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## Analysis of QA/QC Protocols and Value of Data to the Development of Reference Criteria in the Georgia Ecoregions Project

Tracy Jo Ferring  
Columbus State University

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ANALYSIS OF QA/QC PROTOCOLS AND VALUE OF DATA TO THE  
DEVELOPMENT OF REFERENCE CRITERIA IN THE  
GEORGIA ECOREGIONS PROJECT

Tracy Jo Ferring

Columbus State University

College of Science

The Graduate Program in Environmental Science

Analysis of QA/QC Protocols and Value of Data to the Development of  
Reference Criteria in the Georgia Ecoregions Project

A Thesis in

Environmental Science

by

Tracy Jo Ferring

Submitted in Partial Fulfillment  
of the Requirements  
for the Degree of

Master of Science

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I have submitted this thesis in partial fulfillment of the requirements for the degree of Master of Science.

December 9, 2005  
Date

  
Tracy J. Ferring

We approve the thesis of Tracy J. Ferring as presented here.

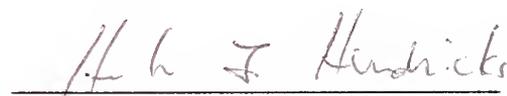
9 Dec 2005  
Date

  
James A. Gore  
Professor of Environmental Science,  
Policy, and Geography  
Thesis Advisor

9 Dec 2005  
Date

  
George E. Stanton  
Dean of Science  
Professor of Biology

12/9/05  
Date

  
Harlan J. Hendricks  
Associate Professor of Biology

**Abstract**

The concept of Measurements Quality Objectives (MQOs), in bioassessment programs is a useful tool in evaluating the consistency of data and limiting variability and potential sources of measurement error. Typical evaluations of data repeatability and/or data quality center on the use of a series of calculations that quantify variability between measures. These calculations provided some indication of not only the quality of the data collected, but also acted as a measure of how representative the biological data were to each ecoregion. The evaluation of the Quality Control data for this project provides a framework for data users and water resource managers to assess the reliability and inherent variability of the proposed biotic indices for the state of Georgia.

In bioassessment programs, it is important to identify natural variability of reference and impaired sites, as well as the variability of the influences anthropogenic stressors. Calculations of variance within the biological parameters measured are necessary for identifying the effects of measurement errors and/or inherent differences between sampling sites in relation to the overall variance of a metric or index on an ecoregional and sub-ecoregional level.

Considering the invertebrate data produced by the Georgia Ecoregions Project, the consistency of all metric categories having average precision measures above the prescribed MQOs for both raw metric values and standardized metric scores may demonstrate that the lotic systems across the state of Georgia naturally have high variability from year-to-year and spatially within catchments. This in turn may indicate that the established precision thresholds of the MQOs may not be indicative of the data quality for this specific project.

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## Introduction

In recognizing the need to improve water quality conditions of the surface waters of the United States, mandates have been set forth by congress through the Federal Water Pollution Control Act, and more specifically, through the requirements of the Clean Water Act (CWA) (33 U.S.C. § *et seq.*). The primary objective of Section 101(a) of the Clean Water Act is to “restore and maintain the chemical, physical, and biological integrity of the nation’s waters,” (CWA, § 101(a), 33 U.S.C., 1251(a), 1999). Recent recommendations from the Environmental Protection Agency (EPA) (USEPA 1987) have determined a need to identify and establish biological standards for surface waters. To meet the primary objective of Section 101(a), the Clean Water Act requires states to develop water quality criteria based on biological assessment (“bioassessment”). These mandated biological criteria (“biocriteria”) are to be used to enforce water quality parameters and to assess possible nonpoint sources (NPS) of pollution (CWA, § 319, 33 U.S.C., §1329, 1999).

Biological monitoring has been mandated by Section 319 of the Clean Water Act as the appropriate tool for assessing the ecological integrity of streams and rivers throughout the United States. “Biological monitoring can be defined as the systematic use of biological responses to evaluate changes in the environment with the intent to use this information in a quality control program. These changes are often due to anthropogenic sources....” (Matthews *et al.* 1982) Anthropogenic influences in an aquatic ecosystem can take on many forms. Most obviously, effluent discharges typical of industry and/or wastewater treatment facilities are known and common sources of “point-source” pollution in aquatic ecosystems. But the core of biomonitoring, through

the use of bioassessment protocols, changes the focus to NPS pollutants and the interaction of widespread chemical or physical degradation and typical land use patterns on a regional basis. The effects of silviculture, agriculture, and urban development on aquatic resources can be difficult to quantify, but through established biological monitoring and assessment protocols, the effects of NPS pollutants can be identified (Barbour *et al.* 1996).

In response to the bioassessment requirements of the Clean Water Act, the EPA has published two major guidelines for the development and use of bioassessment protocols, as well as the interpretation of the resulting biocriteria: *Biological criteria: Technical Guidance for Streams and Small Rivers* (Gibson *et al.* 1996) and *Rapid Bioassessment Protocols for Use in Streams and Wadeable Rivers: Periphyton, Benthic Macroinvertebrates, and Fish* (Barbour *et al.* 1999).

The Rapid Bioassessment Protocol (RBP) is a stepwise methodology for collecting and analyzing biological, chemical, and physical habitat data from stream ecosystems in order to provide a biological framework for water resource managers when assessing water quality issues. Typically, the result of a prescribed bioassessment protocol is the characterization of “biocriteria” that quantify a level of impairment, whether minimal or extreme, in an aquatic system (Fore *et al.* 1996). Bioassessment protocols and the resulting biocriteria are an effective way to assess water quality because of the integration of chemical and physical parameters affecting the biological community (Karr 1990).

The concept of biocriteria has been developed to address the needs of developing

biological sampling as a prime component of surface water management programs. Biocriteria are a series of numeric values derived from the presence and/or absence of taxonomic groups in an ecosystem or region that, in turn, describe the biological status of the chemical and/or physical conditions present. Accurate characterization of the biological condition involves a method that can evaluate patterns of biotic responses from the individual organism to the ecosystem level (Karr *et al.* 1986).

Traditionally, biomonitoring had been utilized to quantify “before-and-after” impacts from a known disturbance. Biomonitoring, as currently implemented, can be used to predict impacts to an aquatic system prior to major anthropogenic impairments within a watershed (Rosenberg and Snow 1977), as well as to serve as a template, ensuring compliance to statutory requirements as set by the EPA through the Clean Water Act. For compliance measures, biological criteria can be applied to evaluate the effects of effluent discharges or other human-induced changes within a catchment and to document that water quality standards have, or have not, been violated (Roper 1985).

To date, the composition of benthic macroinvertebrate communities is the basis of approximately 90% of rapid bioassessment programs for running waters in the United States (Southerland and Stribling 1995). The use of macroinvertebrates for characterizing the biological integrity of a waterbody is advantageous for a number of reasons. The large numbers of invertebrate species present in aquatic systems provide an assortment of biological responses to induced stresses (Hellowell 1986). The sessile nature and limited dispersal ability of aquatic macroinvertebrates make them ideal indicators of not only ambient water quality conditions (Hawkes 1979), but also as a

gauge for spatial and temporal environmental disturbances, both natural and anthropogenic.

The physical and chemical changes that can occur in a catchment, as a result of anthropogenic influences, will have direct and indirect effects not only on the aquatic communities present, but also on the habitat structure and the functional food web of the lotic ecosystem (Townsend and Riley 1999). Although some benthic macroinvertebrate taxa are widespread throughout the stream ecosystem and utilize many different habitats, there are also many groups that are more restricted to one specific habitat (Pardo and Armitage 1997). Having distinctive habitat and biological requirements, macroinvertebrate assemblages can be used in a predictive manner in biomonitoring programs. The absence and/or presence of certain taxonomic groups and species within a stream can be indicative of pollution levels in both impaired and unimpaired catchments (Ravera 2001; Cairns and Pratt 1993).

Through the use of bioassessment protocols, an array of biological metrics are developed to characterize typical aquatic communities, representing both minimally impaired (“reference”) streams and impaired streams. The “multi-metric” approach to characterizing water quality is effective because of its analysis of a number of biological responses in macroinvertebrate communities. However, there are a number of factors to consider when identifying those metrics that are most characteristic of the biological condition in stream, catchment, or region.

The most difficult part of developing a multi-metric approach for assessing water quality parameters for a stream, catchment, and/or ecoregion, is to determine which

biological metrics are diagnostic of the responses of benthic macroinvertebrate assemblages to anthropogenic changes within that catchment. The initial approach to resolving this problem requires a delineation of biological, chemical, and morphological variability across a geographic area. Omernik (1987) has developed an aquatic and terrestrial map of ecoregions for the United States. This map provides the framework for grouping ecosystems, based upon patterns of topography, geology and soil, and land use. This ecoregional grouping is intended to minimize variability within similar regions, as well as maximize variability between dissimilar regions.

The theory behind the use of the ecoregion concept is that adjoining land forms with similar geologic features, soil types, vegetation, and climatic influences will most likely possess similar biological communities (Omernik 1995; Hughes 1995; Omernik and Gallant 1990). This concept is useful in conjunction with bioassessment programs because it can be used to characterize and predict natural variations among systems within similar geographic regions, as well as to detect responses to disturbances based on some reference condition (Hughes and Larsen 1988).

With the variable geology and vegetation patterns across the state of Georgia, it should be expected that a variety of macroinvertebrate assemblages will reflect the ambient water quality and habitat structure of those systems. Similarly, any degradation of habitat and deviation from typical water quality in a region should be reflected by changes in the composition of the macroinvertebrate community. Characterizing a representative macroinvertebrate community in minimally impaired catchments serves as a reference point for other stream ecosystems that have been subjected to some sort

anthropogenic stress.

A reference condition, as prescribed by bioassessment protocols, is defined as “the condition that is representative of a group of minimally disturbed sites organized by physical, chemical and biological characteristics” (Reynoldson *et al.* 1997). The biological condition of a stream, or group of streams, that are classified as “reference” then serve as the point of comparison for all other streams within a catchment and/or ecoregion. The chemical, physical, and biological attributes of a reference stream can then be used to identify levels of impairment in streams that are known to be altered. The differences between the biological condition of a reference and impaired site can be quantified through a series of biological metrics. These metrics are then used to develop a ranking system to identify streams that have acceptable or degraded water quality per EPA standards.

To accurately assess the effects of anthropogenic influences, natural variability within these geographical boundaries must be characterized. In Georgia, there is a very distinctive geological, vegetative, and geomorphological transition from the northwest region to the southeast region (*see* Table 1). This change in ecoregional character dictates a variety of stream morphologies with variable habitat structures and water chemistries. The final determination of a series of metrics must somehow account for natural biological variability within and across ecoregional boundaries (MDEQ 2003).

In 1999, Columbus State University (CSU) was selected to create a rapid bioassessment program for the state of Georgia. Funding for this project was provided through a grant from the Georgia Department of Natural Resources, Environmental

**Table 1.** Descriptions of the primary Georgia ecoregions

Ecoregion Code and Ecoregion Name	Geology	Climate	Principal Land Use/ Dominant Vegetation
<b>45</b> Piedmont	Metamorphic	Mesic-Xeric	Silviculture and Urban Mixed Forest
<b>65</b> Southeastern Plains	Sedimentary (Cretaceous- Miocene)	Mesic-Xeric	Agriculture and Silviculture Pine Forest
<b>66</b> Blue Ridge Mountains	Metamorphic	Mesic-Submesic	Hardwood Forest
<b>67</b> Ridge and Valley	Sedimentary (Paleozoic)	Mesic-Submesic	Agriculture Hardwood Forest
<b>68</b> Cumberland Plateau	Sedimentary (Paleozoic)	Mesic-Submesic	Agriculture Hardwood Forest
<b>75</b> Southern Coastal Plain	Sedimentary  (Pliocene- Pleistocene)	Mari-time	Agriculture and Silviculture  Pine Forest

Ecoregion delineation per Omernik (1987) and ecoregion descriptions per Wharton (1989).

Protection Division (GAEPD), via sponsorship of the United States Environmental Protection Agency (more specifically, United States Environmental Protection Agency Clean Water Act, Section 319(h) FY 98 - Element 1) funding. The resulting "Georgia Ecoregions Project" consisted of four phases of biological, chemical, and physical data collection to characterize water quality conditions across the state. The final

analysis of this data will be used to establish biocriteria relevant to each geographic region of Georgia.

Ultimately, the resulting biocriteria and numerical ranking system derived from this project can be used to evaluate the possible sources and effects of NPS pollution and the effectiveness of Best Management Practices (BMPs) to control NPS inputs, as well as to assess the level of impacts from Total Maximum Daily Loads (TMDLs) in aquatic systems. These established biocriteria can also be used to identify regions or specific catchments in need of restoration, as well as to characterize the sources of impairment and to monitor trends over time (Barbour *et al.* 1999).

Through the use of Geographical Information Systems (GIS) software, (*i.e.* ArcView), land use data, and best professional judgment, efforts were made to identify and locate as many potential “reference” and “impaired” sampling sites as possible (Olson 2002, Gore *et al.* 2004). This process was necessary in order to adequately illustrate the inherent biological, chemical, and physical variability of streams throughout the state within their catchments and ecoregional boundaries. The goal was to collect physical and chemical data from a minimum of ten streams specific to each sub-ecoregion identified for Georgia, five sites being classified as reference, (or minimally impaired), and five sites being classified as impaired, based on land use parameters within the catchment. A statistical summary of land use within the ecoregions of Georgia is provided in Appendix A.

In conjunction with these proposed reference and impaired sites for sampling, additional samples were collected as dictated by the Quality Assurance Project Plan

(QAPP) (CSU 2000). Throughout each phase of the Georgia Ecoregion Study, there were a number of duplicate samples taken to satisfy the Quality Assurance/Quality Control (QA/QC) requirements of the QAPP. These duplicate samples were taken in order to assess the repeatability and precision of the collected data, as well as to assess the training and level of effort between and among field teams. In this paper, QC data are assessed in terms of Measurement Quality Objectives (MQOs) as outlined by the QAPP, but address, more specifically, the amount and degree of variability present in the wadeable stream ecosystems across the state of Georgia, and how these samples affect initial characterization of the reference condition.

There were two designations for QC samples collected to satisfy the QAPP document: (1) a spatial, "duplicate reach" QC sample, and (2) a temporal, "phase" QC sample. According to QAPP procedures, the QC type of duplicate sampling is performed in order to assess the precision and accuracy of the field teams and the representativeness of the data as some measure of "data quality" in bioassessment programs. It is important to analyze the consistency of field teams to ensure that personnel are properly trained so that the collection of biological data are free from bias and error, but more importantly, for this paper, the objective of analyzing the additional biological data was to determine if the sites chosen to characterize the biological condition were true representations of the biological community in that stream.

During development of the reference condition for Georgia, the additional data collected through the QC samples were not used in the creation of overall metric scores, or in characterization of the final biological index. Thus, it became important to

determine the point that the data thoroughly and accurately reflected the composition of the macroinvertebrate community. There have been numerous studies of the effect of sample size on the variability of biotic indices in bioassessment programs (Li *et al.* 2001; Metzeling and Miller 2001). Increase in sample size will result in the increase of number of individuals collected, but, more importantly, also corresponds to an increase in the number of taxa in the system being sampled. It has also been demonstrated that increasing the size of the sampling area, (whether it be sampling more than one riffle or a combination of habitats to constitute one sample), has an effect on the range of variance (Hannaford and Resh 1995, Norris *et al.* 1993). When considering bioassessment protocols, it raises the question of determining what important taxa may have been missed and how these excluded taxa may influence the range of variability of the metrics used to determine the reference and impaired condition.

