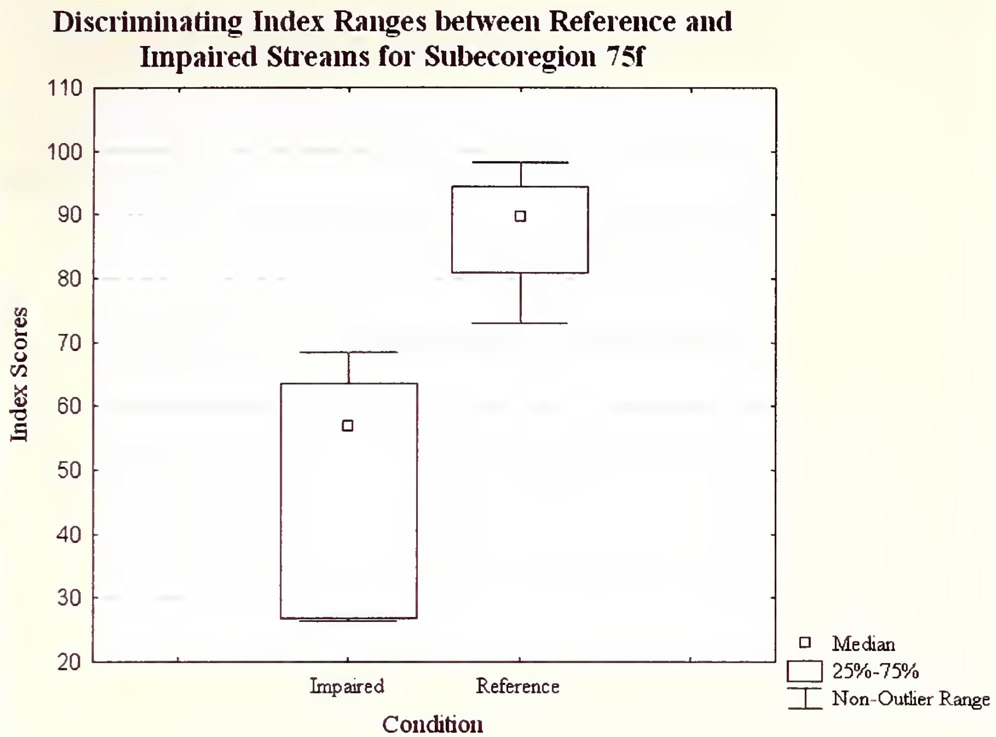


Figure 78: Discriminating Index Ranges between Reference and Impaired Streams for Subecoregion 75f

## Subecoregion 75h (Bacon Terraces)

Ammonia, nitrite, nitrate-nitrite, and total phosphorus in the water column were not found to be significantly different between reference and impaired sites (Table 92). Due to the limited number of sediment samples for this subecoregion, analysis was not performed. However, the sediment data was used in analysis at the ecoregion level. Cluster analysis did not distinguish between reference and impaired macroinvertebrate communities, with the exception of one reference site (Figure 79). No nutrient parameters were significantly correlated with the macroinvertebrate multi-metric index scores (Table 93).

| Table 92: Mann - Whitney U - Test for Subecoregion 75h<br>Red marked tests are significant at p <.05000 | | | | | |
|---|---|---|---|---|---|
| | Z | p-level | Z adjusted | p-level | 2*1sided exact p |
| Ammonia | -1.09545 | 0.273323 | -1.10554 | 0.268926 | 0.329004 |
| Nitrite | -1.27802 | 0.201244 | -1.34040 | 0.180117 | 0.246753 |
| Nitrate-Nitrite | -0.63901 | 0.522817 | -0.64047 | 0.521870 | 0.536797 |
| Total Phosphorus | -0.73030 | 0.465209 | -0.84584 | 0.397642 | 0.536797 |

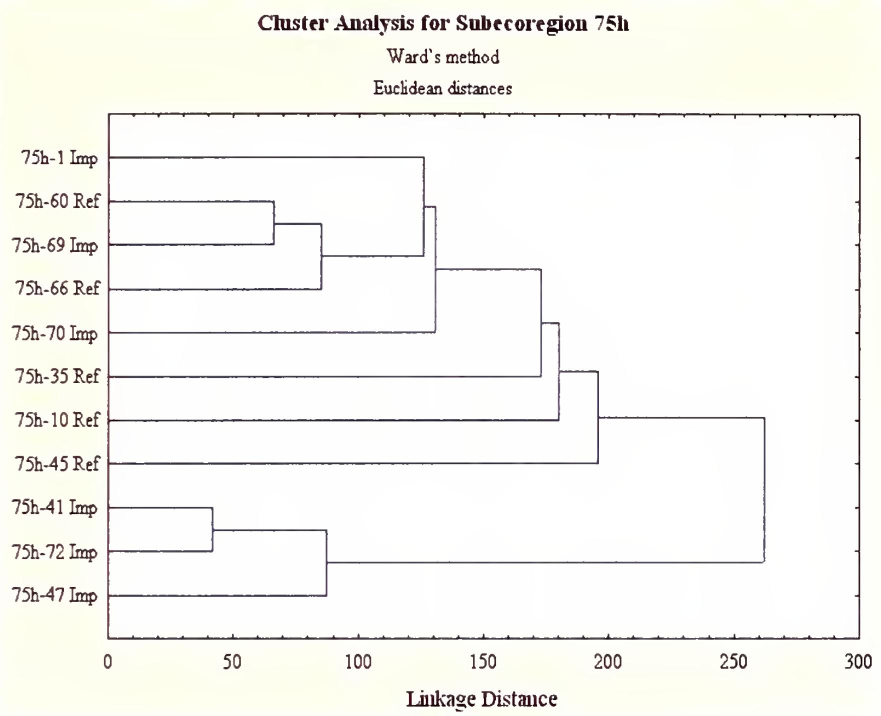


Figure 79: Cluster Analysis for Subecoregion 75h

| Table 93: Spearman Rank Correlation Test - Nutrient Concentration vs. Index Score for Subecoregion 75h<br>Red marked tests are significant at p<.05000 | | | | | |
|---|---|---|---|---|---|
| **Variable** | Ammonia | Nitrite | Nitrate-Nitrite | Total Phosphorus | TP Sediment |
| Index Score | 0.293590 | 0.324177 | 0.232347 | 0.473815 | -0.400000 |

Discrimination efficiency between reference and impaired sites was 100%, with all impaired sites being classified correctly based on the reference condition. The box and whisker plot illustrated no overlap between reference and impaired sites, thus

indicating complete discrimination for the metrics (Figure 80).

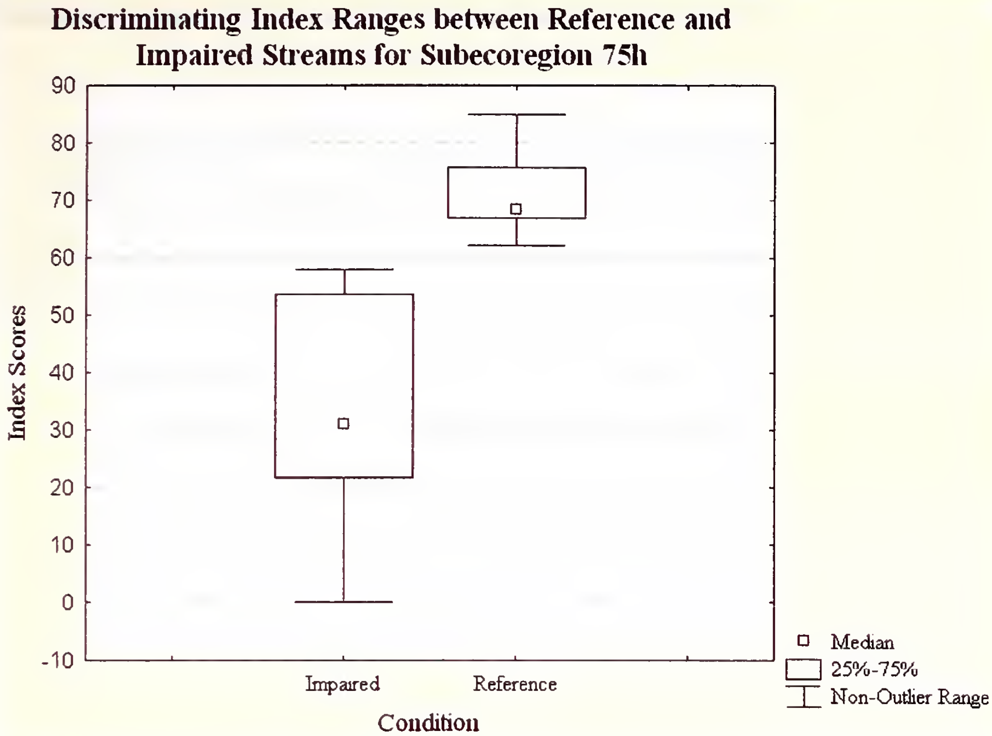


Figure 80: Discriminating Index Ranges between Reference and Impaired Streams for Subecoregion 75h

**Subecoregion 75j (Sea Islands/Coastal Marsh)**

Since 75f-124 is a tidal stream, this site was included with 75j sites for analysis. Total phosphorus in the water column was significantly different between reference and impaired sites (Table 94). Ammonia, nitrite, and nitrate-nitrite in the water column were not found to be significantly different between reference and impaired sites. Due to the limited number of sediment samples for this subecoregion, analysis was not performed. However, the sediment data were used in analysis at the ecoregion level. Cluster analysis did not distinguish between reference and impaired macroinvertebrate communities (Figure 81). Nitrite concentrations were positively correlated with the macroinvertebrate multi-metric index scores (Table 95). Figure 82 is the scatter plot for nitrite vs. index score.