There have been a number of papers analyzing variability in data using RBP protocols (see reviews by Hannaford and Resh 1995), but the majority of these have centered on specific habitat types such as riffles and runs (see, for example, Feminella 2000), based upon the assumption that swifter water habitats yield the highest species richness and abundance of invertebrates (Hynes 1970; Allan 1995). Also common in these previous studies has been the use of "in-field" subsampling of macroinvertebrates as the basis for characterizing variability in the data sets (Metzeling and Miller 2001). Logically there is some question about bias resulting from "in-field" subsampling of macroinvertebrates, as there may be a tendency to choose the larger, more obvious organisms for analysis, resulting in skewed final metrics and biotic indices calculated for

a stream.

Additionally, given that many macroinvertebrates have very specific habitat requirements, it is to be expected that metric results would vary as a function of the range of particular habitats being sampled. There are numerous species that thrive in habitats such as tree roots along stream banks and woody debris (*i.e.* snags), an especially productive habitat type in low-gradient stream systems typical of southern Georgia (Benke *et al.* 1985). The sampling of multiple habitats in bioassessment protocols provides a better biological “picture” of the faunal communities that are subject to changes in habitat structure and water quality.

The research described here addressed a number of questions. With regards to the Georgia Ecoregions QC data, does the inclusion of additional taxonomic data change the range of variability, and what criteria define the reference or impaired condition? Second, will the restriction or expansion of those ranges of variability create difficulties in interpretation of anthropogenic stressors on the biotic community? Likewise, does the range of variability within the identified metrics and biotic indices hinder the decision making process for water resource managers? The answers to these questions might indicate that increasing the sample size, (*e.g.* increasing the number of reference and impaired sites samples, and/or increasing the reach length), in RPB programs may better characterize natural variability within and between ecoregions, and also reduce the variability of the final metrics used to characterize the reference condition and water quality, as well as more narrowly defining numerical criteria of stream health (Gore *et al.* 2005).

## Materials and Methods

The Georgia Ecoregions Project consisted of four phases of ecoregional delineation, sample collection, and data analysis. Phase 1 consisted of identifying and delineating ecoregional boundaries, as established by Omernik (1987). The sub-ecoregions used in this study were from the Level III and IV Sub-Ecoregions of Georgia (Griffith *et al.* 2001) as illustrated in Figure 1. Phase 2 consisted of evaluating candidate reference streams based upon abiotic factors (*i.e.* surrounding land use patterns, physical habitat quality, and water chemistry). In that phase, criteria were established to characterize a “reference” stream in terms of water quality and biological (primarily, macroinvertebrate) assemblages (Olson 2002). The reference condition was essential to provide a “benchmark” by which impairment status would be characterized.

In Phases 3 and 4 of this project, the process of identifying and sampling reference and impaired sites was continued in order to collect as much biological data as possible and accurately reflect water quality and macroinvertebrate assemblages characteristic of the defined ecoregions. To minimize the effect of temporal variability between sampling years/phases, a sampling season (or “index period”) between August and February was determined to be the most indicative of the aquatic communities for the Georgia Ecoregions Project.

At each sampling site, a series of physical and chemical sampling protocols were performed to collect data relevant to the habitat quality, water quality, and macroinvertebrate communities that are representative of the biological condition of the site, as well as being indicative of ecoregional character, (*i.e.* geology, vegetation,

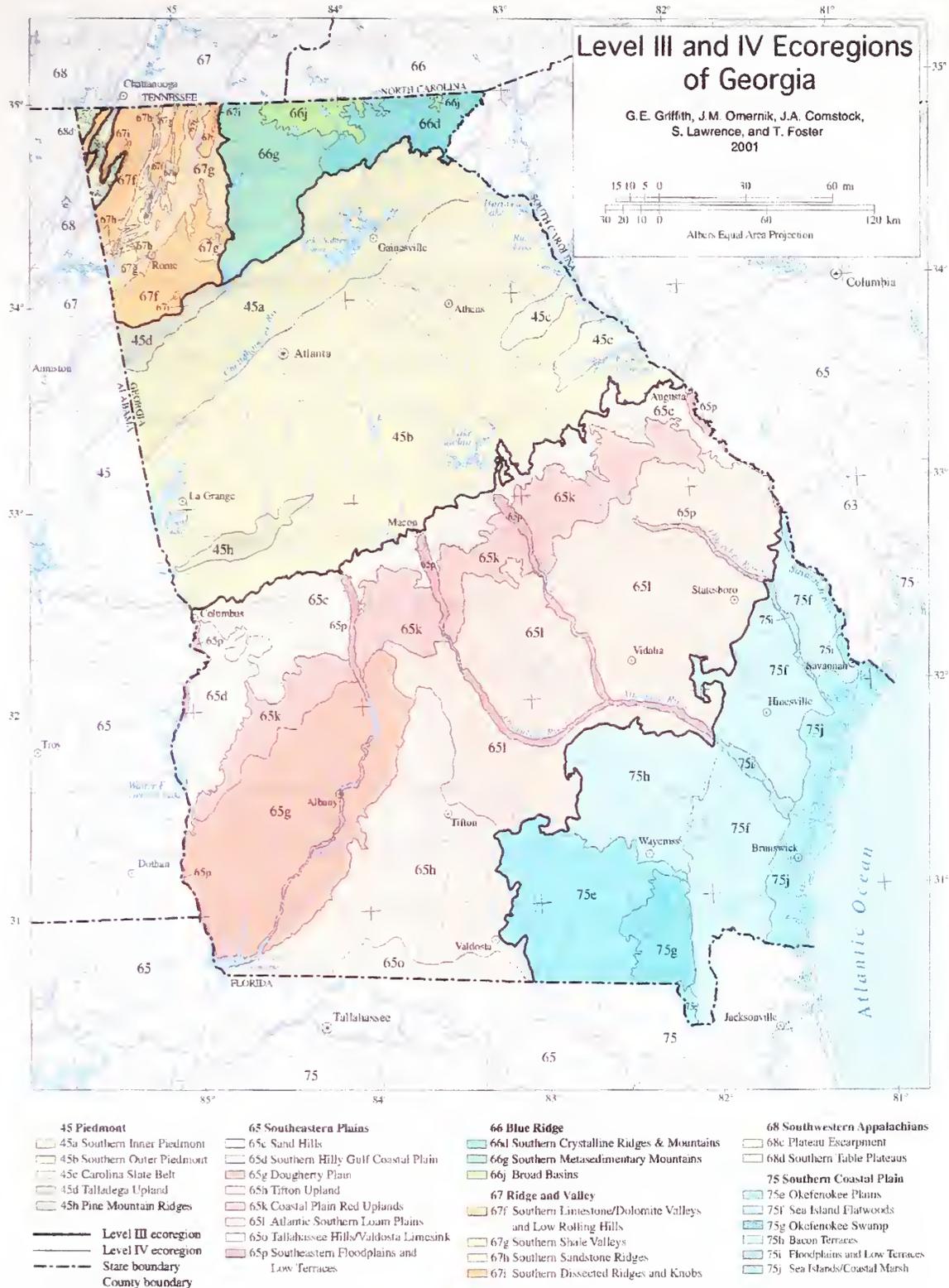


Figure 1. Level III and IV Sub-Ecoregions of Georgia.

climate etc.) , and land use patterns, (*i.e.* silviculture, urbanization, etc.). Chemical and biological sampling of the selected sites was performed using the following procedures. These sampling protocols are described further, in more detail in Columbus State University's (CSU) QAPP as Standard Operating Procedures (SOPs) and generally follow the recommendations of the RBP (Barbour *et al.* 1999):

1. Benthic macroinvertebrate communities were collected using the Georgia DNR's 20-Jab Method (CSU 2000). Table 2 summarizes the level of effort prescribed for various habitat types that are characteristic of high- and low-gradient stream systems. Macroinvertebrates collected from these habitat types were composited into a single sample and returned to the lab for further processing. Macroinvertebrates were identified to the lowest practicable taxonomic level and enumerated.
2. Water chemistry was measured both *in situ*, using a Hydrolab H-20 probe, and by grab samples that were analyzed at a later time in the CSU laboratory. The water chemistry parameters that were analyzed for this project are listed in Appendix B.
3. The physical properties of the streams were also recorded. Those properties included a streambed cross section, velocity, substrate size and composition using a modified Wolman Pebble Count (Bevenger and King 1995). Additional observations included the extent of canopy cover, presence of oils and/or odors, adjacent land use along the stream channel, bank erosion, and types of deposits, (*see* Appendix C).

4. Visually-based habitat assessments were also completed for each site using the EPA's Rapid Bioassessment Protocol habitat assessment methods and forms, (*see* Appendix D). Similar to the partitioning of habitat types between stream gradients for the sampling of macroinvertebrate communities, the habitat assessment forms used to characterize physical and geomorphological attributes of a stream ecosystem were also categorized by gradient classification.

**Table 2\*.** Prioritized list of habitat types for sampling and sample reallocation for the modified 20-jab method.

<b>HIGH GRADIENT STREAMS</b>		
<b>Priority</b>	<b>Habitat Type</b>	<b>Number of Samples</b>
1	Fast Riffle	3
2	Slow Riffle	3
3	Snags	5
4	Undercut Banks/Rootwads	3
5	Leaf Packs	3
6	Sand	3
7	Macrophytes (if any)	3
<b>LOW GRADIENT STREAMS</b>		
<b>Priority</b>	<b>Habitat Type</b>	<b>Number of Samples</b>
1	Woody debris/Snags	8
2	Undercut Banks/Rootwads	6
3	Leaf Packs	3
4	Sand	3
5	Macrophytes (if any)	3

\*From Columbus State University's Quality Assurance Project Plan document (CSU2000).

Each macroinvertebrate sample collected in the field was preserved in 70% ethanol until further processing. The macroinvertebrate samples were evenly spread out

on a Caton gridded screen for subsampling (Caton 1991). Using random number generation, squares within the grid system were designated for removal and subsequent examination. Each grid square was placed in a "white-pan" and examined for the presence of macroinvertebrates. All organisms from the square were removed and again preserved in 70% ethanol. For each macroinvertebrate sample, the goal was to collect a total of 200 organisms. Squares for the grid were continuously selected until the required number of organisms was collected. Macroinvertebrates were then identified to the lowest possible taxonomic level and entered into a database developed by Tetra Tech (1999) known as the Ecological Data Application System (EDAS) for metric analysis.

The QAPP describes the procedures that were used in data collection and their rationale, as well as a series of activities and reporting procedures that were used to document data quality. As prescribed by the QAPP document for the Georgia Ecoregions Project, a number of sites were designated for additional sampling. To address QC/QA protocols related to data quality, ten percent of all the designated sampling sites were required to have duplicate sampling performed. These duplicate samples fell into two designations: "spatial" (200 meter QC) and "temporal" (Phase QC).

Quality control samples that were designated as "spatial" essentially "doubled" the length of the reach designated for sampling. Once the primary sample reach of one hundred meters was established, and all RBP sampling requirements satisfied, the immediate, next one hundred meter reach was sampled. "Temporal" QC's were sites that were sampled in succeeding phases of the ecoregions project, where the originally established sample site reach was resampled in a subsequent "index period". This

sampling approach addressed two possible variations within a stream ecosystem: (1) the variability of the distribution of habitats longitudinally within a catchment, and (2) changes of the macroinvertebrate communities over time.

All QC sites were randomly chosen via a random number generation function in Microsoft's Excel program. As a result of this random number generation, there was some unevenness in the number of duplicate reference- and impaired-site samples, as well as the number of spatial- and temporal-QC samples collected. Additionally, the total number of QC sites collected for this project was not evenly distributed throughout each ecoregion and subcoregion. A list of all spatial and temporal QCs samples collected per ecoregion is provided in Appendix E, combined with GIS maps to illustrate their geographic locations throughout the state of Georgia.

As sites were sampled and taxonomic identifications were completed, all of the physical, chemical, and biological data gathered were entered into the EDAS database for further analysis. The calculated macroinvertebrate metrics encompass a number of benthic macroinvertebrate community structures and functions that characterize the ecological status of the aquatic system being analyzed. An assortment of approximately 65 metrics, from five major structural and functional groups (*i.e.*, taxonomic richness, community composition, tolerant/intolerant organisms, functional feeding groups, and life habit) were calculated for each stream sampled. A list of all metrics considered in developing biocriteria for the Georgia Ecoregions Project has been compiled in Table 3. A brief description of each metric groups and its significance to characterizing ambient water quality conditions follows:

- **Taxonomic Richness** – metrics included in this group evaluated the number of individual taxa within larger taxonomic groups (*i.e.* number of families or genera within an order of aquatic invertebrates such as Ephemeroptera, Diptera, *etc.*). Typically, high values of taxonomic richness are indicative of better water quality and a healthier lotic ecosystem.
- **Community Composition** – these metric values are expressed as percentages, representing a proportion of individuals in a sample belonging to some specific taxonomic group. Higher percentages of those organisms that have been known to tolerate degraded conditions (*i.e.* Diptera) are assumed to be characteristic of impaired water quality.
- **Tolerant/Intolerant Taxa** – this group of metrics are represented by the tolerance levels of biota to stress. In systems with high anthropogenic stress, taxa classified as intolerant to pollution are assumed to be the first organisms to be eliminated from the ecosystem, becoming less abundant. Concurrently, those taxa with higher tolerance to pollution impacts are assumed to dominate the system.
- **Functional Feeding Group** – these metrics reflected the dominant feeding mode of the biological community in the sample. The ecological responses of organisms with specialized or generalized feeding habits are assumed to be indicative of pollution or anthropogenic disturbances. For example, the abundance of “shredders” and “filterers” can be affected when organic materials become scarce or unsuitable.

- **Life Habit** – the metrics include measures of taxa richness and composition that described the locomotive and positioning mechanisms of benthic macroinvertebrates (*i.e.* burrowing, swimming, *etc.*). This group of metrics are probably the most difficult to characterize responses to anthropogenic stressors, as there has been no definitive indication of how these communities identified by life habit respond to increased or decreased perturbations in the ecosystem. (Kerans and Karr 1994).