**Table 94: Mann - Whitney U - Test for Subecoregion 75j**

Red marked tests are significant at p <.05000

| | Z | p-level | Z adjusted | p-level | 2*1sided exact p |
|---|---|---|---|---|---|
| Ammonia | 1.337940 | 0.180917 | 1.346713 | 0.178074 | 0.197116 |
| Nitrite | -0.457716 | 0.647157 | -0.466744 | 0.640683 | 0.653903 |
| Nitrate-Nitrite | -0.563343 | 0.573202 | -0.608711 | 0.542717 | 0.604730 |
| Total Phosphorus | 2.006910 | 0.044760 | 2.045114 | 0.040844 | 0.042964 |

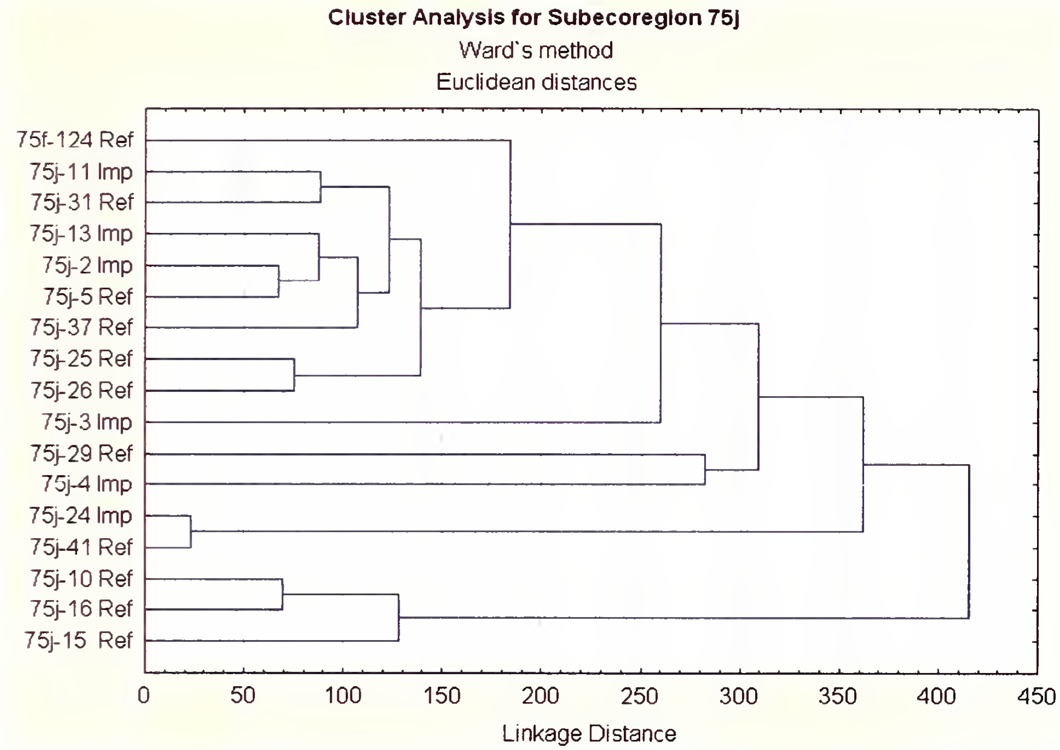


Figure 81: Cluster Analysis for Subecoregion 75j

**Table 95: Spearman Rank Correlation Test - Nutrient Concentration vs. Index Score for Subecoregion 75j**

Red marked tests are significant at p<.05000

| **Variable** | Ammonia | Nitrite | Nitrate-Nitrite | Total Phosphorus | TP Sediment |
|---|---|---|---|---|---|
| Index Score | 0.253600 | 0.484038 | 0.359242 | 0.279904 | -0.428571 |

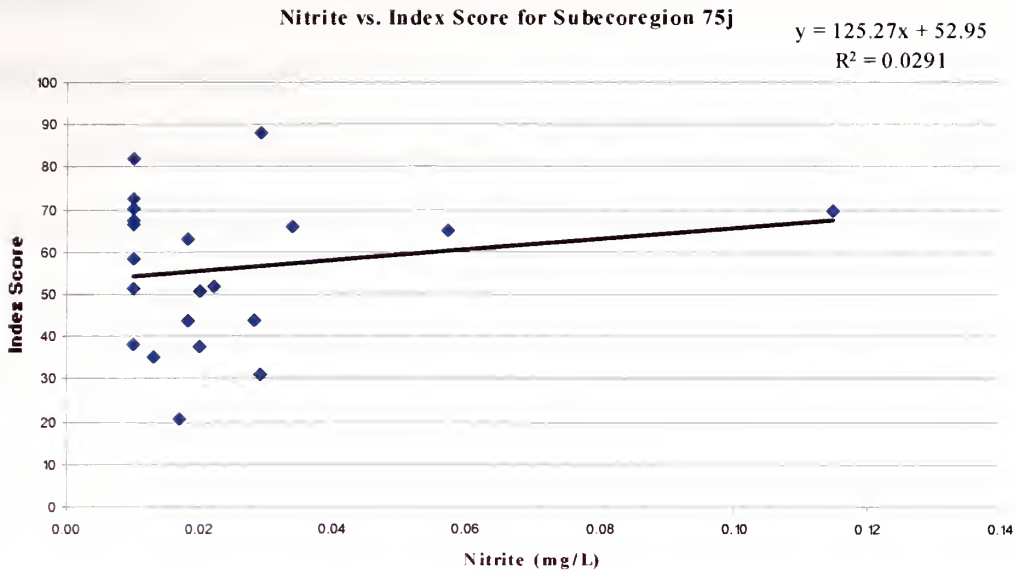


Figure 82: Nitrite vs. Index Score for Subecoregion 75j

Discrimination efficiency between reference and impaired sites was 70%, with seven of the ten impaired sites being classified correctly based on the reference condition. The box and whisker plot illustrated some overlap of interquartile range between reference and impaired sites (Figure 83).

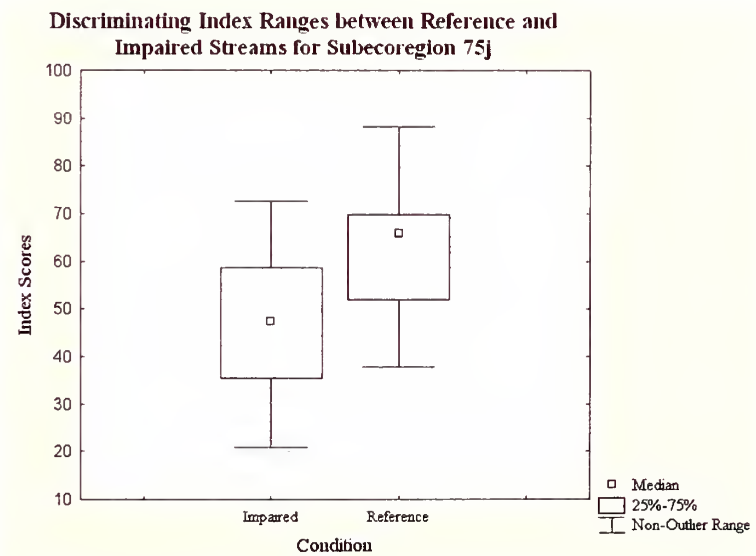


Figure 83: Discriminating Index Ranges between Reference and Impaired Streams for Subecoregion 75j

## DISCUSSION

### Ecoregion 66 (Blue Ridge)

Nitrite concentrations were significantly different between reference and impaired sites and were correlated with the macroinvertebrate multi-metric index scores for the Blue Ridge ecoregion. However the correlation coefficient between metric scores and nitrite concentrations was 0.54 and scatter plot illustrated a horizontal line (Figure 3), thus indicating nitrite concentrations were not significantly correlated with the multi-metric index scores.

Total phosphorous concentrations, in sediment, were significantly different between reference and impaired sites and were correlated with the macroinvertebrate multi-metric index score for the Blue Ridge ecoregion. However the correlation coefficient between total phosphorus concentrations in sediment and the index scores was –0.49 and most concentrations were on the lower end of the scatter plot (Figure 4), thus indicating total phosphorus concentrations in sediment were not significantly correlated with the multi-metric index scores.

Both nitrite concentrations and total phosphorus in sediment concentrations were significantly different between reference and impaired sites, thus indicating a possible source of nutrient loading. However a correlation between the concentrations and index scores was not found. Further research should be conducted to determine if individual metric scores could be correlated with nutrient loading.

In the Blue Ridge ecoregion, the impaired sites have a larger proportion of urbanized and pasture lands, which may contribute to nitrite loading in the water column and phosphorous loading in sediment, which may come from excessive applications of fertilizers, manure, or by atmospheric deposition. Phosphorus exists in the atmosphere as fine-grained particulate matter and this sorbed phosphorus can enter natural waters by both dry fallout and rainfall (EPA 1999b). Possible sources of atmospheric phosphorus are fine spray from water which contains phosphorus, mainly from oceanic sources, which can be only a few kilometers from the sea or hundreds of kilometers; fine particles from Earth's crust; aerosols from plants, from living or dead plant material; and from the burning of fossil fuels (Newman 1995). Lightning discharge also releases small amounts of nitrogen as atmospheric deposition, in the troposphere (Faure 1998). During lightning discharge NO is converted to $NO_3^-$ by a photochemical reaction, which is removed form the atmosphere by rain or snow. Nitrate produced by lightning, in the atmosphere, is assimilated by plants.

**Blue Ridge Subecoregions**

Total phosphorous concentrations were significantly different between reference and impaired sites for the Southern Crystalline Ridges and Mountains (66d), but were not significantly correlated with the macroinvertebrate multi-metric index scores. This indicates that total phosphorus in the water column could be a potential source of nutrient loading for streams in this subecoregion; however, total phosphorus did not indicate a correlation between concentrations and macroinvertebrate taxa, for this particular index. In this case the multi-metric index scores did not discriminate between reference and impaired sites, but did detect nutrient loading. Further research should be conducted to determine if individual metric scores could be correlated with nutrient loading.

In the Southern Metasedimentary Mountains subecoregion (66g), nitrate-nitrite concentrations were significantly different between reference and impaired sites, but were not significantly correlated with the macroinvertebrate multi-metric index scores. This indicated that nitrate-nitrite in the water column could be a potential source of impairment for this subecoregion; however, nitrate-nitrite did not demonstrate a correlation between concentrations and macroinvertebrate taxa, for this particular index. Further research should be conducted to determine if individual metric scores could be correlated with nutrient loading.

Total phosphorus concentrations in the water column, for the Southern Metasedimentary Mountains (66g), was negatively correlated with the index scores, however the correlation coefficient was –0.66 and there were no mid-range concentrations (Figure 9). This indicated there was no significant trend between the multi-metric index scores and the total phosphorus concentrations for this subecoregion. Further research should be conducted to determine if individual metric scores could be correlated with nutrient loading.

Total phosphorous concentrations in the water column were significantly different between reference and impaired sites for the Broad Basin subecoregion (66j) and were correlated with the macroinvertebrate multi-metric index scores. The correlation coefficient between total phosphorus concentrations and index scores was 0.79 and the scatter plot indicated a linear relationship (Figure 12), which indicated total phosphorus concentrations were significantly correlated with the index scores. Total phosphorus concentrations in the water column were positively correlated with the macroinvertebrate multi-metric index scores. As total phosphorus increased, the index scores increased, thus implying an improvement in stream health.

Additionally, for the Broad Basin subecoregion (66j), nitrite concentrations were positively correlated and total phosphorus concentrations in sediment were negatively correlated with the macroinvertebrate multi-metric index scores. The correlation coefficient between nitrite concentrations and index scores was 0.66 and the scatter plot illustrated a horizontal line (Figure 13), thus indicating that nitrite concentrations were not significantly correlated with the index scores. Further research should be conducted to determine if individual metric scores could be correlated with nutrient loading.

Total phosphorous concentrations in sediment were significantly correlated with the macroinvertebrate multi-metric index scores for the Broad Basin subecoregion (66j). The correlation coefficient between total phosphorus concentrations and index scores was –0.84 and the scatter plot indicated a linear relationship (Figure 14), thus indicating total phosphorus concentrations in sediment were significantly correlated with the index scores. Total phosphorus concentrations in the sediment were negatively correlated with the macroinvertebrate multi-metric index scores. As total phosphorus concentrations increased, the index scores decreased, thus implying a decline in stream health.

Among the three Blue Ridge subecoregions, reference sites have a larger percentage of forested land, while the impaired sites contained higher proportions of agriculture, silviculture (as percent barren land), and urbanization. These changes in land use probably accounted for the significant differences in phosphorus concentrations in the Southern Crystalline Ridges & Mountains (66d) and the Broad Basins (66j) subecoregions. Similarly, there were significant differences in nitrate-nitrite concentrations in the Southern Metasedimentary Mountains (66g) subecoregion. Since the Southern Metasedimentary Mountains subecoregion was more dominated by silviculture than the other two subecoregions, nitrogen was probably not as rapidly processed in the areas that were barren. Fertilizer and atmospheric inputs, as well as other urbanization impacts, affected all three of the subecoregions.

**Ecoregion 67 (Ridge and Valley)**

Total phosphorous concentrations in the water column were significantly different between reference and impaired sites and were correlated with the macroinvertebrate multi-metric index scores for the Ridge and Valley ecoregion. However the correlation coefficient between total phosphorus concentrations and the index scores was 0.62 and the scatter plot (Figure 17) did not indicate a distinctive pattern, thus indicating total phosphorus concentrations in the water column were not significantly correlated with the multi-metric index scores. Total phosphorus concentrations were significantly different between reference and impaired sites, thus indicating a possible source of nutrient loading. However a correlation between the concentrations and index scores was not found. Further research should be conducted to determine if individual metric scores could be correlated with nutrient loading.

Similar to the Blue Ridge, forestry predominated in the Ridge and Valley ecoregion. A majority of the reference sites had a larger percentage of forested land, while the impaired sites had a smaller percentage of the forested land. No other trends in land use are apparent. Total phosphorus may come from excessive applications of fertilizers, manure, or by atmospheric deposition. Phosphorus exists in the atmosphere as fine-grained particulate matter and this sorbed phosphorus can enter natural waters by both dry fallout and rainfall (EPA 1999b). Possible sources of atmospheric phosphorus are fine spray from water which contains phosphorus, mainly from oceanic sources, which can be only a few kilometers from the sea or hundreds of kilometers; fine particles from Earth's crust; aerosols from plants, from living or dead plant material; and from the burning of fossil fuels (Newman 1995).

**Ridge and Valley Subecoregions**

Total phosphorus concentrations in the water column for the Southern Limestone/Dolomite Valleys and Low Rolling Hills and Southern Dissected Ridges and Knobs (67f&i) subecoregion were correlated with the multi-metric index scores. The correlation coefficient between total phosphorus concentrations and index scores was 0.70, but the scatter plot (Figure 20) illustrated only one point on the upper end of the concentration, thus without this point the correlation would not be considered significant. This indicated total phosphorus concentrations in the water column were not significantly correlated with index scores. Further research should be conducted to determine if individual metric scores could be correlated with nutrient loading.

Total phosphorus concentrations in the water column and in sediment were correlated with the index scores for the Southern Shale Valleys (67g) subecoregion. Total phosphorus concentrations in the water column versus index scores had a correlation coefficient of 0.70 and indicated a linear regression for the scatter plot (Figure 23). This indicated a significant correlation between total phosphorus concentrations in the water column and the multi-metric index scores. Total phosphorus concentrations in the water column were positively correlated with the macroinvertebrate index score. As total phosphorus increased, the index scores increased, thus implying an improvement in stream health.

Total phosphorus in sediment versus the index scores had a correlation coefficient of -0.67 and the scatter plot (Figure 24) did not indicate a distinctive pattern for the Southern Shale Valleys (67g) subecoregion. The scatter plot had one point for the higher concentrations and the other points were in the lower concentration range. This indicated that total phosphorus concentrations in sediment were not significantly correlated with the index scores. Further research should be conducted to determine if individual metric scores could be correlated with nutrient loading.

For the Southern Sandstone Ridges (67h) subecoregion, no nutrient parameters were found to be significantly different between reference and impaired sites. Also for this subecoregion, no nutrient parameters were significantly correlated with the multi-metric index scores. Further research should be conducted to determine if individual metric scores could be correlated with nutrient loading.

Only four reference and two impaired streams were ultimately sampled in the Southern Sandstone Ridges (67h) subecoregion. As a result, this small sample size may be the reason no nutrient differences were detected. Among the Southern Limestone/Dolomite Valleys and Low Rolling Hills & Southern Dissected Ridges and Knobs (67f&i) subecoregion, reference sites had a higher percentage of forested lands than impaired sites while impaired sites had a higher percentage of urbanization. However, significant differences in nutrient concentrations were not detected for this subecoregion.

The proportion of forested lands decreased from reference to impaired sites in the Southern Shale Valleys (67g) subecoregion. Total phosphorous loading may come from excessive applications of fertilizers, manure, or by atmospheric deposition. Phosphorus exists in the atmosphere as fine-grained particulate matter and this sorbed phosphorus can enter natural waters by both dry fallout and rainfall (EPA 1999b). Possible sources of atmospheric phosphorus are fine spray from water which contains phosphorus, mainly from oceanic sources, which can be only a few kilometers from the sea or hundreds of kilometers; fine particles from Earth's crust; aerosols from plants, from living or dead plant material; and from the burning of fossil fuels (Newman 1995). Lightning discharge also releases small amounts of nitrogen as atmospheric deposition, in the troposphere (Faure 1998). During lightning discharge NO is converted to $NO_3^-$ by a photochemical reaction, which is removed form the atmosphere by rain or snow. Nitrate produced by lightning, in the atmosphere, is assimilated by plants.

These three subecoregions, which are part of the Ridge and Valley ecoregion, did not have a large agricultural component since the region is dominated by steep valleys and relatively high gradient streams. Thus, there is very little difference between the physical, chemical, and biological characteristics of the reference and impaired sites and it should not be surprising that little difference in nutrient loading was detected in this area.

**Ecoregion 68 (Southwestern Appalachians) and Subecoregion 68c&d**

Total phosphorus concentrations in the water column for the Southwestern Appalachians ecoregion were correlated with the multi-metric index scores. The correlation coefficient between total phosphorus concentrations and index scores was 0.73 and the scatter plot (Figure 29) indicated a linear regression, thus indicating total phosphorus concentrations and the index scores were significantly correlated. Total phosphorus concentrations in the water column were positively correlated with the macroinvertebrate index score. As total phosphorus increased, the index scores increased, thus implying an improvement in stream health.

The Southwestern Appalachians ecoregion did not have a distinguishable difference in land uses between reference and impaired stream sites. This ecoregion is small and the resulting small sample size had low variability in conditions. The small sample size may be the reason most nutrient parameters were not found to be significantly different between reference and impaired sites. However total phosphorus in the water column was found to enhance the macroinvertebrate community. Total phosphorus inputs may come from fertilizer and atmospheric inputs, as well as other urbanization impacts.

**Ecoregion 45 (Piedmont)**

Total phosphorus concentrations in the water column were significantly different between reference and impaired sites and were correlated with the macroinvertebrate multi-metric index scores for the Piedmont ecoregion. However the correlation coefficient between metric scores and total phosphorus concentrations was 0.45 and scatter plot did not indicate a distinctive pattern (Figure 32). There was only one concentration on the higher end of the plot and the other concentrations all clumped together on the lower end of the plot, thus indicating total phosphorus concentrations were not significantly correlated with the multi-metric index scores.

In the Piedmont ecoregion, reference sites were more influenced by the presence of forested lands while the majority of impaired sites were affected by urbanization and agriculture uses. Urbanization effects come from many sources, such as golf courses and lawn care activities. Thus increases in fertilizer, manure, and atmospheric inputs may be the cause of phosphorus increases detected between reference and impaired sites in the Piedmont.

**Piedmont Subecoregions**

For the Southern Inner Piedmont (45a) subecoregion, total phosphorus concentrations in the water column were significantly different between reference and impaired sites and were correlated with the multi-metric index scores. The correlation coefficient between the index score and total phosphorus concentrations was 0.87 and the scatter plot illustrated (Figure 35) a reasonable correlation. Total phosphorus concentrations in the water column were positively correlated with the macroinvertebrate index score. As total phosphorus increased, the index scores increased, thus implying an improvement in stream health.

Nitrate-Nitrite concentrations in the Southern Outer Piedmont (45b) subecoregion were significantly correlated between reference and impaired sites. This indicated that nitrate-nitrite loading could be a potential impairment. However nitrate-nitrite was not correlated with the multi-metric index score. Total phosphorus concentrations in the water column, for the Southern Outer Piedmont subecoregion, were correlated with the index scores. However the correlation coefficient between total phosphorus concentrations and index scores was 0.23, thus indicating they were not significantly correlated. Further research should be conducted to determine if individual metric scores could be correlated with nutrient loading.

For the Carolina Slate Belt (45c) subecoregion, no nutrient parameters were found to be significantly different between reference and impaired sites. Also for this subecoregion, no nutrient parameters were significantly correlated with the multi-metric index scores. Further research should be conducted to determine if individual metric scores could be correlated with nutrient loading.

For the Talladega Upland (45d) subecoregion, total phosphorus concentrations in the water column were significantly different between reference and impaired sites and were correlated with the multi-metric index scores. The correlation coefficient between the index scores and total phosphorus concentrations was 0.78 and the scatter plot indicated (Figure 43) a linear relationship. Total phosphorus concentrations in the water column were positively correlated with the macroinvertebrate index score. As total phosphorus increased, the index scores increased, thus implying an improvement in stream health.

For the Pine Mountain Ridge (45h) subecoregion, no nutrient parameters were found to be significantly different between reference and impaired sites. Also for this subecoregion, no nutrient parameters were significantly correlated with the multi-metric index score. Further research should be conducted to determine if individual metric scores could be correlated with nutrient loading.