**Table 3. Predicted Responses of Benthic Macroinvertebrate Metrics to Stress.**

<b>METRIC CATEGORY</b>	<b>METRIC</b>	<b>STRESS RESPONSE</b>
<b>Taxonomic Richness</b>	Total Taxa	Decrease
	Ephemeroptera, Plecoptera, & Trichoptera (EPT) Taxa	Decrease
	Ephemeroptera Taxa	Decrease
	Plecoptera Taxa	Decrease
	Trichoptera Taxa	Decrease
	Coleoptera Taxa	Decrease
	Diptera Taxa	Decrease
	Chironomidae Taxa	Decrease
	Tanytarsini Taxa	Decrease
	Evenness	Decrease
	Margalef's Index	Decrease
	Shannon-Wiener base e	Decrease
	Simpson's 'Diversity	Increase
<b>METRIC CATEGORY</b>	<b>METRIC</b>	<b>STRESS RESPONSE</b>
<b>Community Composition</b>	% Ephemeroptera	Decrease
	% Amphipoda	Decrease
	% Chironomidae	Increase
	% Coleoptera	Decrease
	% Diptera	Increase
	% Gastropoda	Decrease

**Table 3. Predicted Responses of Benthic Macroinvertebrate Metrics to Stress, (cont.)**

<b>METRIC CATEGORY</b>	<b>METRIC</b>	<b>STRESS RESPONSE</b>
<b>Community Composition (cont.)</b>	% Isopoda	Increase
	% NonInsect	Increase
	% Odonata	Increase
	% Plecoptera	Decrease
	% Tanytarsini	Decrease
	% Oligochaeta	Increase
	% Trichoptera	Decrease
	% Chironominae / Total Chironomidae (TC)	Variable
	% Orthoclaadiinae / TC	Decrease
	% Tanypodinae / TC	Increase
	% Hydropsychidae / Total Trichoptera	Increase
	% Hydropsychidae / Total EPT	Increase
	% Tanytarsini / TC	Decrease
	% <i>Cricotopus sp.</i> & <i>Chironomus sp.</i> / TC	Increase
<b>METRIC CATEGORY</b>	<b>METRIC</b>	<b>STRESS RESPONSE</b>
<b>Tolerance/Intolerance</b>	Tolerant Taxa	Increase
	% Tolerant Individuals	Increase
	Intolerant Taxa	Decrease
	% Intolerant Individuals	Decrease
	% Dominant Individuals	Increase
	Dominant Individuals	Increase
	Beck's Index	Decrease
	Hilsenhoff's Biotic Index (HBI)	Increase
North Carolina Biotic Index (NCBI)	Increase	
<b>METRIC CATEGORY</b>	<b>METRIC</b>	<b>STRESS RESPONSE</b>
<b>Functional Feeding Group</b>	% Scraper	Decrease
	Scraper Taxa	Decrease
	% Collector	Decrease
	Collector Taxa	Decrease
	% Predator	Decrease
	Predator Taxa	Decrease

**Table 3. Predicted Responses of Benthic Macroinvertebrate Metrics to Stress, (cont.)**

<b>METRIC CATEGORY</b>	<b>METRIC</b>	<b>STRESS RESPONSE</b>
<b>Functional Feeding Group (cont.)</b>	% Shredder	Decrease
	Shredder Taxa	Decrease
	% Filterer	Increase
	Filterer Taxa	Decrease
<b>METRIC CATEGORY</b>	<b>METRIC</b>	<b>STRESS RESPONSE</b>
<b>Life Habit</b>	Clinger Taxa	Decrease
	% Clinger	Decrease
	Burrower Taxa	Decrease
	Climber Taxa	Decrease
	Sprawler Taxa	Decrease
	Swimmer Taxa	Decrease

The use of various metrics from these groups resulted in a “multi-metric” approach to assess the health of a stream ecosystem. This variety of biological data includes many ecologically significant factors in aquatic systems that are then compiled into a single biotic index relevant to each ecoregion. Final biotic indices were comprised of five to seven metrics, with the metrics being chosen with at least one representative from each of the five metric categories mentioned above.

Metric values calculated by EDAS were separated by ecoregion and subecoregion, as well as by impairment status, within the ecoregional designation. These raw metric scores were initially used to distinguish which metrics were to be considered as candidates for the final biotic index. A series of analyses was performed to assess the ability of each metric to discriminate between the characteristics of a “reference” stream and an “impaired” stream. For those sites designated as reference streams, inter-quartile

ranges of the distribution of metric values for each metric category were calculated. These percentile values then served as templates for the calculations of “discrimination efficiency”, metric score standardization, and the final biotic index for each ecoregion and subecoregion.

Raw metrics scores for both reference and impaired streams sites were used to calculate the discrimination efficiency (DE) of each metric. DE was calculated by the following formula (Stribling *et al.* 2000):

$$\text{DE} = (a/b) \times 100$$

For those metrics values that typically decrease with increased stress, **a** = the number of impaired streams that scored below the 25<sup>th</sup> percentile of the distribution of reference metric values; whereas for those metrics values that increase in response to increasing stress, **a** = the number of impaired streams that scored above the 75<sup>th</sup> percentile of the distribution of the reference metric values, and **b** = the total number of impaired sites sampled specific to the ecoregion and/or subecoregion analyzed. No metric with a DE value below 0.5 was considered as a candidate for inclusion in the final biotic index.

Those metrics that exhibited high DEs were then evaluated with box-and-whisker plots (Tukey 1970) to compare the distribution of metric values for reference and impaired stream classes. Box-and-whisker plots graphically demonstrate the range of variation not only within the stream class (*i.e.* reference or impaired), but also between the stream classes (*i.e.* reference vs. impaired) (Gibson *et al.* 1996). The best performing metrics, and those that were to be considered as candidates for the final index, demonstrated minimal variation within stream classes and maximized variation between

stream classes (Barbour *et al.* 1999). The metrics deemed to be candidates were then analyzed by Pearson-Product-Moment-Correlation to determine which metrics may be redundant with one another (Steel and Torrie 1980). Redundant metrics were not used when determining the final biotic index for each ecoregion.

To incorporate raw metric values into an index, the data were first standardized, thus creating a metric “score”. Standardization of the raw metric values normalized each metric to a similar scale so that they could be compared to one another. The method of standardization of the raw metric values was dependent upon the stress response of the metric. For metric values that were known to decrease as a response increasing stress, the standardized score was calculated as:

$$\text{Standardized Metric Score} = (c/d) \times 100$$

Where **c** = the raw metric value for a site, and **d** = the 95<sup>th</sup> percentile of the distribution of the reference metric values. For metric values that were known to increase as a response to increasing stress, the standardized score was calculated as:

$$\text{Standardized Metric Score} = [(e-c)/(e-f)] \times 100$$

Where **c** = the raw metric value for a site; **e** = the maximum observed value among all streams (*i.e.*, reference and impaired); and **f** = the 5<sup>th</sup> percentile of the distribution of the reference metric values.

Standardized metric scores of the initial candidate metrics were then combined from each metric category to create a candidate biotic index. A series of indices was first calculated to see which combination of metrics determined the best index to describe the biotic factors that represented the reference and impaired condition for specific

ecoregions. Index Scores were assembled from the standardized scores in an additive manner as:

$$\text{Index Score} = (g+h+i+j+k\dots)/n$$

Where **g, h, i, j, k...** = the standardized metric score of the candidate metrics, and **n** = the total number of standardized metrics included in the index. All candidate indices contained five to seven metrics that are scored on a 0 to 100 point scale. The initial candidate indices were then analyzed for DEs and compared by box-and-whisker plots. The final candidate index was distinguished by the highest DE and the best box-and-whisker separation. A final biotic index was developed for each ecoregion and subecoregion of Georgia (Gore *et al.* 2004, Middleton, 2005). Although the final biotic index was guided by statistical considerations (*i.e.*, DEs, inter-quartile ranges, *etc.*), the choices of the final metrics were also based upon their ability to accurately represent ecoregional characteristics and the ability to typify a response to anthropogenic stress (Barbour *et al.* 1999).

There are many important factors to consider when developing biocriteria for stream ecosystems. In the process of collecting biological data, field methods cannot predict if the information being collected is an accurate portrayal of the ecosystem under investigation (ITFM 1995). The properties of a given field sample may be known, but typically biological data are collected with the intent of answering questions relating to much larger spatial and temporal scales (Barbour *et al.* 1999). The consistency of field methods and level of effort in collecting biological data are the key to obtaining information that is representative of field conditions at that point in time, but truly

accurate assessments of the biological data are hindered because the natural variability of the ecosystem cannot be controlled (Resh and Jackson 1993).

In the same vein as the step-wise process for identifying an ecoregional biotic index from raw metric values, the QC samples collected for this project were considered for their precision and representativeness of the biological condition. With RBPs and the use of multimetric assessment methods, the precision of the total bioassessment score is as important as the precision of the individual metrics that comprise the score (Diamond *et al.* 1996). Typically, when considering wide scale bioassessment programs, some form of criterion is established to assess the quality of the data that has been collected. These criteria are commonly referred to as Data Quality Objectives (DQOs), and/or Measurement Quality Objectives (MQOs). These are qualitative and quantitative parameters developed by data analysts and resource managers to evaluate data accuracy and quality.

For the Georgia Ecoregions Project, there was a number of QA/QC objectives defined in the QAPP. In my research, only a few of these parameters were examined. Specifically, analysis centered upon three measurement parameters associated with the collection of benthic macroinvertebrates: metric values, standardized metric scores, and bioassessment scores. Not only were these parameters analyzed with regards to the prescribed MQOs, but the QC data were also evaluated for their relevance and value to characterizing the reference and impaired conditions, as defined by the biotic index.

Typical evaluations of data repeatability and/or data quality center on the use of a series of calculations that quantify variability between measures. The following

relationships are typical of bioassessment protocols that define measures of acceptable variability (Stribling and Bressler 2004, Barbour *et al.* 1999, USEPA 1995):

- **Relative Percent Difference (RPD)**: used to quantify the proportional difference between two measures as:

$$\text{RPD} = [(C_1 - C_2)/(C_1 + C_2)] \times 100$$

Where  $C_1$  = the larger of the two values being compared, and  $C_2$  = the smaller of the two values being compared (Berger *et al.* 1996).

- **Root Mean Square of Error (RMSE)**: used as an estimate of the standard deviation of a group of observations. The RMSE is determined by performing an analysis of variance between duplicate samples to determine the mean square error (MSE) that is representative of within group variance.
- **Coefficient of Variability (CV)**: calculated by expressing the standard deviation as a percentage of the mean. The coefficient of variability for a population was calculated as:

$$\text{CV} = (\text{RMSE}/Y) \times 100$$

Where  $Y$  was the mean of the dependent variable (*e.g.* metric values, scores, etc.).

Values associated with RPDs and RMSEs characterized some level of precision among the parameters being analyzed. As defined by the QAPP for the Georgia Ecoregions Project, RPDs for metric values, metric scores, and bioassessment scores were defined to indicate some level of data quality (see Table 4). Additionally, RMSEs for these same measurement parameters were developed here. For each raw metric

value and metric score, both RPDs and RMSEs were calculated, while only the candidate metrics used for the development of the biotic index were examined. These calculations provided some indication of not only the quality of the data collected, but also acted as a measure of how representative the biological data were to each ecoregion. This evaluation of the QC data for this project provides a framework for data users and water resource managers to assess the reliability and inherent variability of the proposed biotic indices for the state of Georgia.

**Table 4.** Precision Measurement Quality Objectives for benthic macroinvertebrates as defined in the Georgia Ecoregions Project Quality Assurance Project Plan (QAPP) (see, also, Barbour *et al.* 1999, USEPA 1995).

<u>Measurement Parameter</u>	<u>Precision Level</u>
Metric Values	RPD $\leq$ 20% RMSE = TBD*
Metric Scores	RPD $\leq$ 5% RMSE = TBD*
Bioassessment Scores	RPD $\leq$ 5% RMSE = TBD*

\*TBD, ("to be determined"); RMSE levels developed as a result of this study.

## Results

Precision calculations of the measurement parameters for benthic macroinvertebrate metrics are presented at the primary ecoregional level (refer to Table 1 and Figure 1 for ecoregion and subcoregion classifications and descriptions). For each primary ecoregion, calculations of RPDs and RMSEs, (as required by the QAPP document), and CVs are provided as averages for all QC samples collected, (both reference/impaired and spatial/temporal), inclusive of their ecoregional designation. Average RPDs, RMSEs, and CVs are provided for each raw metric value, standardized metric score, and the final bioassessment scores (biotic indices) developed for each ecoregion. Additionally, average RPD, RMSE, and CV calculations are presented relative to the specific metrics used in the developed biotic indices for each primary ecoregion and subcoregion (Gore *et al.* 2005). At the subcoregional level, average RPD, RMSE, and CV values for raw metric values, standardized metric scores, and bioassessment scores are provided in the associated appendices.

Also included in the appendices for each ecoregion are RPD, RMSE, and CV calculations, (at the ecoregion and subcoregion level), for each stream class (*i.e.* reference vs. impaired), and collectively, for all original and QC sites sampled within the ecoregional designation. Therefore, the averages specific to reference or impaired streams in each ecoregional designation only include data relevant to the designated stream class, whereas the averages provided for the entire ecoregion are inclusive of all original and associated QC samples in the ecoregion designation, both reference and impaired, spatial and temporal.

Also, in conjunction with the required precision measures dictated by the QAPP, RPD values were calculated to compare stream classes, (*i.e.* reference vs. impaired), as well as QC sample type, (*i.e.* spatial vs. temporal). Again, these parameters are summarized for all raw metric values at the primary ecoregional level. Raw data associated with these parameters, as well as site specific metric values for all original and QC sites, are provided on the CD-Rom included in the pocket materials of this research paper.

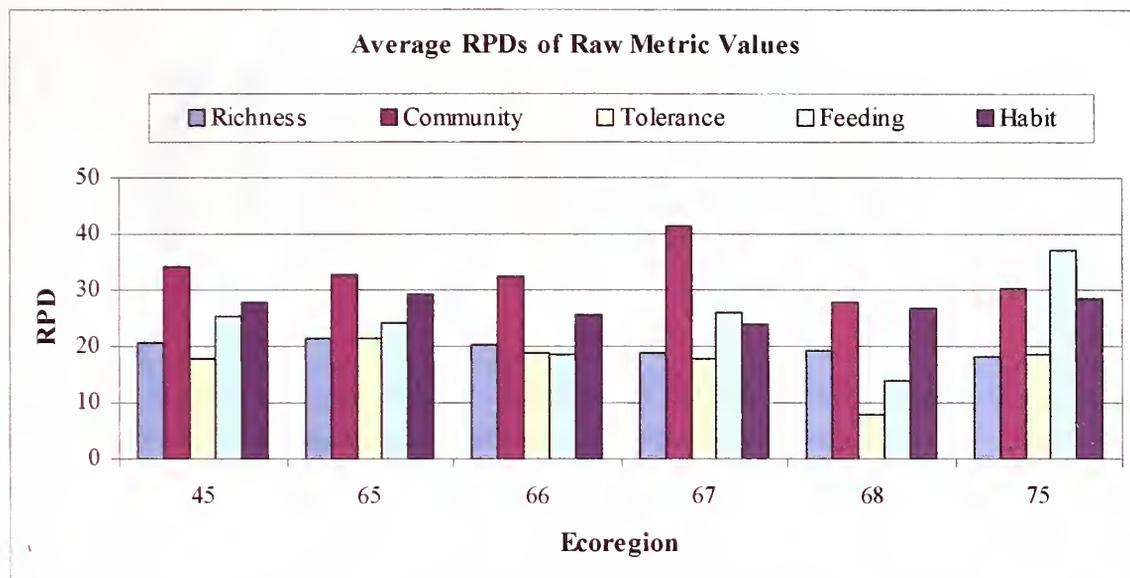
### **RPD Precision Measures for Raw Metric Values and Standardized Metric Scores**

As with determining the validity of certain metrics to determine the biological condition (*e.g.* examining DEs and box-and-whisker plots), the raw metric values were analyzed for RPDs, acting both as a measure of data precision and data uncertainty due to natural variability of the lotic ecosystem. Table 5 contains a summary of RPDs averaged for all metrics within each category. The average RPDs for individual metrics in each metric category, in most cases, were higher than the measurement quality objectives dictated by the Ecoregions QAPP. The RPDs of raw metric values from duplicate

**Table 5.** Average Relative Percent Difference (RPD) for all raw metric values within each metric category and per primary ecoregion designation.

<b>Metric Groups</b>	<b>45</b>	<b>65</b>	<b>66</b>	<b>67</b>	<b>68</b>	<b>75</b>
Taxonomic Richness	20.9	21.4	20.3	19.1	19.2	18.1
Community Composition	34.2	32.7	32.5	41.5	27.9	30.5
Tolerance/Intolerance	18.0	21.3	19.0	18.0	8.0	18.5
Functional Feeding Group	25.5	24.4	18.5	26.1	13.9	37.2
Life Habit	27.7	29.2	25.7	23.8	26.9	28.4

reaches was expected to be in 80 percent agreement (Table 4). This is better illustrated in Figure 2, which demonstrates that RPD values are relatively consistent between the metric categories and ecoregional designation.