Increased urbanization affected impaired sites in the Southern Inner Piedmont (45a) subecoregion. Fertilizer applications and atmospheric inputs probably affected this subecoregion; thus, the significant differences in total phosphorus concentrations for this subecoregion. The Carolina Slate Belt (45c) and Pine Mountain Ridge (45h) subecoregions showed a decrease in forested lands for impaired sites. However, the smallest proportion of forested land for the Carolina Slate Belt is 50% and for Pine Mountain Ridge, is 61%. While there were some differences between reference and impaired sites in these two subecoregions, no significant differences in nutrient concentrations could be detected. There were no significant differences in land use in the Talladega Upland (45d) subecoregion, located in the northern part of the state where there are steep gradients. There were significant differences in phosphorus concentrations between reference and impaired sites and the steep gradients may allow for greater runoff of nutrient laden water into the streams. A greater proportion of urbanization (Atlanta and its suburbs, for example) dominated the impaired streams of the Southern Outer Piedmont (45b) subecoregion and there was a significant difference in nitrate-nitrite concentrations. Urbanized streams are more commonly influenced by increases in nitrogen based fertilizers and could account for the significant differences in nitrate-nitrite concentrations.

**Ecoregion 65 (Southeastern Plains)**

Nitrite concentrations in the water column for the Southeastern Plains were significantly different between reference and impaired sites and were correlated with the multi-metric index scores. However the correlation coefficient between nitrite concentrations and index scores was 0.37 and the scatter plot (Figure 48) was a horizontal line, thus indicating nitrite was not significantly correlated with the index scores. Further research should be conducted to determine if individual metric scores could be correlated with nutrient loading. Nitrite concentrations were significantly different between reference and impaired sites, thus indicating a possible source of nutrient loading.

The Southeastern Plains ecoregion reference sites were predominately influenced by forested lands (natural or plantation) whereas the impaired sites were predominately affected by agriculture, in this case mostly pasturelands. A majority of the impaired streams were also affected by urbanization. Thus, increases in manure and fertilizer use and/or misuse may be the cause of nitrite loading in the Southeastern Plains ecoregion.

**Southeastern Plains subecoregions**

For the Sand Hills (65c) subecoregion, nitrite concentrations were significantly different between reference and impaired sites and total phosphorus concentrations in sediment were correlated with the multi-metric index scores. The correlation coefficient between total phosphorus concentrations in sediment and index scores was 0.77 and the scatter plot (Figure 51) illustrated a reasonable correlation. Total phosphorus concentrations in sediment were positively correlated with the macroinvertebrate index score. As total phosphorus increased, the index scores increased, thus implying an improvement in stream health. Nitrite concentrations were significantly different between reference and impaired sites, thus indicating a possible source of nutrient loading.

For the Southern Hilly Gulf Coastal Plain (65d) subecoregion, total phosphorus concentrations were significantly different between reference and impaired sites. Nitrate-nitrite concentrations and total phosphorus concentrations in the water column were positively correlated with the multi-metric index scores. For nitrate-nitrite concentrations the correlation coefficient between the concentrations and index scores was 0.64 and the scatter plot (Figure 54) illustrated a horizontal line, which indicated nitrate-nitrite was not significantly correlated with the index scores. Total phosphorus concentrations and the index scores had a correlation coefficient of 0.80 and the scatter plot illustrated (Figure 55) a reasonable correlation. Total phosphorus concentrations in the water column were positively correlated with the macroinvertebrate index score. As total phosphorus increased, the index scores increased, thus implying an improvement in stream health.

For the Dougherty Plain (65g) subecoregion, no nutrient parameters were found to be significantly different between reference and impaired sites. Also for this subecoregion, no nutrient parameters were significantly correlated with the multi-metric index score. Further research should be conducted to determine if individual metric scores could be correlated with nutrient loading.

Ammonia concentrations were correlated with the multi-metric index scores for the Tifton Upland (65h) subecoregion. The correlation coefficient between ammonia concentrations and index scores was –0.82 and the scatter plot (Figure 60) illustrated a reasonable correlation. Ammonia concentrations in the water column were negatively correlated with the macroinvertebrate index score. As ammonia increased, the index scores decreased, thus implying a decline in stream health.

For the Coastal Plain Red Uplands (65k) subecoregion, no nutrient parameters were found to be significantly different between reference and impaired sites. Also for this subecoregion, no nutrient parameters were significantly correlated with the multi-metric index scores. Further research should be conducted to determine if individual metric scores could be correlated with nutrient loading.

For the Atlantic Southern Loam Plains (65l) subecoregion, ammonia and nitrite concentrations were positively correlated with the multi-metric index scores. The correlation coefficient between ammonia concentrations and index scores was 0.68 and the scatter plot for ammonia versus the index score (Figure 65) illustrated a horizontal line. This indicated ammonia was not significantly correlated with the index score. The correlation coefficient between nitrite concentrations and index scores was 0.82 and the scatter plot (Figure 66) illustrated a reasonable correlation. Nitrite concentrations in the water column were positively correlated with the macroinvertebrate index score. As nitrite increased, the index scores increased, thus implying an improvement in stream health.

In the Tallahassee Hills/Valdosta Limesink (65o) subecoregion, total phosphorus concentrations were significantly different between reference and impaired sites. For this subecoregion nitrite concentrations were correlated with the multi-metric index scores. The correlation coefficient between nitrite concentrations and index scores was 0.71. However the scatter plot (Figure 69) did not demonstrate a good correlation between nitrite concentrations and index scores, thus indicating nitrite was not significantly correlated with the macroinvertebrate community. Further research should be conducted to determine if individual metric scores could be correlated with nutrient loading.

Forestry decreased in the impaired sites in the Southern Hilly Gulf Coastal Plain (65d) subecoregion. A majority of the reference sites had a larger percentage of forested land, while the impaired sites had a smaller percentage of the forested land. No other trends in land use were apparent. Nitrogen and phosphorus loading may come from excessive applications of fertilizers, manure, or by atmospheric deposition.

Similar to other subecoregions with the same sorts of changes in land use, the Sand Hills (65c) subecoregion had a larger percentage of barren, agriculture, and urbanization land use, combined, than do reference sites. Thus, nitrite inputs may be coming from fertilizer, manure, and atmospheric deposition.

For the Tallahassee Hills/Valdosta Limesink (65o), no evident land use pattern was established. Total phosphorus inputs may be coming from fertilizer, manure, and atmospheric deposition. Also, since this area is a heavy agriculture area, the buffer zones may be reduced, thus allowing total phosphorus to enter the streams rapidly.

In the Atlantic Southern Loam Plains (65l) and Tifton Upland (65h) subecoregions, forestry land use was higher in the reference sites and agriculture was higher in the impaired sites, particularly row cropping. Thus nitrite and ammonia inputs may be coming from fertilizer, manure, and other farm practices.

The Coastal Plain Red Uplands (65k) and Dougherty Plain (65g) subecoregions did not appear to have significant nutrient differences between reference and impaired sites. These subecoregions have been heavily impacted by agricultural activities for decades. In these subecoregions, the reference conditions are probably degraded; already containing high nutrient loads, and contrasts between nutrient concentrations in reference and impaired streams are difficult. These subecoregions, which belong to the

Southeastern Plains ecoregion, also contain both black water and clear water streams. Black and clear water streams may need to have different metric criteria established (Pillai 2005). These subecoregions were also under drought conditions during the sampling index period and there might have been a resulting affect on nutrient loading. Reynolds and Edwards (1995) examined the effects of drought on stream water in the United Kingdom and found that, after a drought, nitrate concentrations increased nearly 13-fold from previously recorded levels. Reynolds *et al.* (1992) concluded that vegetation might become damaged due to drought stress; thus, reducing nutrient uptake and leading to an accumulation of nitrogen in the soil. These large increases in the soil can cause increases in soil water nitrate concentrations. Thus, in my study, streams that received rains prior to sampling may have had influxes of nutrients and did not accurately reflect historical nutrient levels. More research should be conducted on potential differences between black and clear water streams and additional sites should be sampled during non-drought conditions.

The southeastern plains subecoregions were dominated by low gradient streams, which may slow the travel rate of runoff containing fertilizer and other nutrient pollutants. Since nutrients are transported downstream in large quantities by turbulent mixing, then, by reducing diffusion gradients, nutrients come in contact with substrates on the stream bottom (Elwood *et al.* 1983). This makes nutrients available for sorption and use. In low flow conditions, retention and uptake are favored due to a high ratio of streambed area to channel volume, retention devices (*i.e.* debris dams and beaver ponds), and permeable substrates, which allow substantial interstitial flow (Allen 1995). Under high flow conditions, through-flow of inputs and export of stored materials are favored.

Thus, streams in these subecoregions may be able to more efficiently process nutrient loads over a longer period of time.

**Ecoregion 75 (Southern Coastal Plains)**

For the Southern Coastal Plains ecoregion, total phosphorus concentrations in the water column and in sediment were significantly different between reference and impaired sites and were correlated with the multi-metric index scores. The correlation coefficient between total phosphorus concentrations in the water column and index scores was 0.38 and the scatter plot (Figure 72) did not illustrate a clear correlation. Thus total phosphorus concentrations in the water column were not significantly correlated with the index scores. The correlation coefficient between total phosphorus concentrations in sediment and index scores was –0.59 and the scatter plot (Figure 73) did not illustrate a clear correlation. Thus total phosphorus concentrations in sediment were not significantly correlated with the index scores. Further research should be conducted to determine if individual metric scores could be correlated with nutrient loading.

In the Coastal Plains ecoregion, a majority of the reference streams had a large percentage of forested areas and lower percentage of urban land use. The urbanized areas in the Coastal Plains area, especially the city of Savannah, have many golf courses, likely a significant source of fertilizer runoff to area streams. In this ecoregion, many of the impaired streams have been channelized, resulting in a reduced riparian zone to control nutrient and sediment contamination. Nutrient flows are reduced to streams by riparian buffer strips (Vought *et al.* 1994). The major portion of the nitrogen and phosphorus carried by surface runoff can be retained with a buffer strip of 10 to 20 meters (Vought *et* *al*. 1994). A study conducted in the 1970's in the coastal plain of Georgia (Hubbard and Lowrance 1994) concluded the riparian zone is an effective nutrient filter, which was attributed to both denitrification and vegetative uptake. The study also concluded that nitrate was retained, utilized, or transformed 96% of the time in heavily vegetated riparian forests of the Coastal Plain. In the channelized streams, the riparian zone and vegetative bank cover are reduced; thus, nutrients are not taken up by the plants and are able to reach the stream by surface runoff. The increases in fertilizer application combined with significant channelization may be the reason for phosphorus loading in the Coastal Plains.

**Southern Coastal Plains subecoregions**

For the Okefenokee Plains (75e), Sea Island Flatwoods (75f), and Bacon Terrances (75h) subecoregions, no nutrient parameters were found to be significantly different between reference and impaired sites. In these subecoregions, no nutrient parameters were significantly correlated with the multi-metric index scores. Further research should be conducted to determine if individual metric scores could be correlated with nutrient loading.

For the Sea Islands/Coastal Marsh (75j) subecoregion, total phosphorus concentrations were significantly different between reference and impaired sites and nitrite concentrations were correlated with the multi-metric index scores. The correlation coefficient value between nitrite concentrations and index scores was 0.48 and the scatter plot (Figure 82) did not illustrate a clear correlation, thus indicating, that nitrite was not significantly correlated with the index scores. Further research should be conducted to determine if individual metric scores could be correlated with nutrient loading.

Impaired streams in the Sea Islands/Coastal Marsh subecoregion have greater proportions of urbanization land use than reference streams. Increased phosphorus loading may be coming from fertilizer from lawn care applications and atmospheric deposition. Phosphorus exists in the atmosphere as fine-grained particulate matter and this sorbed phosphorus can enter natural waters by both dry fallout and rainfall (EPA 1999b). Possible sources of atmospheric phosphorus are fine spray from water which contains phosphorus, mainly from oceanic sources, which can be only a few kilometers from the sea or hundreds of kilometers; fine particles from Earth's crust; aerosols from plants, from living or dead plant material; and from the burning of fossil fuels (Newman 1995). Many of the impaired streams in this subecoregion have been channelized, allowing nutrients to flow more rapidly into the streams since it is common for the riparian zone and vegetative bank zone to be reduced when channelized.

No nutrient differences were detected for streams in the Okefenokee Plains, Sea Island Flatwoods, and Bacon Terraces subecoregions. These subecoregions have been heavily impaired by urbanization and agriculture and the reference sites are probably degraded and contain high nutrient concentrations. Thus, it is difficult to establish comparisons to impaired streams. These subecoregions, which are included in the Coastal Plain ecoregion, also contain black and clear water streams and these streams were also affected by drought conditions during the sampling index period. In combination, the "noise" from a small and diverse sample size (blackwater and clear water) and increased nutrient concentrations in drought-stricken streams may explain the difficulty in detecting nutrient differences.

**Other Interferences**

The stream ecosystem is a very complex system and there are many factors that could be affecting nutrient availability. For instance, ammonia can act synergistically with other chemicals, resulting in toxic effects to aquatic organisms (Russo 1985). There has been some evidence that a combination of ammonia and copper is more toxic than either toxicant individually (Russo 1985). Similarly, the combination of both ammonia and zinc was greater than that of each chemical separately. The combination of ammonia and nitrate were reported to have additive toxicity, except when ammonia-to-nitrate ratios were very low. Other factors that affect ammonia include pH, dissolved oxygen concentration, temperature, calcium concentration, salinity, fluctuation or intermittency of exposures, and presence of other toxicants (Russo 1985). In some cases physical, chemical, and possibly biological influences were not tested, which could affect the results.

Land use is also another factor that is affecting the outcome of nutrient loading. Nitrogen and phosphorus inputs to streams are reduced in forested watersheds due to the biological and geochemical process that retain these nutrients in the upper soil horizons (Mulholland 1992). When forest vegetation is removed it causes a decrease in transpiration, increase of soil temperature, and increase in soil moisture, which are conditions that favor mineralization of stored soil organic matter (Golladay *et al.* 1992); thus, causing open nutrient cycles and high rates of nutrient loss from forests.

Weathering is one natural aspect that could be interfering with detecting non-point source nutrient loading. In this case, it cannot be determined what percentage comes from natural weathering and non-point source contaminates. Weathering is the breakdown of rock and soil minerals that introduces phosphorus, a mineral nutrient, into the biological components of the environment (EPA 2000). Inorganic phosphate ions, produced by the breakdown of mineral phosphorus, are absorbed by plants from the soil or water. The main reservoir of natural phosphorus comes from natural phosphate deposits, which are released through weathering, leaching erosion, and mining (EPA 1999b). This may interfere with detecting a nutrient parameter since there may be an increase of natural phosphorus in both reference and impaired sites. Watersheds draining phosphorus rich rocks (*i.e.* sedimentary or volcanic) can enrich the stream naturally, thus causing problems associated with increased phosphorus concentrations (EPA 1999b). The breakdown of rock and soil minerals introduces phosphorus, a mineral nutrient, into the biological components of the environment (EPA 2000). Plants from the soil or water absorb inorganic phosphate ions, produced by the breakdown of mineral phosphorus.

The main reservoir of natural phosphorus comes from rocks and natural phosphate deposits, which are released through weathering, leaching erosion, and mining (EPA 1999b). Most of phosphorous in rocks is from apatite, which occurs only as a minor component (Newman 1995). Porter & Fitzsimons (1978), Holland (1978), and Schlesinger (1991) reported phosphorus concentrations for world rocks: continental crust 0.6 – 1.2 mg/g, igneous rocks 0.6 – 1.3 mg/g, and sedimentary rocks: shales – 0.7 mg/g, sandstones 0.4 mg/g, and carbonates 0.2 mg/g. Phosphorus in rocks may interfere with detecting a direct anthropogenic affect from phosphorous loading since there may be an increase of natural phosphorus in both reference and impaired sites (EPA 1999b). Streams containing phosphorus-rich rocks, such as sedimentary or volcanic, can be enriched naturally, thus causing problems associated with increased phosphorus concentrations (EPA 1999b).

Another important issue is the availability of current data. Land use data are very important in this analysis and need to be kept up to date. Several studies (Peterjohn and Correll 1984, Lowrance *et al.* 1985, and Osborne and Wiley 1988) have shown a strong correlation between land use and nutrient concentrations. Nitrate-nitrite and orthophosphate significantly impact predominately in streams in agricultural and urban catchments. For this project, reference and impaired sites were determined and sampling was performed according to 1994 land use data (Gore *et al.* 2004). The 1998 land use data showed some differences from the 1994 data. However, since the data were coded differently, it was difficult to make effective comparisons. Thus, it will be very important to have land use data that are timely and comparable since the choice of reference characteristics is a process that is continually revisited as conditions change.

## CONCLUSION

### Macroinvertebrates

Buikema and Cairns (1980) and Munther (1985) have suggested that macroinvertebrates are very sensitive to environmental perturbations; thus, valuable in early detection of habitat changes (Rinne 1998).

At the community structure level (cluster analysis), only a few subecoregions were found to be significantly different in macroinvertebrate composition between reference and impaired sites. Thus, cluster analysis was not a good indicator of changes in macroinvertebrate distribution due to disturbance. However, all but two ecoregions (Piedmont and Southeastern Plains) and one subecoregion (Sea Islands/Coastal Marsh) were determined to be significantly different between macroinvertebrate taxa in reference and impaired sites when using macroinvertebrate multi-metric indices (a functional approach) rather than strict taxonomic composition values. It would appear, then that these indices are good indicators of changes in macroinvertebrate distribution. The differences found between macroinvertebrates may be able to be used to predict sources of impairment (*i.e.* nitrogen and phosphorus loading).

**Ecoregion Level**

At the Ecoregion level, total phosphorus in the water column was significantly different between reference and impaired sites for the Ridge & Valley (67), Piedmont (45), and Southern Coastal Plains (75). The Blue Ridge (66) and Southeastern Plains (65) ecoregions were significantly different between reference and impaired sites for nitrite concentrations. The Southwestern Appalachians (86) and Piedmont (45) ecoregions did not show significant differences in nutrients between reference and impaired sites. Figure 83 illustrates this distribution across the state of Georgia.

For the Blue Ridge (66), Ridge and Valley (67), Piedmont (45), Southeastern Plains (65), and Southern Coastal Plains (75) ecoregions no nutrient parameters were found to be significantly correlated with changing multi-metric index scores (see Figure 84 for distribution). Total phosphorus in the water column was found to be positively correlated with the index scores for the Southwestern Appalachians (68) ecoregion (see Figure 84 for distribution). In the Southwestern Appalachians ecoregion, there is only one subecoregion for the state of Georgia, resulting in a smaller sample size for analysis. This indicates the subecoregional approach is a better method to use when analyzing nutrient and macroinvertebrate data. This also indicates that, at the ecoregion level, the rapid bioassessment method cannot be used to determine if nutrient loading is impacting the macroinvertebrate community. However, nutrient loading was detected between reference and impaired sites, which varied between ecoregions. Trends between nutrient parameters and macroinvertebrates could not be established using the index scores. The individual metrics that comprise the index scores are different for each ecoregion (see Appendix 14 for metrics). Further research should be conducted to determine if certain individual metrics can predict a relationship between nutrient loading and macroinvertebrate community structure.

For future research, black, clear, tidal, and non-tidal streams should be analyzed separately to determine if different metrics should be used for each stream type. Separation of these stream types may reveal nutrient loading that was not apparent for this research. Since Georgia was under drought conditions during much of the sampling period, streams should be resampled during normal conditions to determine if the drought affected the results. A single grab-sample of chemical conditions may be insufficient to create an adequate analysis of nutrient loading in most ecoregions. It may be necessary to conduct serial sampling of nutrients over time, in order to determine the response of the RBP scores to change in nutrient levels.