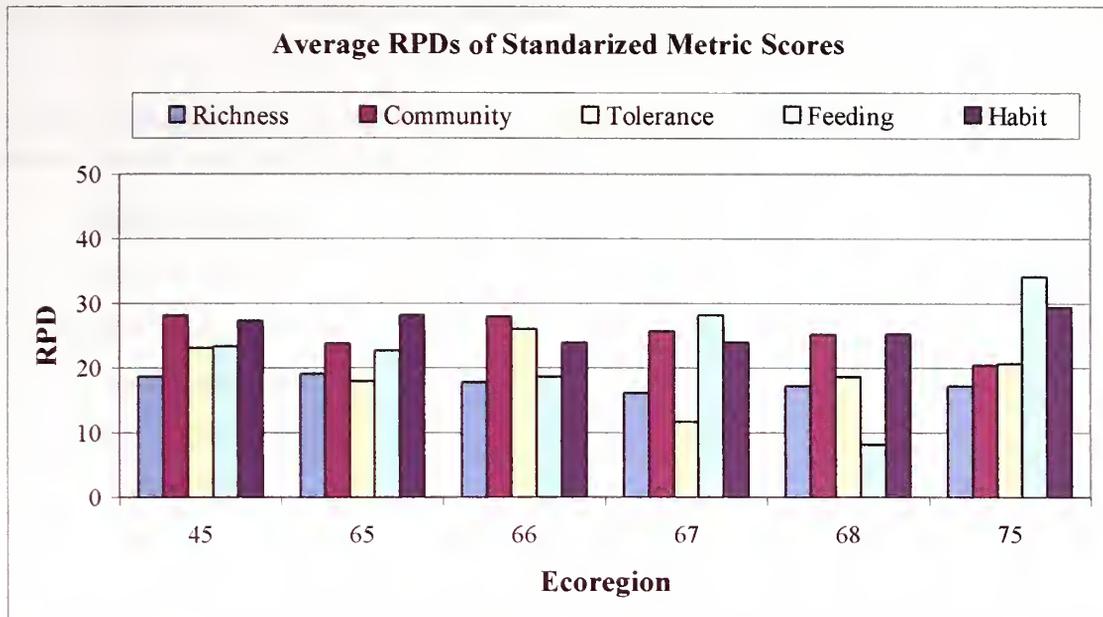


**Figure 2.** Average Relative Percent Difference (RPD) for all raw metrics values within each metric category and per ecoregion designation.

RPDs were also calculated for the standardized metric scores and have been summarized for the primary ecoregions of Georgia in Table 6 and illustrated in Figure 3. Again, these values are higher than the prescribed MQO of 95 percent agreement for standardized metric scores.

**Table 6.** Average Relative Percent Difference (RPD) for all standardized metric scores within each metric category and per primary ecoregion designation.

Metric Groups	45	65	66	67	68	75
Taxonomic Richness	18.7	19.1	17.8	16.2	17.3	17.3
Community Composition	28.2	23.8	28.0	25.7	25.2	20.5
Tolerance/Intolerance	23.1	18.0	26.1	11.6	18.7	20.7
Functional Feeding Group	23.4	22.7	18.6	28.1	8.2	34.2
Life Habit	27.4	28.2	24.0	24.0	25.3	29.4



**Figure 3.** Average Relative Percent Difference (RPD) for all standardized metric scores within each metric category and per primary ecoregion designation.

### RMSE Precision Measures of Raw Metric Values and Standardized Metric Scores

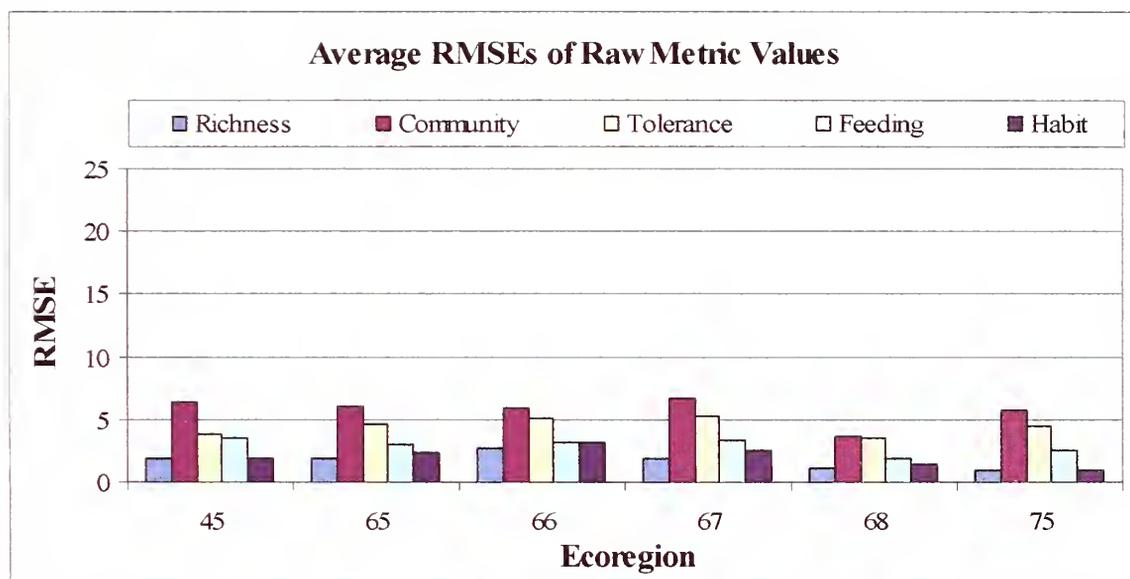
Another precision measurement utilized was the “root mean square error” (RMSE), which is a representation of within group variance, and acted as an estimate of the standard deviation of each population of metric values. Acceptable levels of error associated with RMSEs have not been established or quantified for this project. The values presented in this paper established the ranges of variability on an ecoregional and subcoregional basis. Similar to the precision measurements for the RPDs of raw metric values and standardized metric scores, values for RMSEs presented here are averages of all metrics within each metric category for each primary ecoregion of Georgia. Subcoregional averages of RMSE values are presented in the associated appendices.

The average RMSE was calculated for all raw metrics values as summarized on Table 7 and illustrated in Figure 4. Similarly, average RMSEs for standardized metric

score are presented in Table 8 and illustrated in Figure 5.

**Table 7.** Average Root Mean Square Error (RMSE) for all raw metric values within each metric category and per primary ecoregion designation.

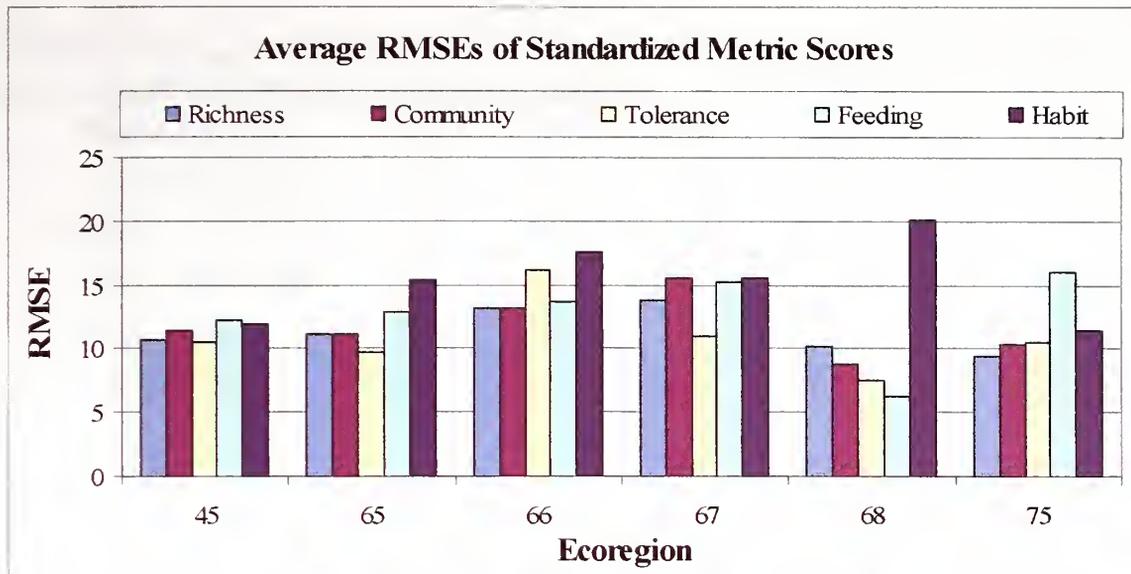
Metric Groups	45	65	66	67	68	75
Taxonomic Richness	1.9	1.9	2.7	1.9	1.1	0.9
Community Composition	6.4	5.9	5.9	6.7	3.7	5.8
Tolerance/Intolerance	3.8	5.1	5.1	5.3	3.5	4.4
Functional Feeding Group	3.5	3.2	3.2	3.3	2.0	2.6
Life Habit	2.0	2.4	3.2	2.5	1.4	0.9



**Figure 4.** Average Root Mean Square Error (RMSE) for all raw metric values within each metric category and per primary ecoregion designation.

**Table 8.** Average Root Mean Square of Error (RMSE) for all standardized metric scores within each metric category per primary ecoregion designation.

Metric Groups	45	65	66	67	68	75
Taxonomic Richness	10.7	11.2	13.2	13.8	10.2	9.4
Community Composition	11.4	11.2	13.2	15.6	8.8	10.4
Tolerance/Intolerance	10.6	9.7	16.2	11.0	7.5	10.6
Functional Feeding Group	12.3	12.9	13.7	15.3	6.2	16.0
Life Habit	12.0	15.4	17.6	15.6	20.1	11.5



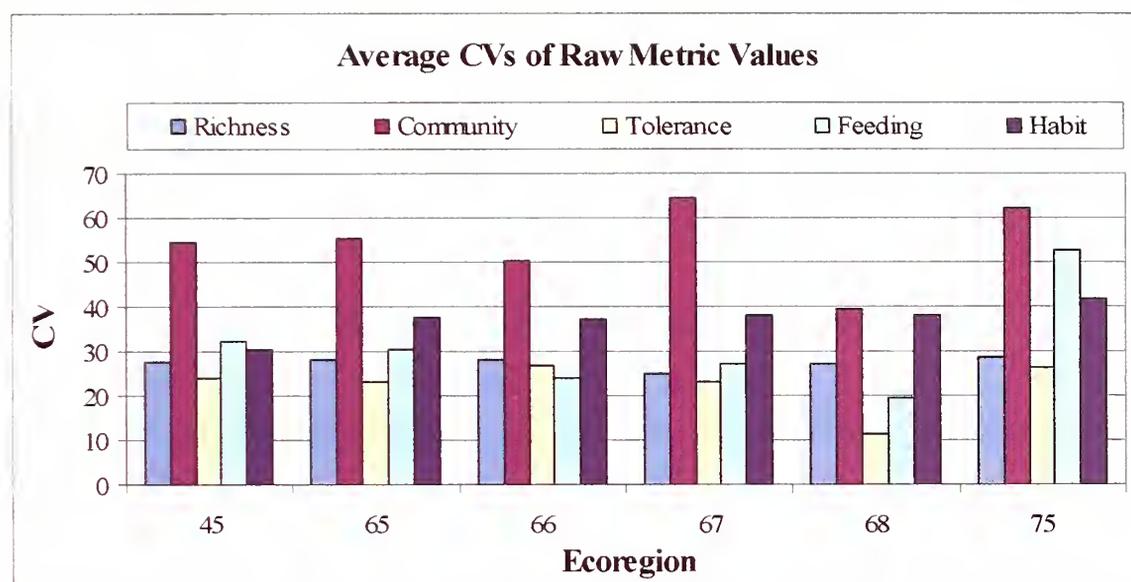
**Figure 5.** Average Root Mean Square Error (RMSE) for all standardized metric scores within each metric category per primary ecoregion designation.

### CV Precision Measures of Raw Metric Values and Standardized Metric Scores

The coefficient of variability (CV) was another measure of variability and precision that was calculated for raw metric values, standardized metric scores, and bioassessment scores. Although not prescribed by the Georgia Ecoregions QAPP with regards to MQOs, CV values were calculated to further illustrate the ranges of variability of metrics within and between each ecoregion. Statistically, as the CV value increases, the precision of the variable examined declines. CV values were calculated for raw metric values (presented in Table 9 and Figure 6), and for standardized metric values (presented in Table 9 and Figure 7). Subcoregional values of CVs for raw metric values and standardized scores are presented in the ecoregional appendices.

**Table 9.** Average Coefficient of Variability (CV) for all raw metric values within each metric category per primary ecoregion designation.

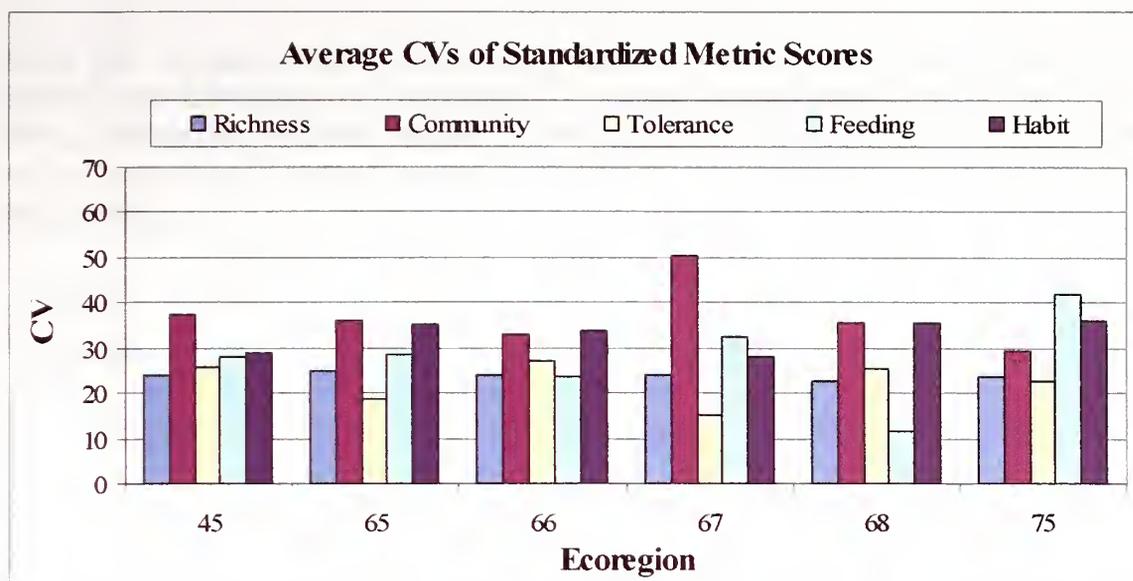
Metric Groups	45	65	66	67	68	75
Taxonomic Richness	27.9	28.0	28.2	25.5	27.1	28.5
Community Composition	54.5	55.6	50.4	64.4	39.5	62.1
Tolerance/Intolerance	24.1	23.3	26.8	23.1	11.3	26.4
Functional Feeding Group	32.3	30.6	24.2	27.3	19.6	52.8
Life Habit	30.3	37.6	37.4	38.1	38.1	41.8



**Figure 6.** Average Coefficient of Variability (CV) for all raw metric values within each metric category and per primary ecoregion designation.

**Table 10.** Average Coefficient of Variability (CV) for all standardized metric values within each metric category and per primary ecoregion designation.