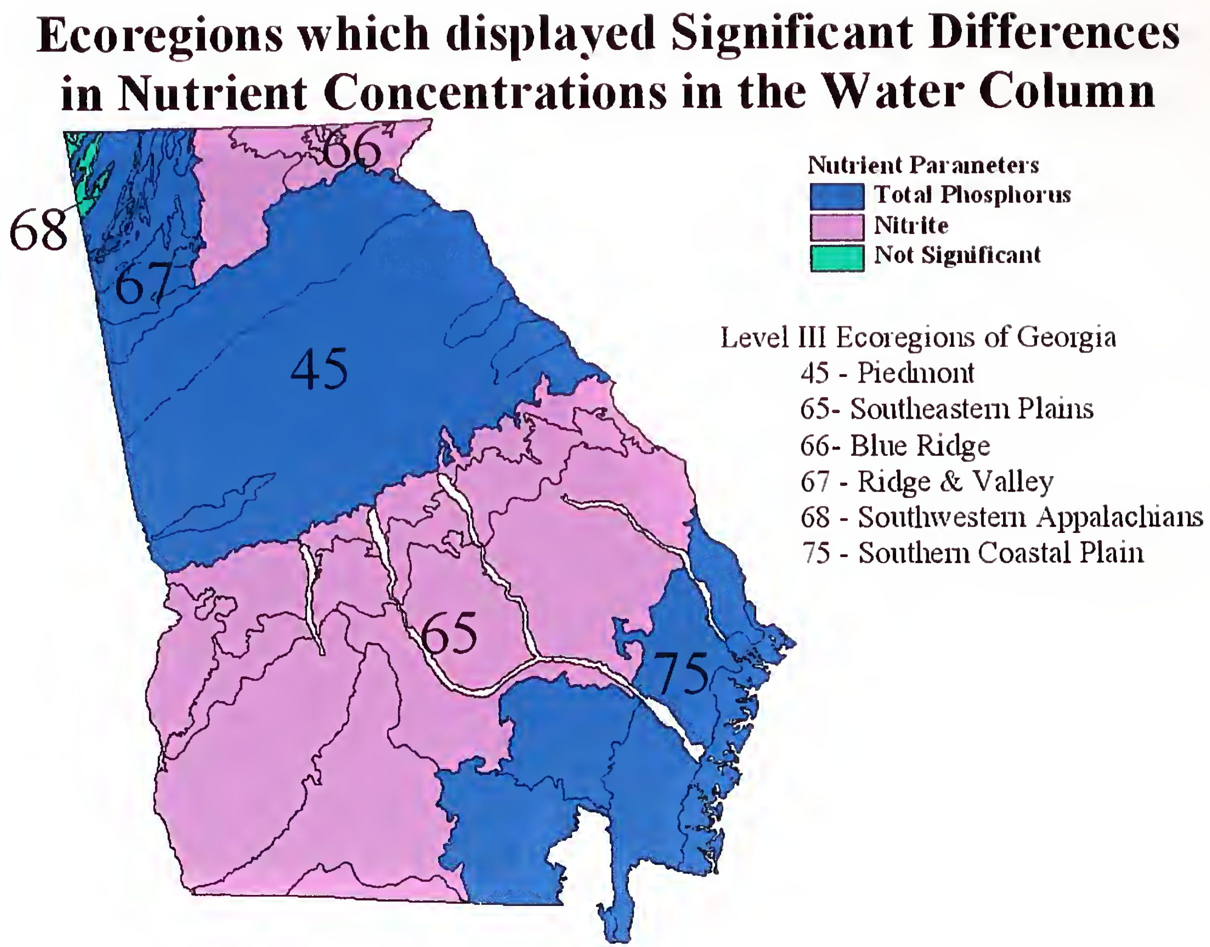


Figure 84: Ecoregions which displayed Significant Differences in Nutrient Concentrations in the Water Column

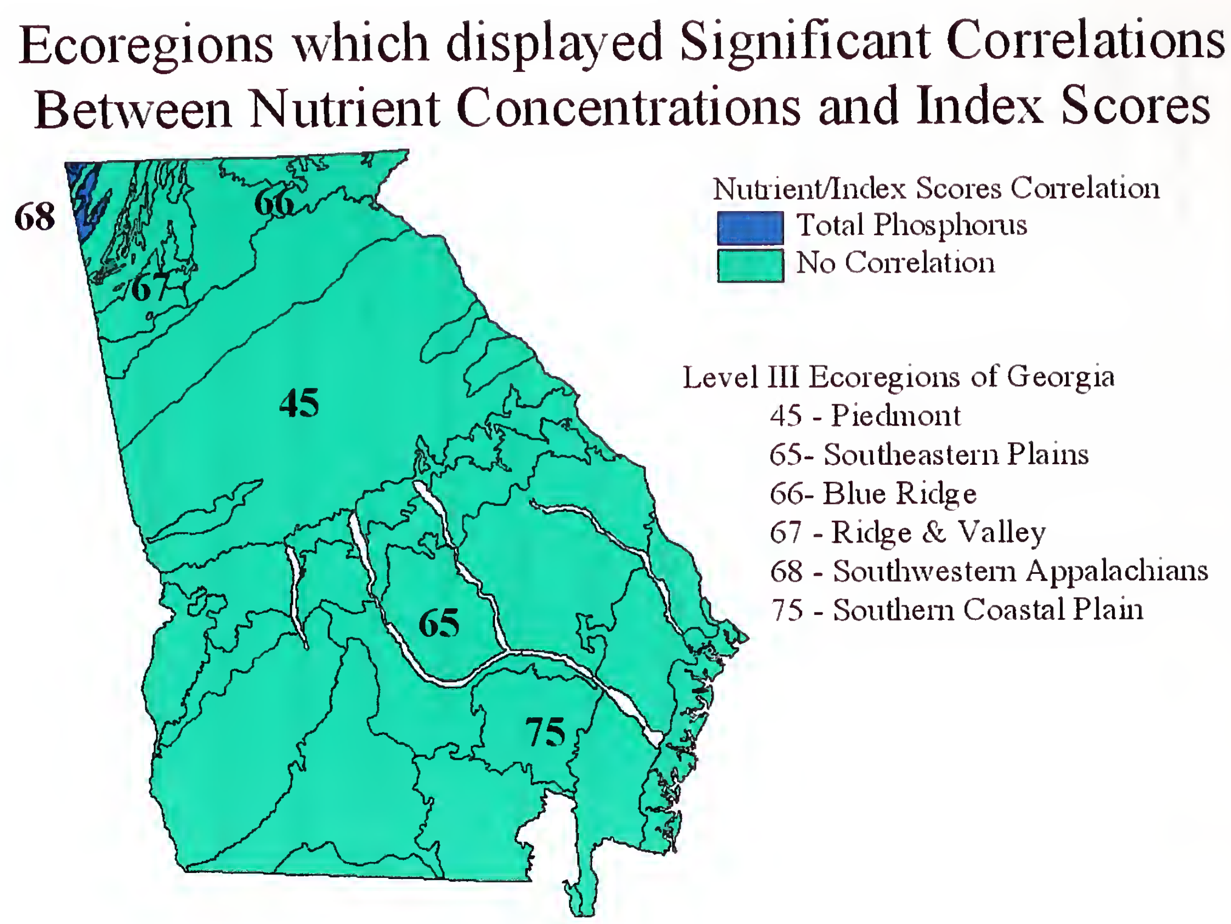


Figure 85: Ecoregions which displayed Significant Correlations Between Nutrient Concentrations and Index Scores

**Subecoregion Level**

Total phosphorus, nitrate-nitrite, and nitrite concentrations in the water column were found to be significantly different between reference and impaired sites in many of the subecoregions (see Figure 85 for distribution). Nitrate-nitrite concentrations were significantly different for reference and impaired streams in the Southern Metasedimentary Mountains (66g) and Southern Outer Piedmont (45b) subecoregions. Nitrite in the water column was found to be significantly different between reference and impaired sites for the Sand Hills (65c) subecoregion. Total phosphorus concentrations were found to be significantly different between reference and impaired sites for seven subecoregions: Southern Crystalline Ridges and Mountains (66d), Broad Basins (66j), Southern Inner Piedmont (45a), Talladega Upland (45d), Southern Hilly Gulf Coastal Plain (65d), Tallahassee Hills/Valdosta Limesink (65o), and Sea Island Flatwoods (75j). No significant differences in nutrient concentrations were found between reference and impaired streams in the remainder of the subecoregions.

Total phosphorus concentrations in the water column was positively correlated with the macroinvertebrate multi-metric index scores for these subecoregions: Broad Basins (66j), Southern Shale Valleys (67g), Plateau Escarpment and Southern Table Plateaus (68c&d), Southern Inner Piedmont (45a), Talladega Upland (45d), and Southern Hilly Gulf Coastal Plain (65d) (see Figure 86 for distribution). Ammonia concentrations were negatively correlated with the macroinvertebrate multi-metric index scores for the Tifton Upland (65h) subecoregion (see Figure 86 for distribution). For the Atlantic Southern Loam Plains (65l), nitrite concentrations were positively correlated with the macroinvertebrate multi-metric index scores (see Figure 86 for distribution). No nutrient parameters were found to be significantly correlated with the index scores for the other subecoregions for this research.

The rapid bioassessment method detected total phosphorus loading in the water column and correlations between concentrations and index scores across the state of Georgia. However, as total phosphorus concentrations increased in the water column, the index scores increased. This may indicate total phosphorus concentrations ranging from 0.01 to 1.2 mg/L were not impairing the macroinvertebrate community, but improving the stream health.

Ammonia and nitrite concentrations were only found to be significantly correlated with the macroinvertebrate multi-metric index scores in one subecoregion, respectively. Nitrate-nitrite concentrations were not found to be significantly correlated with the macroinvertebrate multi-metric index scores for any of the subecoregions. For these nutrient parameters, the rapid bioassessment method was not detecting a correlation between macroinvertebrates and nutrient parameters. However, nutrient loading was detected between reference and impaired sites.

The individual metrics that comprise the index scores were different for each subecoregion (see Appendix 14 for metrics). Further research should be conducted to determine if individual metrics can predict a relationship between nutrient loading and macroinvertebrate community structure. While total phosphorus in the water column was detected in six of the subecoregions, the rapid bioassessment method (macroinvertebrate multi-metric index score) is not recommended to determine if nutrient parameters are correlated with the macroinvertebrate community.

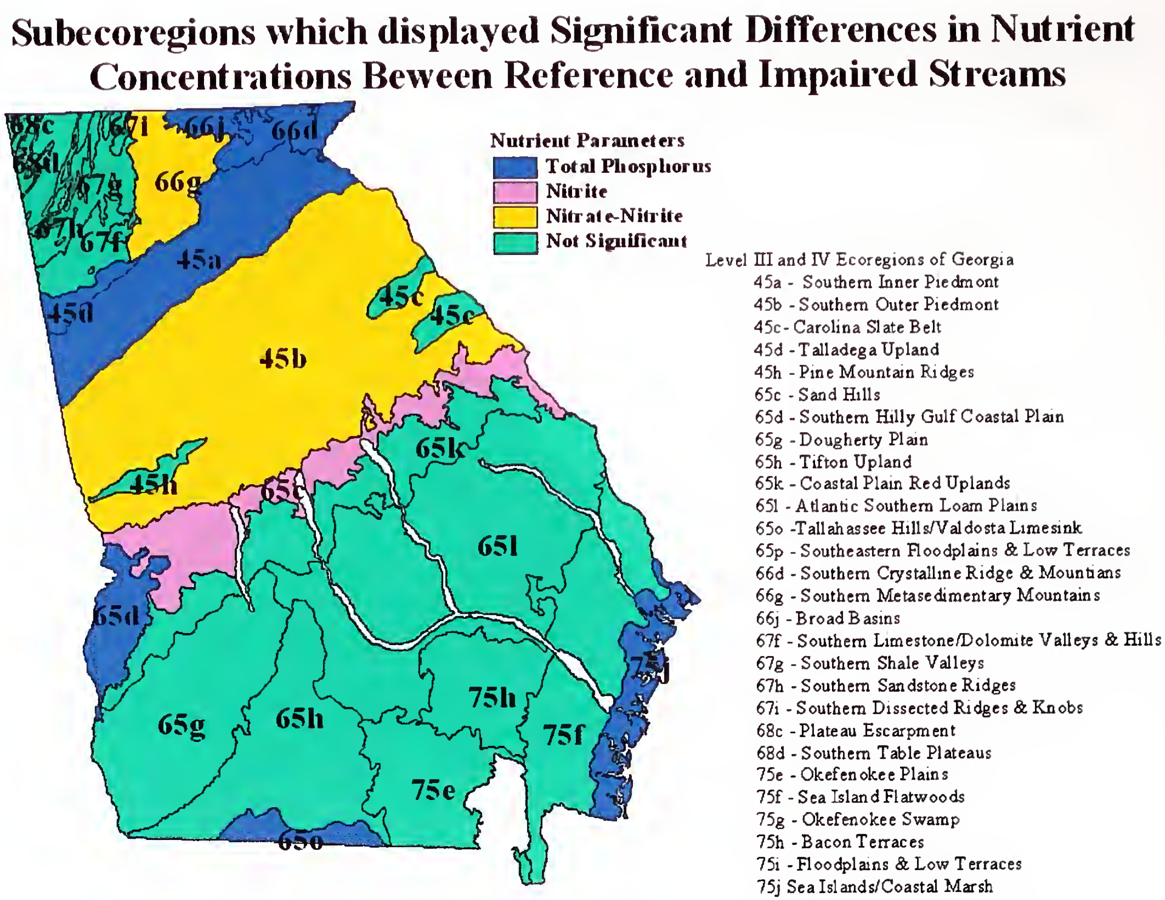


Figure 86: Subecoregions which displayed Significant Differences in Nutrient Concentrations Between Reference and Impaired Streams

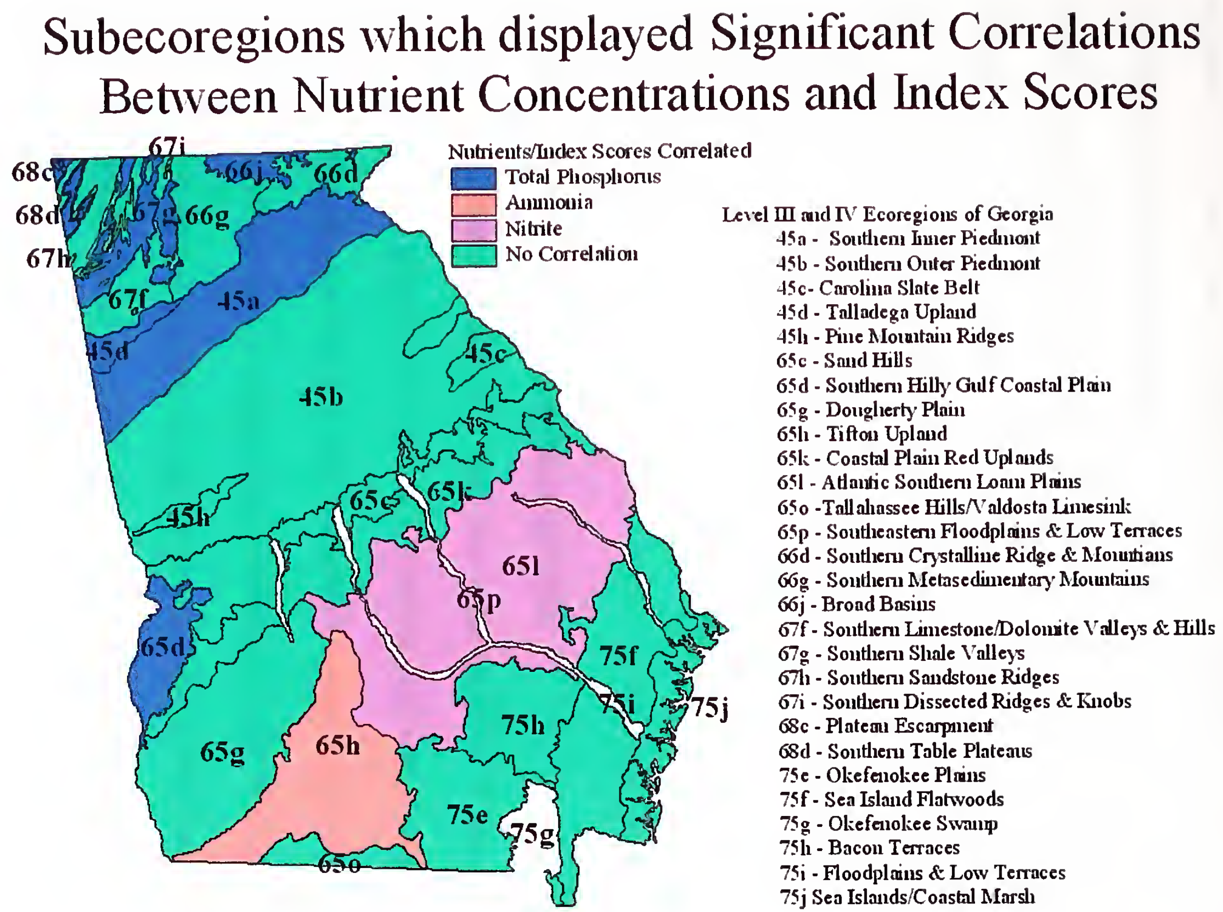


Figure 87: Subecoregions which displayed Significant Correlations Between Nutrient Concentrations and Index Scores

For future research, black, clear, tidal, and non-tidal streams should be analyzed separately to determine if different metrics should be used for each stream type. Separation of these stream types may reveal nutrient loading that was not apparent before. Since Georgia was under drought conditions during much of the sampling period, streams should be resampled during normal conditions to determine if the drought affected these results. A single grab-sample of chemical conditions may be insufficient to create an adequate analysis of nutrient loading in most ecoregions. It may be necessary to conduct serial sampling of nutrients over time, in order to determine the response of the RBP scores to change in nutrient levels.

**Ecoregional versus Subecoregional Approach**

The Piedmont ecoregion covers a large portion of the state of Georgia and stretches from the Atlantic coast to the western border with Alabama. Within this large ecoregion, subecoregions have a great variability in characteristics and impairments. The Southern Outer Piedmont (Atlanta area) is affected mostly by urbanization. The Carolina Slate Belt and Pine Mountain Ridge subecoregions contain less impaired areas. The Southern Inner Piedmont and Talladega Upland subecoregions are affected by forestry and agriculture. This ecoregion is also a transitional area for the state. The northern half of the ecoregion is similar to the Ridge and Valley and Blue Ridge, while the more southern potions of the ecoregion are like the Southeastern Plains. With this amount of ecoregional variability, the subecoregional level is the only appropriate level for macroinvertebrate metric analysis. These variations across the ecoregion make it difficult to develop a multi-metric index for the whole ecoregion.

The Southeastern Plains ecoregion contains both black and clear water streams. Pillai (2005) suggests that a significant difference exists between these stream types and that the development of a separate multi-metric index for each reference stream type must be derived. In addition, some subecoregions were variously affected by drought conditions during the sampling period. Thus, a multi-metric macroinvertebrate index for the entire ecoregion is not appropriate.

The Sea Islands/Coastal Marsh subecoregion also contains both black and clear water streams. In addition, this subecoregion contains both tidal and non-tidal streams. Tolerance values, functional feeding groups, and habit have not been determined for the brackish and marine species found in the tidal communities. With the resulting small sample size of blackwater, clear water, tidal and non-tidal streams, clear distinction could not be made between reference and impaired sties for macroinvertebrate communities.

Although, nutrient concentrations and metric indices were significantly different between reference and impaired streams at the ecoregion level and subecoregion level (within the same ecoregion), the subecoregional level provided a better understanding of sources of impairment since the streams were physicochemically and biologically more similar and had similar impairments. Thus, the subecoregional approach should continue to be used by the Georgia Department of Natural Resources (GADNR) in developing criteria for stream reference condition and for identification of potential sources of impairment from high nutrient concentrations.

**Sediment**

Sediment particles greater than two millimeters in diameter, which may consist of shells, rocks, and other detrital materials, are usually not a source of bioavailable contaminants (Maher *et al.* 1999). Since the clay/silt particles, less than 63 micrometers, have a high specific surface area and because of surface coatings of iron and manganese oxides and natural organics, these particles are more likely to adsorb organic and trace metal contaminants. Thus, nutrients bound to clay and silt particles are most often associated with anthropogenic contaminants. Substrates of streams in the Coastal Plains (75) ecoregion are predominately silt/clay particles. These substrates allow phosphorus to be bound in the sediment and I detected a significant difference in bound phosphorus between reference and impaired sites (see Figure 87 for distribution). In the Blue Ridge (66) ecoregion, total phosphorus was also determined to be significantly different between reference and impaired sites (see Figure 87 for distribution). Bedrock, and boulders dominate the Blue Ridge, and the streams also contain sand and silt/clay particles. The other ecoregions and subecoregions did not have a significantly detectable total phosphorus concentration in sediment between reference and impaired sites.

Since sediments represent a potential source of contaminants to the overlying water, there can be a significant influence on overall water quality (Maher *et al.* 1999). Diffusion of contaminants to the water column from sediments occurs when the concentration in the pore water exceeds the concentration in the overlying water, thus increasing the contaminant levels in the water column.

Total phosphorus in sediment was negatively correlated with the macroinvertebrate multi-metric index for the Broad Basins (66j) subecoregion (see Figure

88 for distribution). This indicated that, as phosphorus concentrations increased, the index scores decreased, thus implying a decline in stream health. However for the Sand Hills (65c) subecoregion, the opposite affect was observed, total phosphorus in sediment being positively correlated with the macroinvertebrate multi-metric index (see Figure 88 for distribution). In the Broad Basins subecoregion, the area was a mix of gravel, cobble, boulder, bedrock, sand and silt. However in the Sand Hills subecoregion the subecoregion was dominated by sand and also contained a smaller percentage of silt, gravel, and cobble. The other differences between the two subecoregions were in the macroinvertebrate community composition due to change in available habitat. The Broad Basins subecoregion contains a mix of Coleoptera, Plecoptera, Trichoptera, Ephemeroptera, and Diptera taxa. The Sand Hills subecoregion also contained the same taxa, but had greater numbers of Diptera, particularly Chironomidae. Chironomidae live in and on the sediment, thus are found more frequently in the Sand Hills versus the Broad Basins subecoregion. Total phosphorus in sediment was not found to be significantly correlated with the index score at ecoregion level or to any of the other subecoregions (see Figure 88 for distribution).

The individual metrics that comprise the index scores were different for each subecoregion (see Appendix 14 for metrics). Further research should be conducted to determine if individual metrics can predict a relationship between nutrient loading and macroinvertebrate community structure. Currently, the RBP would not be recommended to determine if total phosphorus concentrations in sediment are influencing the macroinvertebrate community.