Metric Groups	45	65	66	67	68	75
Taxonomic Richness	23.9	25.1	24.0	24.2	22.9	23.7
Community Composition	37.2	36.3	33.0	50.2	35.7	29.4
Tolerance/Intolerance	25.9	18.7	27.0	15.3	25.6	22.6
Functional Feeding Group	28.1	28.7	23.7	32.4	11.6	42.0
Life Habit	28.9	35.4	33.9	27.9	35.8	36.2



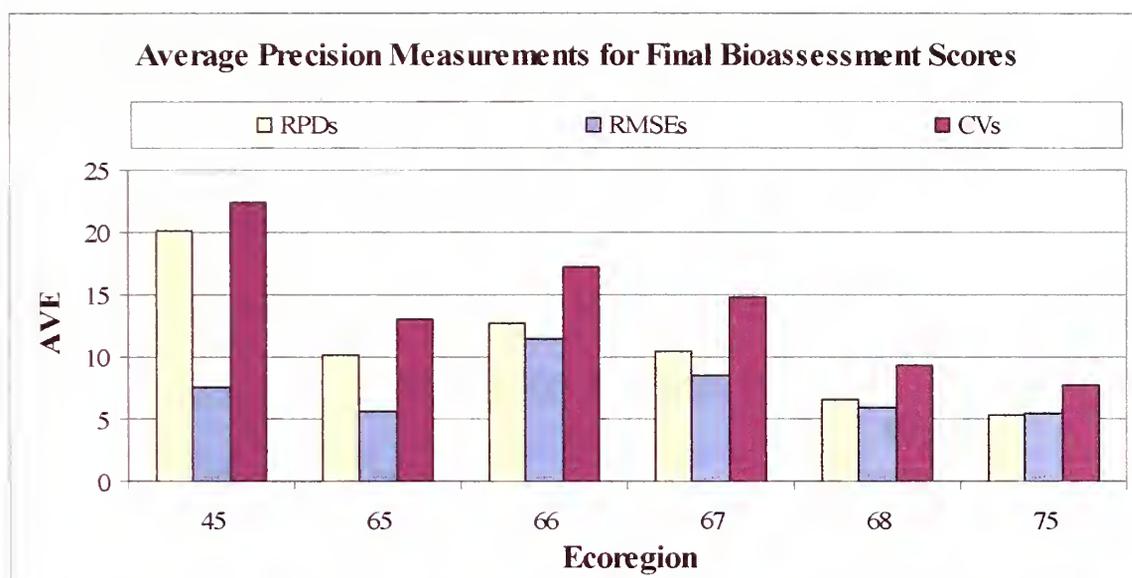
**Figure 7.** Average Coefficient of Variability (CV) for all standardized metric scores within each metric category and per primary ecoregion designation.

### Precision Measures for Bioassessment Scores

The final precision measures for evaluation of the variability of metrics within and between ecoregional designations centered upon the final bioassessment scores that constitute the biotic indices developed for each ecoregion. The average values calculated for RPDs, RMSEs, and CVs presented in Table 11 were inclusive of only those metrics that were determined to be indicative of community assemblages that exhibited responses to anthropogenic stress and were descriptive of the reference and impaired condition. In conjunction with Table 11, Figure 8 illustrates the ecoregional averages of RPD, RMSE, and CV values for the final bioassessment scores used in the development of the biotic index. Comparisons of RPD, RMSE, and CV values for final bioassessment metrics for each ecoregion and their corresponding subcoregions are also presented in Tables 12 to 65 and illustrated in Figures 9 to 13.

**Table 11.** Average Relative Percent Difference (RPD), Root Mean Square Error (RMSE), and Coefficient of Variability (CV) values for final bioassessment scores used in the development of biotic indices for the primary ecoregions of Georgia. (Averages are inclusive of only the metrics used to develop the final biotic index for each ecoregion designation.)

	45	65	66	67	68	75
<b>RPDs</b>	20.1	10.1	12.7	10.4	6.7	5.4
<b>RMSEs</b>	7.6	5.7	11.5	8.6	6.0	5.6
<b>CVs</b>	22.5	13.1	17.2	14.9	9.4	7.7



**Figure 8.** Comparison of Average Relative Difference (RPD), Root Mean Square of Error (RMSE), and Coefficient of Variability (CV) values for final bioassessment scores used in the development of biotic indices for the primary ecoregions of Georgia. (Averages are inclusive of only the metrics used to develop the final biotic index for each ecoregion designation.)

### Comparison of Precision Measures and Discrimination Efficiencies of Ecoregional and Subcoregional Biotic Indices

The final biotic indices developed from the Georgia Ecoregions study at the ecoregional and subcoregional level are presented in the following tables. These biotic indices were taken from the Georgia Ecoregions numerical index report (Gore *et al.*

2005) submitted to Georgia's DNR for establishing stream ecosystem rankings and implementing guidelines for TMDL permitting with the purpose of maintaining the ecological integrity of Georgia's freshwater lotic systems. In addition to the following tables that summarize the metrics used in the biotic indices for the primary ecoregions of Georgia, corresponding averages of the precision measures of RPDs, RMSEs, and CVs are also presented for the standardized metric scores that comprised the final additive bioassessment scores. Also included with the precision measures are the discrimination efficiency values specific to each metric.

### Summary of Biotic Indices and Precision Measures for Ecoregion 45

**Table 12.** The Biotic Index of primary Ecoregion 45 developed from the Georgia Ecoregions Project.

#### Biotic Index – Ecoregion 45

Metric	Metric Category
Coleoptera Taxa	Richness
% Chironomidae	Composition
% Plecoptera	
% Intolerant Individuals	Tolerance / Intolerance
North Carolina Biotic Index (NCBI)	

**Table 13.** Average precision measure values of standardized metric scores and discrimination efficiencies (DE) for the metrics that comprise the biotic index for the primary ecoregion 45. (RPD = Relative Percent Difference; RMSE = Root Mean Square of Error; CV = Coefficient of Variability)

#### Precision Measures – Ecoregion 45

Metric	RPD	RMSE	CV	DE
Coleoptera Taxa	32.0	12.8	36.1	0.6
% Chironomidae	34.8	13.2	26.7	0.7
% Plecoptera	29.4	2.7	34.0	0.7
% Intolerant Individuals	67.0	18.8	93.2	0.6
North Carolina Biotic Index (NCBI)	23.5	9.9	17.8	0.6

**Table 14.** The Biotic Index of Subcoregion 45a developed from the Georgia Ecoregions Project.**Biotic Index – Subcoregion 45a**

<b>Metric</b>	<b>Metric Category</b>
Plecoptera Taxa	Richness
% Trichoptera	Composition
% Chironomus Cricotopus/TC	
Tolerant Taxa	Tolerance
% Scraper	Functional Feeding Group
Clinger Taxa	Habitat

**Table 15.** Average precision measure values of standardized metric scores and discrimination efficiencies (DE) for the metrics that comprise the biotic index for Subcoregion 45a. (RPD = Relative Percent Difference; RMSE = Root Mean Square of Error; CV = Coefficient of Variability)**Precision Measures – Subcoregion 45a**

<b>Metrics</b>	<b>RPDs</b>	<b>RMSEs</b>	<b>CVs</b>	<b>DEs</b>
Plecoptera Taxa	17.3	7.6	37.7	0.5
% Trichoptera	31.5	13.4	30.6	0.8
% Chironomus Cricotopus/TC	5.3	6.4	6.8	1.0
Tolerant Taxa	12.2	10.6	14.8	1.0
% Scraper	25.5	7.6	22.0	0.8
Clinger Taxa	10.4	9.9	15.4	0.9

**Table 16.** The Biotic Index of Subcoregion 45b developed from the Georgia Ecoregions Project.**Biotic Index – Subcoregion 45b**

<b>Metric</b>	<b>Metric Category</b>
Coleoptera Taxa	Richness
% Oligochaeta	Composition
% Plecoptera	
Shredder Taxa	Functional Feeding Group
Scraper Taxa	
Swimmer Taxa	Habitat

**Table 17.** Average precision measure values of standardized metric scores and discrimination efficiencies (DE) for the metrics that comprise the biotic index for Subcoregion 45b. (RPD = Relative Percent Difference; RMSE = Root Mean Square of Error; CV = Coefficient of Variability)

**Precision Measures – Subcoregion 45b**

Metrics	RPDs	RMSEs	CVs	DEs
Coleoptera Taxa	44.4	8.0	60.6	0.9
% Oligochaeta	1.2	1.4	1.6	0.8
% Plecoptera	33.3	0.6	141	0.8
Shredder Taxa	49.2	10.7	41.6	0.9
Scraper Taxa	6.7	2.7	10.9	0.9
Swimmer Taxa	83.3	31.0	101	0.9

**Table 18.** The Biotic Index of Subcoregion 45c developed from the Georgia Ecoregions Project.

**Biotic Index – Subcoregion 45c**

Metric	Metric Category
Tanytarsini Taxa	Richness
% Odonata	Composition
% Tanypodinae/ Total Chironomidae	
Dominant Individual	Tolerance
% Intolerant Individuals	
% Shredder	Functional Feeding Group
Swimmer Taxa	Habitat

**Table 19.** Average precision measure values of standardized metric scores and discrimination efficiencies (DE) for the metrics that comprise the biotic index for Subcoregion 45c. (RPD = Relative Percent Difference; RMSE = Root Mean Square of Error; CV = Coefficient of Variability)

**Precision Measures – Subcoregion 45c**

Metrics	RPDs	RMSEs	CVs	DEs
Tanytarsini Taxa	0.0	0.0	0.0	0.6
% Odonata	99.8	35.4	141	0.6
% Tanypodinae/ TC	5.4	6.5	7.6	0.6
Dominant Individual	25.6	8.6	36.2	0.6
% Intolerant Individuals	100	13.2	141	0.8
% Shredder	0.0	0.0	0.0	1.0
Swimmer Taxa	100	17.7	141	0.4

**Table 20.** The Biotic Index of Subcoregion 45d developed from the Georgia Ecoregions Project.

**Biotic Index – Subcoregion 45d**

Metric	Metric Category
Coleoptera Taxa	Richness
% Tanypodinae/ Total Chironomidae	Composition
% Odonata	
North Carolina Biotic Index (NCBI)	Tolerance
% Tolerant Individuals	
Shredder Taxa	Functional Feeding Group

**Table 21.** Average precision measure values of standardized metric scores and discrimination efficiencies (DE) for the metrics that comprise the biotic index for Subcoregion 45d. (RPD = Relative Percent Difference; RMSE = Root Mean Square of Error; CV = Coefficient of Variability)

**Precision Measures – Subcoregion 45d**

Metrics	RPDs	RMSEs	CVs	DEs
Coleoptera Taxa	20.0	8.0	28.3	0.8
% Tanypodinae/ TC	0.0	0.0	0.0	1.0
% Odonata	11.8	14.9	16.7	0.8
NCBI	5.1	4.4	7.2	1.0
% Tolerant Individuals	15.9	17.1	22.5	1.0
Shredder Taxa	14.3	16.5	20.2	0.4

**Table 22.** The Biotic Index of Subcoregion 45h developed from the Georgia Ecoregions Project.

**Biotic Index – Subcoregion 45h**

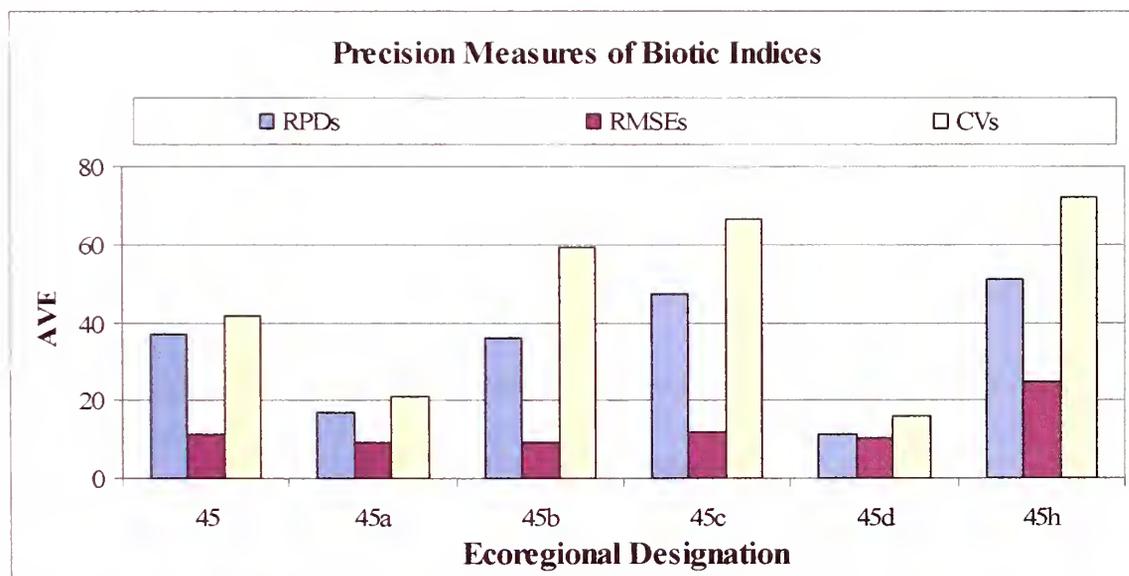
Metric	Metric Category
Plecoptera Taxa	Richness
% Ephemeroptera	Composition
% Plecoptera	
% Intolerant Individuals	Tolerance
% Scraper	Functional Feeding Group
% Clinger	Habitat

**Table 23.** Average precision measure values of standardized metric scores and discrimination efficiencies (DE) for the metrics that comprise the biotic index for Subcoregion 45h. (RPD = Relative Percent Difference; RMSE = Root Mean Square of Error; CV = Coefficient of Variability)

**Precision Measures – Subcoregion 45h**

Metrics	RPDs	RMSEs	CVs	DEs
Plecoptera Taxa	18.9	16.2	39.7	1.0
% Ephemeroptera	100	39.6	141	0.8
% Plecoptera	31.0	20.5	44.3	0.8
% Intolerant Individual	100	33.4	141	0.8
% Scraper	41.0	25.1	44.5	0.6
% Clinger	14.6	12.4	21.7	0.6

Figure 9 illustrates the comparison of the precision measures of RPD, RMSE, and CV for the biotic indices of Ecoregion 45 and its Subcoregions.



**Figure 9.** Comparison of Average Relative Difference (RPD), Root Mean Square of Error (RMSE), and Coefficient of Variability (CV) values of final metrics used in the development of biotic indices for Ecoregion 45 and its Subcoregions. (Averages are inclusive of only the standardized metrics used to develop the final biotic index for each ecoregion designation.)

### Summary of Biotic Indices and Precision Measures for Ecoregion 65

**Table 24.** The Biotic Index of primary Ecoregion 65 developed from the Georgia Ecoregions Project.

#### Biotic Index – Ecoregion 65

Metric	Metric Category
% Coleoptera	Composition
% Oligochaeta	
Intolerant Taxa	Tolerance
% Intolerant Individuals	
% Predator	Functional Feeding Group
% Clinger	Habitat

**Table 25.** Average precision measure values of standardized metric scores and discrimination efficiencies (DE) for the metrics that comprise the biotic index for the primary Ecoregion 65. (RPD = Relative Percent Difference; RMSE = Root Mean Square of Error; CV = Coefficient of Variability)

#### Precision Measures – Ecoregion 65

Metric	RPD	RMSE	CV	DE
% Coleoptera	40.7	14.1	43.9	0.5
% Oligochaeta	5.3	6.6	7.1	0.6
Intolerant Taxa	32.4	9.3	23.7	0.5
% Intolerant Individuals	42.6	13.7	46.2	0.6
% Predator	23.1	9.3	29.8	0.5
% Clinger	30.3	14.4	39.6	0.5

**Table 26.** The Biotic Index of Subcoregion 65c developed from the Georgia Ecoregions Project.

#### Biotic Index – Subcoregion 65c

Metric	Metric Category
% Trichoptera	Composition
Tolerant Taxa	Tolerance
Intolerant Taxa	
% Scraper	Functional Feeding Group
Clinger Taxa	Habitat

**Table 27.** Average precision measure values of standardized metric scores and discrimination efficiencies (DE) for the metrics that comprise the biotic index for Subcoregion 65c. (RPD = Relative Percent Difference; RMSE = Root Mean Square of Error; CV = Coefficient of Variability)

**Precision Measures – Subcoregion 65c**

Metrics	RPDs	RMSEs	CVs	DEs
% Trichoptera	39.5	25.0	65.5	0.7
Tolerant Taxa	6.6	6.5	9.0	0.8
Intolerant Taxa	28.1	6.7	12.5	0.8
% Scraper	58.6	22.1	70.6	0.9
Clinger Taxa	24.3	19.2	26.7	0.6

**Table 28.** The Biotic Index of Subcoregion 65d developed from the Georgia Ecoregions Project.

**Biotic Index – Subcoregion 65d**

Metric	Metric Category
Plecoptera Taxa	Richness
% Chironomidae	Composition
% Hydropsychidae/ EPT	
% Filterer	Functional Feeding Group
Swimmer Taxa	Habitat

**Table 29.** Average precision measure values of standardized metric scores and discrimination efficiencies (DE) for the metrics that comprise the biotic index for Subcoregion 65d. (RPD = Relative Percent Difference; RMSE = Root Mean Square of Error; CV = Coefficient of Variability)

**Precision Measures – Subcoregion 65d**

Metrics	RPDs	RMSEs	CVs	DEs
Plecoptera Taxa	9.5	7.9	12.0	0.7
% Chironomidae	8.6	10.0	12.4	0.7
% Hydropsychidae/ EPT	13.2	14.9	18.6	0.6
% Filterer	43.1	30.7	54.0	0.7
Swimmer Taxa	12.5	11.4	26.0	0.6

**Table 30.** The Biotic Index of Subcoregion 65g developed from the Georgia Ecoregions Project.

**Biotic Index – Subcoregion 65g**

Metric	Metric Category
EPT Taxa	Richness
% Oligochaeta	Composition
% Intolerant Individuals	
HBI	Functional Feeding Group
Filterer Taxa	
Clinger Taxa	Habitat

**Table 31.** Average precision measure values of standardized metric scores and discrimination efficiencies (DE) for the metrics that comprise the biotic index for Subcoregion 65g. (RPD = Relative Percent Difference; RMSE = Root Mean Square of Error; CV = Coefficient of Variability)

**Precision Measures – Subcoregion 65g**

Metrics	RPDs	RMSEs	CVs	DEs
EPT Taxa	0.0	0.0	0.0	1.0
% Oligochaeta	100	18.1	141	1.0
% Intolerant Individuals	0.0	0.0	0.0	1.0
HBI	15.2	4.1	21.5	1.0
Filterer Taxa	33.3	10.6	47.1	0.8
Clinger Taxa	20.0	7.2	28.3	1.0

**Table 32.** The Biotic Index of Subcoregion 65h developed from the Georgia Ecoregions Project.

**Biotic Index – Subcoregion 65h**

Metric	Metric Category
Tanytarsini Taxa	Richness
Shannon-Wiener base e	
% Oligochaeta	Composition
% Tanytarsini	
NCBI	Tolerance
% Predator	Functional Feeding Group
Clinger Taxa	Habitat

**Table 33.** Average precision measure values of standardized metric scores and discrimination efficiencies (DE) for the metrics that comprise the biotic index for Subcoregion 65h. (RPD = Relative Percent Difference; RMSE = Root Mean Square of Error; CV = Coefficient of Variability)

**Precision Measures – 65h**

Metrics	RPDs	RMSEs	CVs	DEs
Tanytarsini Taxa	64.8	48.0	92.7	0.8
Shannon-Wiener base e	11.4	13.4	15.6	0.7
% Oligochaeta	7.3	7.8	8.9	0.9
% Tanytarsini	41.4	10.1	38.1	1.0
NCBI	14.5	14.0	19.0	0.8
% Predator	3.4	2.8	3.5	0.6
Clinger Taxa	25.1	23.2	30.2	0.9

**Table 34.** The Biotic Index of Subcoregion 65k developed from the Georgia Ecoregions Project.

**Biotic Index – Subcoregion 65k**

Metric	Metric Category
% Gastropoda	Composition
% Orthocladiinae/Total Chironomidae	
% Coleoptera	
% Hydropsychidae/Total Trichoptera	
% Filterer	Functional Feeding Group
% Collector	

**Table 35.** Average precision measure values of standardized metric scores and discrimination efficiencies (DE) for the metrics that comprise the biotic index for Subcoregion 65k. (RPD = Relative Percent Difference; RMSE = Root Mean Square of Error; CV = Coefficient of Variability)

**Precision Measures – Subcoregion 65k**

Metrics	RPDs	RMSEs	CVs	DEs
% Gastropoda	25.0	14.1	23.5	0.8
% Orthocladiinae/Total Chironomidae	63.9	25.3	79.3	0.6
% Coleoptera	28.2	7.2	24.5	0.6
% Hydropsychidae/Total Trichoptera	50.0	35.4	70.7	0.6
% Filterer	27.2	4.1	7.5	0.6
% Collector	13.4	9.5	16.3	0.9

**Table 36.** The Biotic Index of Subcoregion 65l developed from the Georgia Ecoregions Project.**Biotic Index – Subcoregion 65l**

<b>Metric</b>	<b>Metric Category</b>
EPT Taxa	Richness
Diptera Taxa	
% EPT	Composition
% Trichoptera	
HBI	Tolerance
Predator Taxa	Functional Feeding Group
Clinger Taxa	Habitat

**Table 37.** Average precision measure values of standardized metric scores and discrimination efficiencies (DE) for the metrics that comprise the biotic index for Subcoregion 65l. (RPD = Relative Percent Difference; RMSE = Root Mean Square of Error; CV = Coefficient of Variability)**Precision Measures – 65l**

<b>Metrics</b>	<b>RPDs</b>	<b>RMSEs</b>	<b>CVs</b>	<b>DEs</b>
EPT Taxa	75.0	12.1	56.6	0.8
Diptera Taxa	9.4	9.1	14.7	0.6
% EPT	67.0	3.0	8.5	0.8
% Trichoptera	66.7	7.8	141	0.9
HBI	19.4	11.2	25.5	0.6
Predator Taxa	8.5	4.5	8.3	0.7
Clinger Taxa	6.7	4.2	17.7	0.8

**Table 38.** The Biotic Index of Subcoregion 65o developed from the Georgia Ecoregions Project.**Biotic Index – Subcoregion 65o**

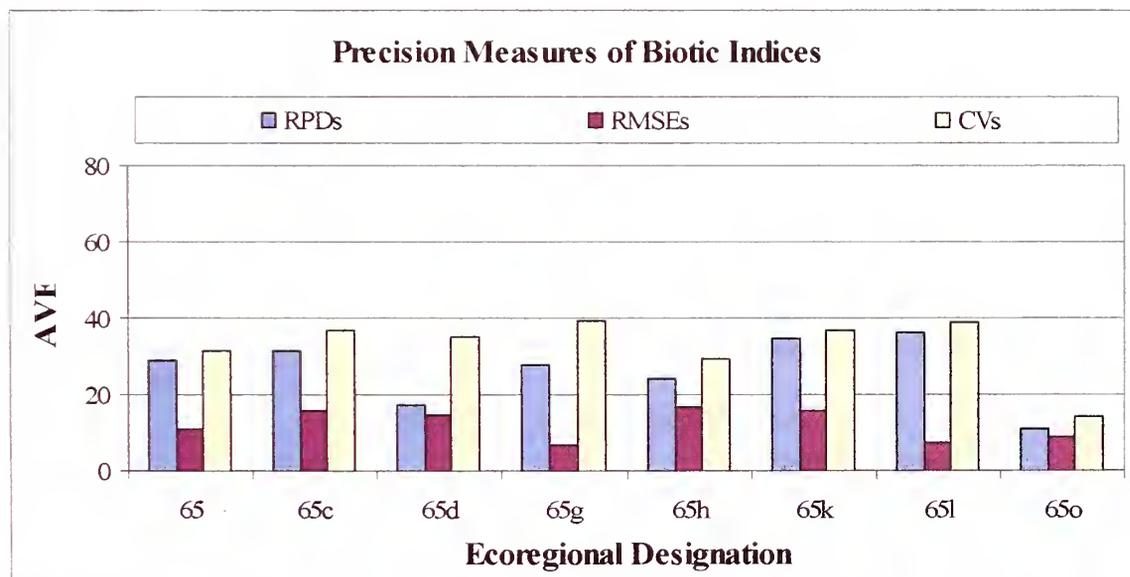
<b>Metric</b>	<b>Metric Category</b>
Chironomidae Taxa	Richness
% Oligochaeta	Composition
Beck's Index	Tolerance
NCBI	
Scraper Taxa	Functional Feeding Group
Burrower Taxa	Habitat
Sprawler Taxa	

**Table 39.** Average precision measure values of standardized metric scores and discrimination efficiencies (DE) for the metrics that comprise the biotic index for Subcoregion 65o. (RPD = Relative Percent Difference; RMSE = Root Mean Square of Error; CV = Coefficient of Variability)

**Precision Measures – Subcoregion 65o**

Metrics	RPDs	RMSEs	CVs	DEs
Chironomidae Taxa	14.4	13.5	19.4	0.8
% Oligochaeta	3.0	4.0	4.2	0.8
Beck's Index	9.4	8.1	10.7	0.4
NCBI	19.2	16.0	27.7	0.6
Scraper Taxa	0.0	0.0	0.0	0.8
Burrower Taxa	18.3	10.4	25.9	0.6
Sprawler Taxa	11.4	9.8	11.0	0.8

Figure 10 illustrates the comparison of the precision measures of RPD, RMSE, and CV for the biotic indices of Ecoregion 65 and its Subcoregions.



**Figure 10.** Comparison of Average Relative Difference (RPD), Root Mean Square of Error (RMSE), and Coefficient of Variability (CV) values of final metrics used in the development of biotic indices for Ecoregion 65 and its Subcoregions. (Averages are inclusive of only the standardized metrics used to develop the final biotic index for each ecoregion designation.)

### Summary of Biotic Indices and Precision Measures for Ecoregion 66

**Table 40.** The Biotic Index of primary Ecoregion 66 developed from the Georgia Ecoregions Project.

#### Biotic Index – Ecoregion 66

Metric	Metric Category
Plecoptera Taxa	Richness
Simpson's Index	
% Trichoptera	Composition
% Intolerant Individuals	Tolerance
NCBI	
Predator Taxa	Functional Feeding Group
Burrower Taxa	Habitat

**Table 41.** Average precision measure values of standardized metric scores and discrimination efficiencies (DE) for the metrics that comprise the biotic index for primary Ecoregion 66. (RPD = Relative Percent Difference; RMSE = Root Mean Square of Error; CV = Coefficient of Variability)

#### Precision Measures – Ecoregion 66

Metric	RPD	RMSE	CV	DE
Plecoptera Taxa	31.2	18.8	33.9	0.7
Simpson's Index	20.4	14.0	20.1	0.6
% Trichoptera	14.9	11.5	16.5	0.5
% Intolerant Individuals	24.2	17.3	30.1	0.6
NCBI	22.7	12.8	20.2	0.7
Predator Taxa	15.7	15.7	21.1	0.8
Burrower Taxa	21.4	20.1	26.6	0.5

**Table 42.** The Biotic Index of Subcoregion 66d developed from the Georgia Ecoregions Project.

#### Biotic Index – Subcoregion 66d

Metric	Metric Category
Diptera Taxa	Richness
% Plecoptera	Composition
% Odonata	
% Dominant Individuals	Tolerance
% Shredder	Functional Feeding Group
Clinger Taxa	Habitat

**Table 43.** Average precision measure values of standardized metric scores and discrimination efficiencies (DE) for the metrics that comprise the biotic index for Subcoregion 66d. (RPD = Relative Percent Difference; RMSE = Root Mean Square of Error; CV = Coefficient of Variability)

**Precision Measures – Subcoregion 66d**

Metrics	RPDs	RMSEs	CVs	DEs
Diptera Taxa	4.1	18.5	26.8	0.8
% Plecoptera	20.2	10.8	24.3	0.6
% Odonata	50.7	42.3	72.6	1.0
% Dominant Individuals	3.8	18.3	22.5	0.6
% Shredder	8.6	15.5	42.8	0.8
Clinger Taxa	13.8	16.8	20.4	0.6

**Table 44.** The Biotic Index of Subcoregion 66g developed from the Georgia Ecoregions Project.

**Biotic Index – Subcoregion 66g**

Metric	Metric Category
EPT Taxa	Richness
% Chironomidae	Composition
% Tanypodinae/Total Chironomidae	
NCBI	Tolerance
% Dominant Individuals	
Scraper Taxa	Functional Feeding Group
% Clinger	Habitat

**Table 45.** Average precision measure values of standardized metric scores and discrimination efficiencies (DE) for the metrics that comprise the biotic index for Subcoregion 66g. (RPD = Relative Percent Difference; RMSE = Root Mean Square of Error; CV = Coefficient of Variability)

**Precision Measures – Subcoregion 66g**

Metrics	RPDs	RMSEs	CVs	DEs
EPT Taxa	5.4	10.9	15.7	0.9
% Chironomidae	35.9	15.1	36.9	0.9
% Tanypodinae/Total Chironomidae	35.4	14.0	52.3	0.9
NCBI	41.8	18.4	28.9	0.7
% Dominant Individuals	39.4	17.0	25.0	0.7
Scraper Taxa	13.0	11.3	18.8	0.9
% Clinger	4.9	10.9	18.4	0.7

**Table 46.** The Biotic Index of Subcoregion 66j developed from the Georgia Ecoregions Project.

**Biotic Index – Subcoregion 66j**

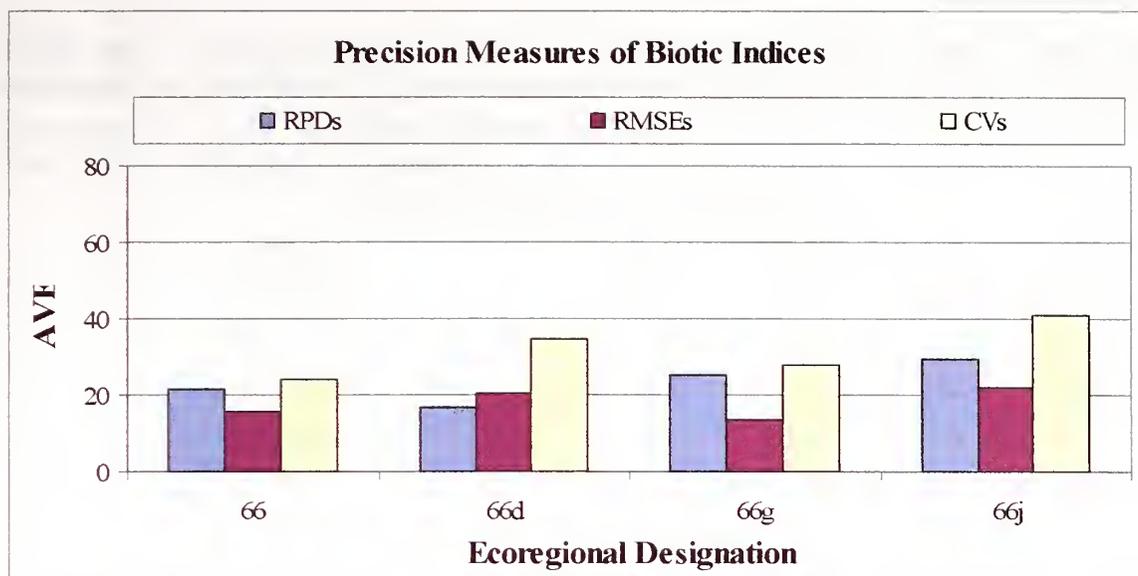
<b>Metric</b>	<b>Metric Category</b>
Simpson's Diversity Index	Richness
Margalef's Index	
% Tanytarsini	Composition
% Intolerant Individuals	Tolerance
Predator Taxa	Functional Feeding Group
Sprawler Taxa	Habitat

**Table 47.** Average precision measure values of standardized metric scores and discrimination efficiencies (DE) for the metrics that comprise the biotic index for Subcoregion 66j. (RPD = Relative Percent Difference; RMSE = Root Mean Square of Error; CV = Coefficient of Variability)

**Precision Measures – Subcoregion 66j**

<b>Metrics</b>	<b>RPDs</b>	<b>RMSEs</b>	<b>CVs</b>	<b>DEs</b>
Simpson's Diversity Index	38.9	26.8	46.7	0.8
Margalef's Index	10.4	12.1	14.3	0.8
% Tanytarsini	63.5	39.4	93.0	0.8
% Intolerant Individuals	29.9	22.7	41.8	0.6
Predator Taxa	16.1	14.5	22.0	0.8
Sprawler Taxa	19.2	18.6	29.7	0.6

Figure 11 illustrates the comparison of the precision measures of RPD, RMSE, and CV for the biotic indices of Ecoregion 66 and its Subcoregions.