## Ecoregions with Significant Differences in Sediment-Bound Phosphorus

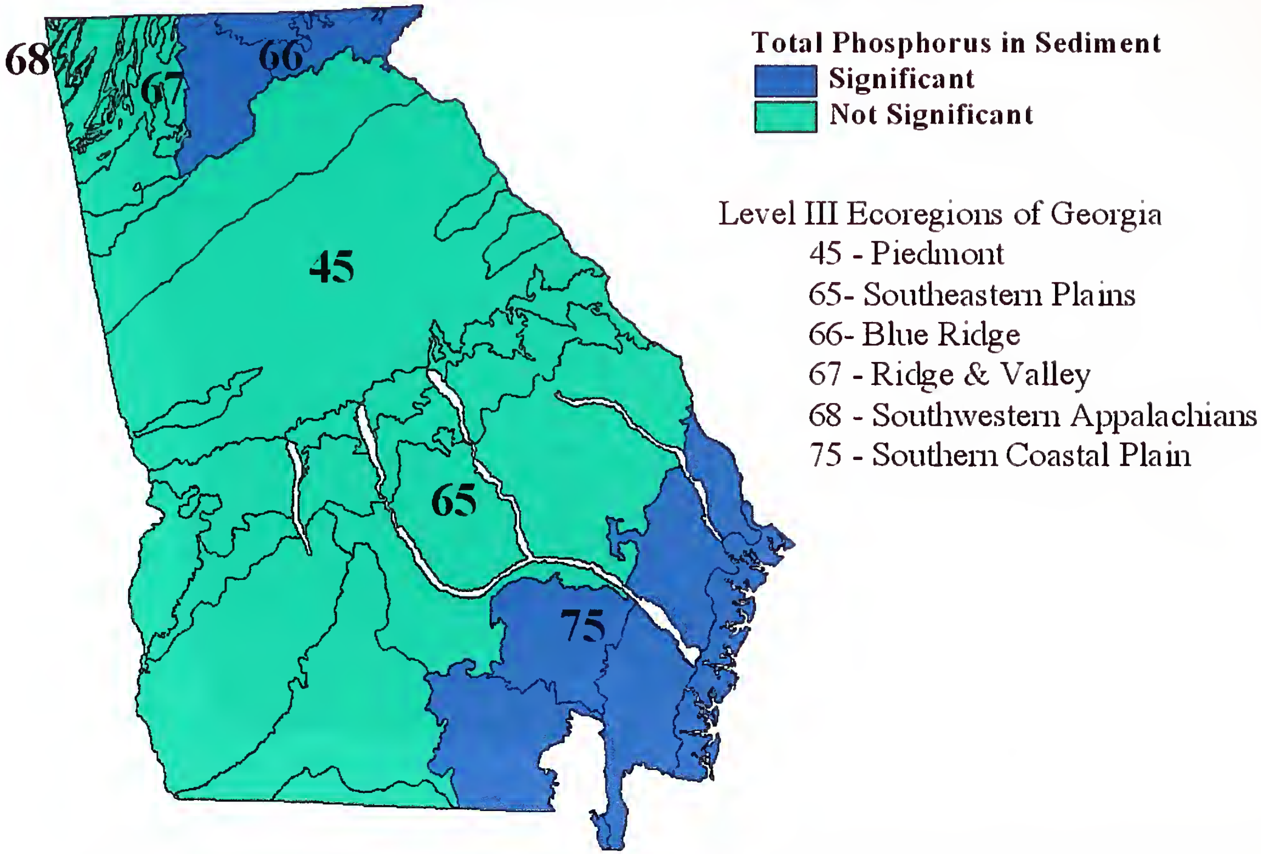


Figure 88: Ecoregions with Significant Differences in Sediment-Bound Phosphorus

## Subecoregions which displayed Significant Correlations Between Sediment-Bound Phosphorus and Index Scores

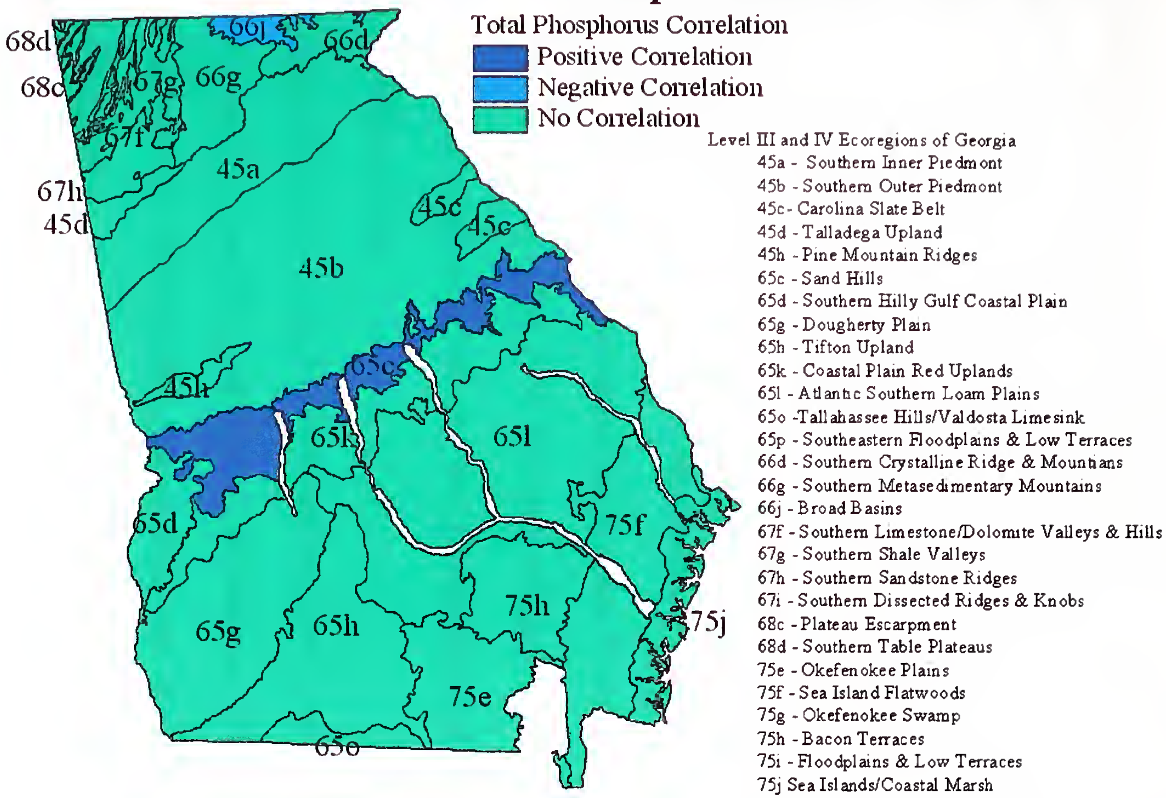


Figure 89: Subecoregions which displayed Significant Correlations Between Sediment-Bound Phosphorus and Index Scores

For future research, black, clear, tidal, and non-tidal streams should be analyzed separately to determine if different metrics should be used for each stream type. Separation of these stream types may reveal nutrient loading that was not apparent before. Since Georgia was under drought conditions during much of the sampling period, streams should be resampled during normal conditions to determine if the drought affected these results.

## Final Thoughts

The *Water Quality Inventory* 1996 report to congress has stated that 40% of streams and rivers surveyed, nation-wide, were impaired by nutrient loading and that no well-defined standards have been proposed to determine if nutrients impair flowing water (Dodds and Welch 2000). The EPA has been charged with establishing maximum acceptable levels of nutrients in streams and developing total maximum daily loads (TMDL's) of those nutrients.

Nutrient criteria are needed for many reasons related to adverse effects on humans and domestic animals, aesthetic impairment, interference with human use, negative impacts on aquatic life, and excessive nutrient input into downstream systems (Dodds and Welch 2000). Thus, it will be important to develop a method to determine what areas are affected by nutrient loading. Then, better recommendations on best management practices to control these nutrient impairments can be developed.

From the results of this study, the Rapid Bioassessment Protocol (RBP) may be used as an indication of nutrient loading as a potential source of impairment for the state of Georgia. Metric analysis determined that macroinvertebrate distribution is significantly different between reference and impaired sites. The results in only a few cases indicated a correlation between nutrient loading and the distribution of macroinvertebrates. These results indicate that nutrient loading is one of the conditions affecting the water quality in both reference and impaired sties. However, the RBP cannot be used to determine if nutrients are affecting the macroinvertebrate community directly. Further research should be conducted to determine if individual metric indices can be used to determine if there is a trend between macroinvertebrates and nutrients.

There are several reasons why a nutrient difference may not have been detected in this study. With a larger sample size for some ecoregions/subecoregions, differences in nutrient concentrations may have been detected. This was especially true in regions where more research needs to be done to determine separate reference conditions for black water and clear water, as well as tidal and non-tidal streams. Since drought affected these streams during the research period, extra research should be accomplished once the streams have maintained a normal water level over a longer period of time, at least a period of one year. In addition, other chemical (*i.e.* metals, salinity, pH, etc.) and physical (*i.e.* substrate, current, temperature, etc.) interferences to nutrient measurement need to be accounted for in future analyses.

However, since nutrient loading was detected in some areas across the state, the results from this study can be used as a tool for developing, evaluating, and monitoring priorities for total maximum daily loads (TMDL's) (Section 303[d] of CWA). The results of this study can also be used to detect macroinvertebrate differences that may be able to predict a source of impairment (*i.e.* nitrogen and phosphorus loading).

The results of this study may help to identify non-point source management needs and programs (as required by Section 319 of CWA) for the state of Georgia. For example, commonly used BMP's in the United States include sediment and erosion control, nutrient management pesticide management, livestock grazing management, irrigation water management, confined animal facilities, buffer strips, and constructed/engineered wetlands (Caruso 2000). Point sources can be controlled by single management techniques, but no single method is likely to be effective in restoring water-quality conditions in streams suffering from diffuse source impacts (Osborne and Kovacic 1993). While no single method can be used, the nutrient loading results from this study and continued monitoring after emplacement can determine which BMP's will be most effective.

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**APPENDICIES 1 - 42: CD-ROM Containing Appendices and Other Data**

Appendices 1-42 are contained on this CD. For the list of appendices, see table of contents. This CD also contains chemistry raw data values and standard curves for the nutrient parameters. The CD also contains EDAS (Ecological Data Application System) (Version 3.32K) analysis program, which contains all physical, chemical, land use, and biological data. To use this program, you must have Access 2000 or must update to the latest version of EDAS. EDAS can be acquired from Tetra Tech, Inc. (http://wwwttwater.com/edas.html) A newer copy of the data may be available from the Georgia Department of Natural Resources, Atlanta, Georgia. The CD also contains photographs of most of