**Figure 11.** Comparison Average Relative Difference (RPD), Root Mean Square of Error (RMSE), and Coefficient of Variability (CV) values of final metrics used in the development of biotic indices for Ecoregion 66 and its Subcoregions. (Averages are inclusive of only the standardized metrics used to develop the final biotic index for each ecoregion designation.)

### Summary of Biotic Indices and Precision Measures for Ecoregions 67 and 68

**Table 48.** The Biotic Index of primary Ecoregion 67 developed from the Georgia Ecoregions Project.

#### Biotic Index – Ecoregion 67

Metric	Metric Category
EPT Taxa	Richness
Plecoptera Taxa	
% Plecoptera	Composition
% Isopoda	
HBI	Tolerance
Clinger Taxa	Habitat

**Table 49.** Average precision measure values of standardized metric scores and discrimination efficiencies (DE) for the metrics that comprise the biotic index for primary Ecoregion 67. (RPD = Relative Percent Difference; RMSE = Root Mean Square of Error; CV = Coefficient of Variability)

**Precision Measures – Ecoregion 67**

Metric	RPD	RMSE	CV	DE
EPT Taxa	17.4	13.5	24.6	0.8
Plecoptera Taxa	26.3	25.7	61.6	0.8
% Plecoptera	38.5	19.9	130	0.8
% Isopoda	4.8	6.4	7.0	0.7
HBI	8.6	6.7	9.6	0.8
Clinger Taxa	7.8	7.0	9.9	0.7

**Table 50.** The Biotic Index of Subcoregion 67f&i developed from the Georgia Ecoregions Project.

**Biotic Index – Subcoregion 67f&i**

Metric	Metric Category
EPT Taxa	Richness
Plecoptera Taxa	
% EPT	Composition
NCBI	Tolerance
Scraper Taxa	Functional Feeding Group
% Clinger	Habitat

**Table 51.** Average precision measure values of standardized metric scores and discrimination efficiencies (DE) for the metrics that comprise the biotic index for Subcoregion 67f&i. (RPD = Relative Percent Difference; RMSE = Root Mean Square of Error; CV = Coefficient of Variability)

**Precision Measures – 67f&i**

Metrics	RPDs	RMSEs	CVs	DEs
EPT Taxa	17.1	16.2	24.9	1.0
Plecoptera Taxa	23.1	25.3	57.5	1.0
% EPT	19.7	17.9	30.4	1.0
NCBI	7.6	9.3	10.3	0.8
Scraper Taxa	18.3	18.8	22.9	0.8
% Clinger	8.7	11.7	15.3	1.0
Clinger Taxa	10.9	8.3	12.1	0.7

**Table 52.** The Biotic Index of Subcoregion 67h developed from the Georgia Ecoregions Project.

**Biotic Index – Subcoregion 67h**

<b>Metric</b>	<b>Metric Category</b>
Plecoptera Taxa	Richness
% Gastropoda	Composition
% Tolerant Individuals	Tolerance
HBI	
Scraper Taxa	Functional Feeding Group
Swimmer Taxa	Habitat

**Table 53.** Average precision measure values of standardized metric scores and discrimination efficiencies (DE) for the metrics that comprise the biotic index for Subcoregion 67h. (RPD = Relative Percent Difference; RMSE = Root Mean Square of Error; CV = Coefficient of Variability)

**Precision Measures – 67h**

<b>Metrics</b>	<b>RPDs</b>	<b>RMSEs</b>	<b>CVs</b>	<b>DEs</b>
Plecoptera Taxa	55.6	47.8	78.6	0.5
% Gastropoda	0.0	0.0	0.0	1.0
% Tolerant Individuals	19.5	19.7	27.5	1.0
HBI	0.6	0.3	0.9	1.0
Scraper Taxa	20.0	12.6	28.3	1.0
Swimmer Taxa	33.3	35.4	47.1	1.0

**Table 54.** The Biotic Index of primary Ecoregion 68 developed from the Georgia Ecoregions Project.

**Biotic Index – Ecoregion 68**

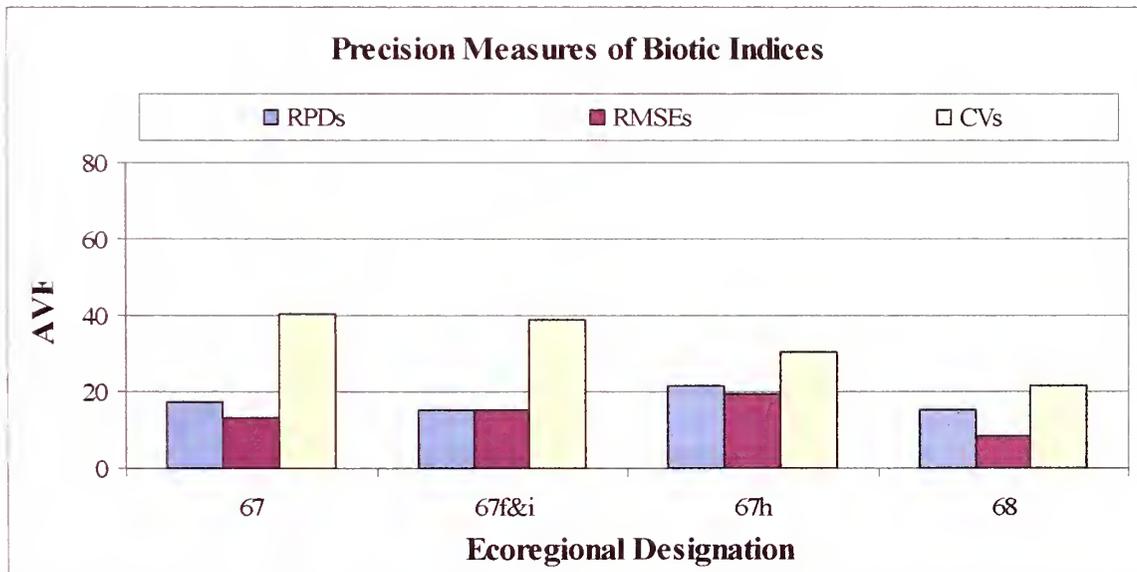
<b>Metric</b>	<b>Metric Category</b>
Plecoptera Taxa	Richness
% Hydropsychidae/Total Trichoptera	Composition
% Tanypodinae/Total Chironomidae	
NCBI	Tolerance
Scraper Taxa	Functional Feeding Group
% Clinger	Habitat

**Table 55.** Average precision measure values of standardized metric scores and discrimination efficiencies (DE) for the metrics that comprise the biotic index for primary Ecoregion 68. (RPD = Relative Percent Difference; RMSE = Root Mean Square of Error; CV = Coefficient of Variability)

**Precision Measures – Ecoregion 68**

Metric	RPD	RMSE	CV	DE
Plecoptera Taxa	0.0	0.0	0.0	0.8
% Hydropsychidae/Total Trichoptera	7.8	6.0	11.1	0.6
% Tanypodinae/Total Chironomidae	47.2	9.0	66.7	0.8
NCBI	1.8	2.8	4.5	1.0
Scraper Taxa	33.3	31.7	47.1	0.8
% Clinger	1.0	1.3	1.4	0.6

Figure 12 illustrates the comparison of the precision measures of RPD, RMSE, and CV for the biotic indices of Ecoregion 67 and its Subcoregions, as well as Ecoregion 68 which consisted solely of one Subcoregion (*i.e.* 68c&d).



**Figure 12.** Comparison of Average Relative Difference (RPD), Root Mean Square of Error (RMSE), and Coefficient of Variability (CV) values of final metrics used in the development of biotic indices for Ecoregion 67 and its Subcoregions, and Ecoregion 68 which consisted of one Subcoregion (c&d). (Averages are inclusive of only the standardized metrics used to develop the final biotic index for each ecoregion designation.)

### Summary of Biotic Indices and Precision Measures for Ecoregions 75

**Table 56.** The Biotic Index of primary Ecoregion 75 developed from the Georgia Ecoregions Project.

#### Biotic Index – Ecoregion 75

Metric	Metric Category
% Non-Insect	Composition
% Oligochaeta	
% Odonata	
% Tanypodinae/Total Chironomidae	
Tolerant Taxa	Tolerance
% Tolerant Individuals	

**Table 57.** Average precision measure values of standardized metric scores and discrimination efficiencies (DE) for the metrics that comprise the biotic index for primary Ecoregion 75. (RPD = Relative Percent Difference; RMSE = Root Mean Square of Error; CV = Coefficient of Variability)

#### Precision Measures – Ecoregion 75

Metric	RPD	RMSE	CV	DE
% Non-Insect	22.2	9.9	19.3	0.6
% Oligochaeta	6.0	6.2	6.8	0.7
% Odonata	2.5	3.2	3.4	0.5
% Tanypodinae/Total Chironomidae	3.2	3.4	3.7	0.5
Tolerant Taxa	24.1	17.7	34.1	0.6
% Tolerant Individuals	24.6	13.8	26.8	0.5

**Table 58.** The Biotic Index of Subcoregion 75e developed from the Georgia Ecoregions Project.

#### Biotic Index – Subcoregion 75e

Metric	Metric Category
% Non-Insect	Composition
% Oligochaeta	
% Isopoda	
% Odonata	
% Tolerant Individuals	Tolerance
% Filterer	Functional Feeding Group

**Table 59.** Average precision measure values of standardized metric scores and discrimination efficiencies (DE) for the metrics that comprise the biotic index for Subcoregion 75e. (RPD = Relative Percent Difference; RMSE = Root Mean Square of Error; CV = Coefficient of Variability)

**Precision Measures – Subcoregion 75e**

Metrics	RPDs	RMSEs	CVs	DEs
% Non-Insect	18.1	11.4	21.4	0.9
% Oligochaeta	8.2	7.1	8.9	0.9
% Isopoda	28.4	19.9	33.3	0.6
% Odonata	20.2	21.6	27.2	0.6
% Tolerant Individuals	28.0	7.2	28.2	0.6
% Filterer	41.6	35.8	56.1	0.6

**Table 60.** The Biotic Index of Subcoregion 75f developed from the Georgia Ecoregions Project.

**Biotic Index – Subcoregion 75f**

Metric	Metric Category
% Oligochaeta	Composition
% Tanypodinae/Total Chironomidae	
Tolerant Taxa	Tolerance
% Filterer	Functional Feeding Group

**Table 61.** Average precision measure values of standardized metric scores and discrimination efficiencies (DE) for the metrics that comprise the biotic index for Subcoregion 75f. (RPD = Relative Percent Difference; RMSE = Root Mean Square of Error; CV = Coefficient of Variability)

**Precision Measures – Subcoregion 75f**

Metrics	RPDs	RMSEs	CVs	DEs
% Oligochaeta	10.3	11.6	14.5	0.7
% Tanypodinae/Total Chironomidae	1.9	2.7	2.7	0.8
Tolerant Taxa	50.0	44.0	70.7	0.8
% Filterer	0.0	0.0	0.0	0.7

**Table 62.** The Biotic Index of Subcoregion 75h developed from the Georgia Ecoregions Project.

**Biotic Index – Subcoregion 75h**

Metric	Metric Category
% Oligochaeta	Composition
% Tolerant Individuals	Tolerance
HBI	
% Shredder	Functional Feeding Group
Collector Taxa	
% Filterer	

**Table 63.** Average precision measure values of standardized metric scores and discrimination efficiencies (DE) for the metrics that comprise the biotic index for Subcoregion 75h. (RPD = Relative Percent Difference; RMSE = Root Mean Square of Error; CV = Coefficient of Variability)

**Precision Measures – Subcoregion 75h**

Metrics	RPDs	RMSEs	CVs	DEs
% Oligochaeta	2.4	3.4	3.5	0.8
% Tolerant Individuals	9.8	12.7	13.9	0.8
HBI	27.7	29.3	39.2	0.5
% Shredder	100	15.8	141	0.8
Collector Taxa	4.3	4.5	6.1	0.5
% Filterer	5.2	6.5	7.3	0.5

**Table 64.** The Biotic Index of Subcoregion 75j developed from the Georgia Ecoregions Project.

**Biotic Index – Subcoregion 75j**

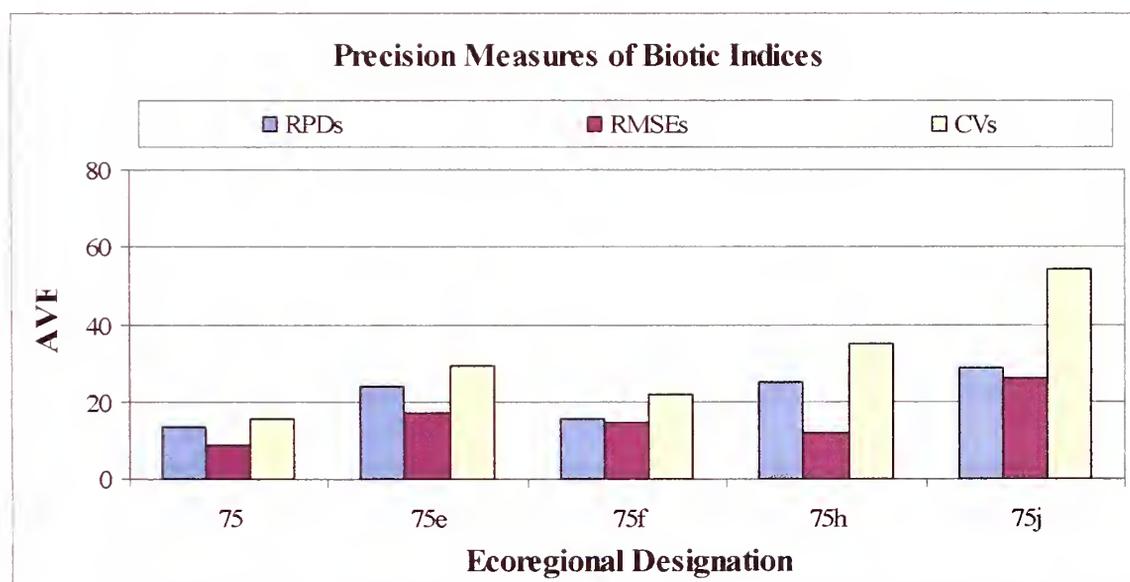
Metric	Metric Category
% Non-Insect	Composition
% Oligochaeta	
% Tolerant Individuals	Tolerance
Shredder Taxa	Functional Feeding Group

**Table 65.** Average precision measure values of standardized metric scores and discrimination efficiencies (DE) for the metrics that comprise the biotic index for Subcoregion 75j. (RPD = Relative Percent Difference; RMSE = Root Mean Square of Error; CV = Coefficient of Variability)

**Precision Measures – Subcoregion 75j**

Metrics	RPDs	RMSEs	CVs	DEs
% Non-Insect	38.0	37.6	107	0.6
% Oligochaeta	7.5	13.0	14.2	0.5
% Tolerant Individuals	35.4	34.1	47.5	0.6
Shredder Taxa	33.3	20.4	49.0	0.5

Figure 13 illustrates the comparison of the precision measures of RPD, RMSE, and CV for the biotic indices of Ecoregion 75 and its Subcoregions.



**Figure 13.** Comparison of Average Relative Difference (RPD), Root Mean Square of Error (RMSE), and Coefficient of Variability (CV) values of final metrics used in the development of biotic indices for Ecoregion 75 and its Subcoregions. (Averages are inclusive of only the metrics used to develop the final biotic index for each ecoregion designation.)

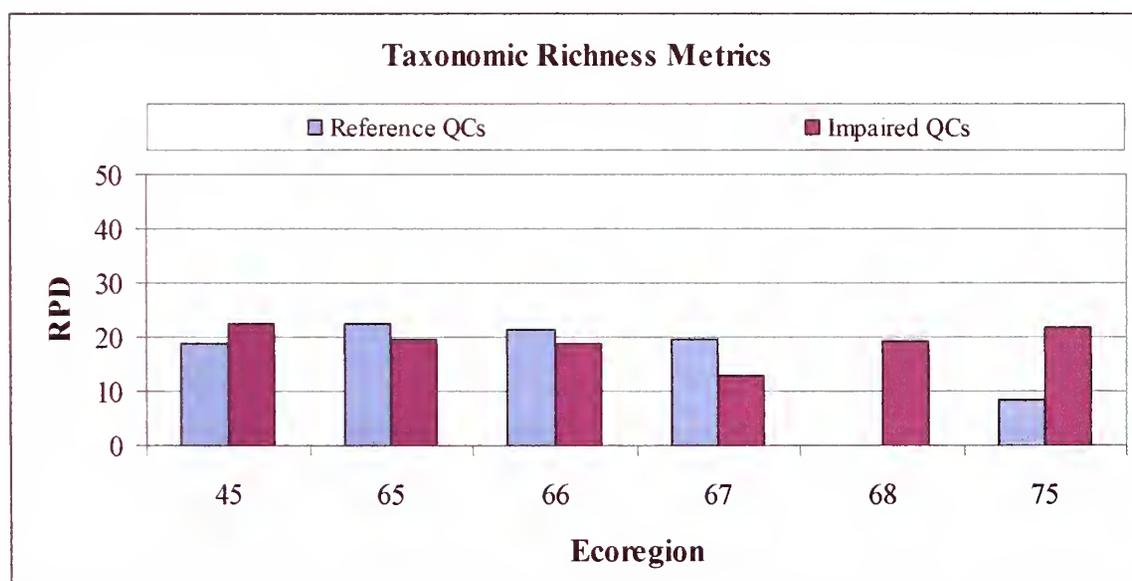
### **Comparison of Relative Percent Difference by Stream Class Designation**

Apart from the requirements of the QAPP to analyze data quality and the variability of the biological metrics used in the development of the biological indices at the ecoregional and subcoregional level, the range of variability between and among stream classes was also considered. Although the final determination of metrics that represented the biological condition was ultimately based on each metric's ability to distinguish differences in the characteristics of a reference or impaired stream ecosystem, it was interesting to note the measures of variability of the stream classes themselves. Specifically, the precision measure of relative percent difference was considered to illustrate the variability of raw metric values calculated for reference and impaired streams separately.

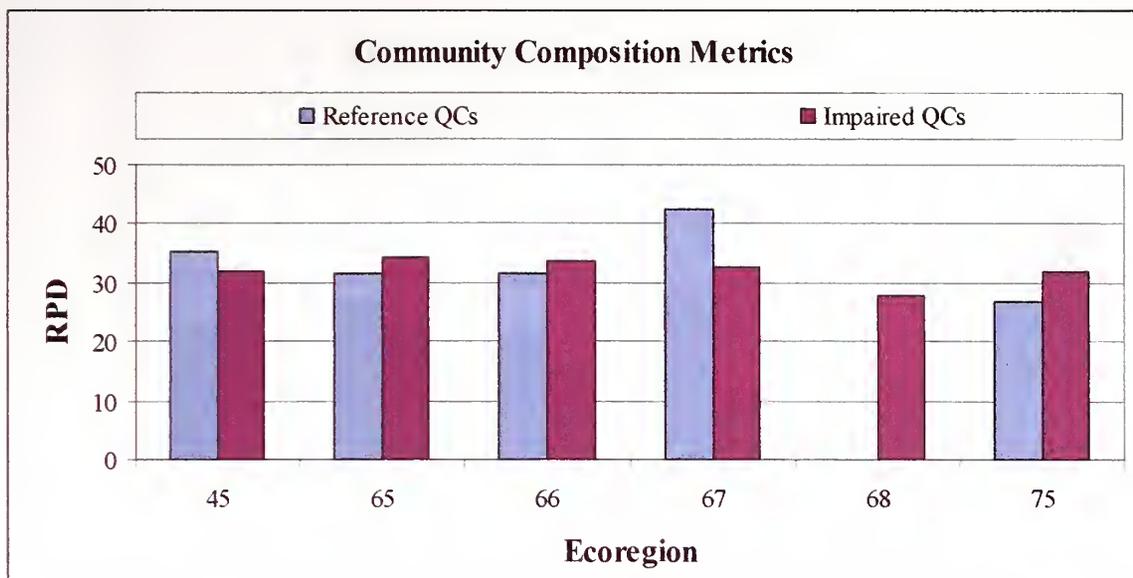
The RPD precision measures of raw metric values for reference and impaired stream classes are presented at the ecoregional level and per metric group are summarized in Table 66. Additionally, the differences in variability between the stream classes are illustrated in two manners: (1) each metric category is compared individually between ecoregional designations, and (2) each metric category is compared to each other per primary ecoregion designation. The RPD values presented in Table 66 are illustrated in Figures 14 to 24. These illustrations demonstrate variability for each metric category in relation to other metric categories, as well as the variability of each metric category within each ecoregional designation. Metric specific calculations of RPDs for each stream class at the ecoregional and subcoregional level are included on the CD-Rom in the pocket materials of this research paper.

**Table 66.** Average Relative Percent Difference (RPD) of Quality Control (QC) sites per stream class and per primary ecoregion. Values are averaged for all raw metric values within the metric group category. (“na” denotes no QC sample was collected for the stream class designation.)

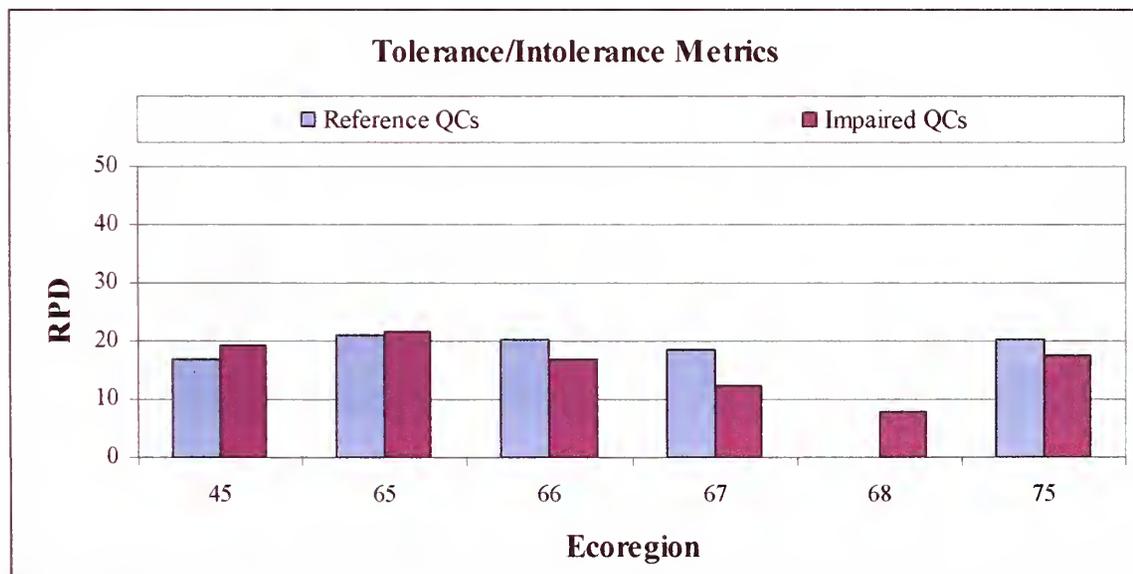
Metric Groups	Class	45	65	66	67	68	75
Taxonomic Richness	Reference	19.0	22.7	21.3	19.7	na	8.6
	Impaired	22.6	19.5	18.9	13.0	19.2	21.9
Community Composition	Reference	35.5	31.6	31.6	42.5	na	26.7
	Impaired	32.0	34.3	33.6	32.5	27.9	32.0
Tolerance/Intolerance	Reference	16.8	21.0	20.5	18.6	na	20.3
	Impaired	19.1	21.8	17.0	12.3	8.0	17.8
Functional Feeding Group	Reference	22.4	26.4	21.4	27.7	na	26.5
	Impaired	28.1	21.4	14.6	11.9	13.9	41.4
Life Habit	Reference	23.8	26.5	29.8	24.7	na	8.6
	Impaired	31.2	33.1	20.3	15.7	26.9	36.3



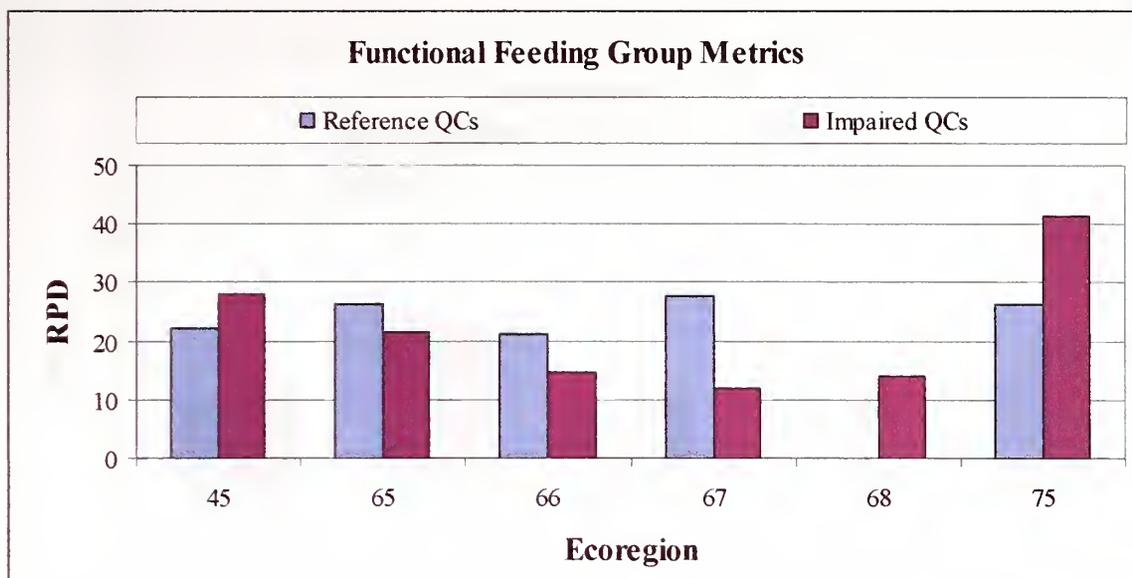
**Figure 14.** Comparison of Relative Percent Difference (RPD) values averaged for all raw metric values of the taxonomic richness metrics per ecoregion designation and for stream class Quality Control (QC) samples. (No Reference QC samples were collected for Ecoregion 68.)



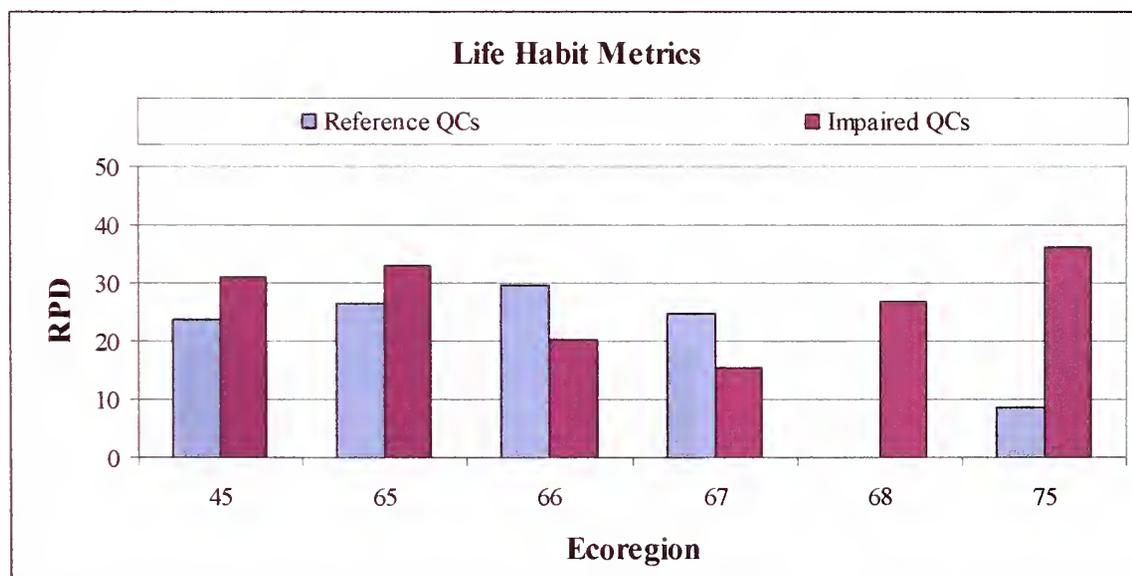
**Figure 15.** Comparison of Relative Percent Difference (RPD) values averaged for all raw metric values of the community composition metrics per ecoregion designation and for stream class Quality Control (QC) samples. (No Reference QC samples were collected for Ecoregion 68.)



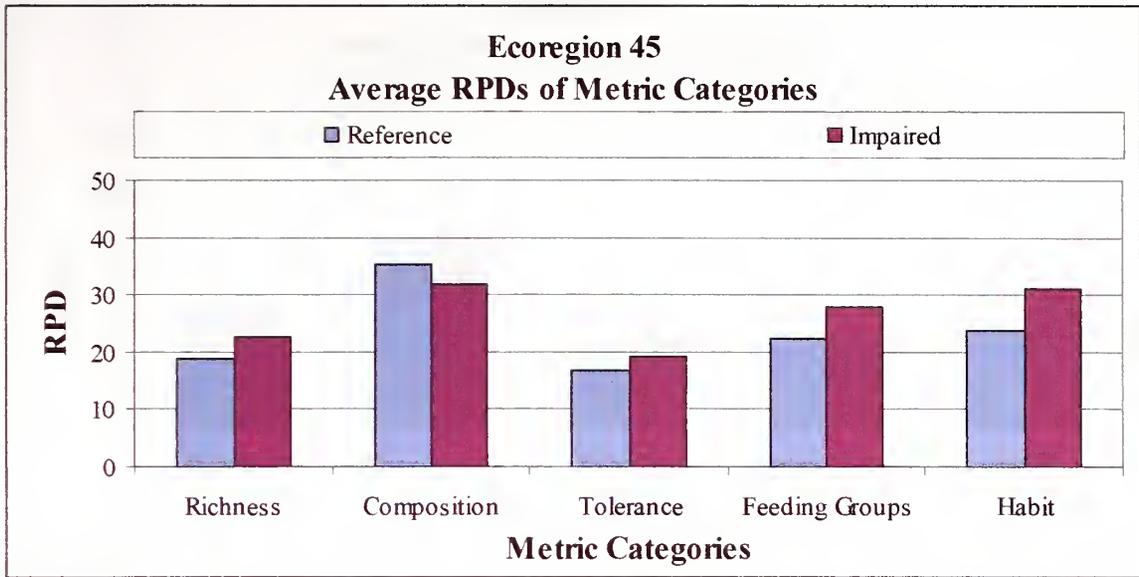
**Figure 16.** Comparison of Relative Percent Difference (RPD) values averaged for all raw metric values of the tolerant/intolerant individuals metrics per ecoregion designation and for stream class Quality Control (QC) samples. (No Reference QC samples were collected for Ecoregion 68.)



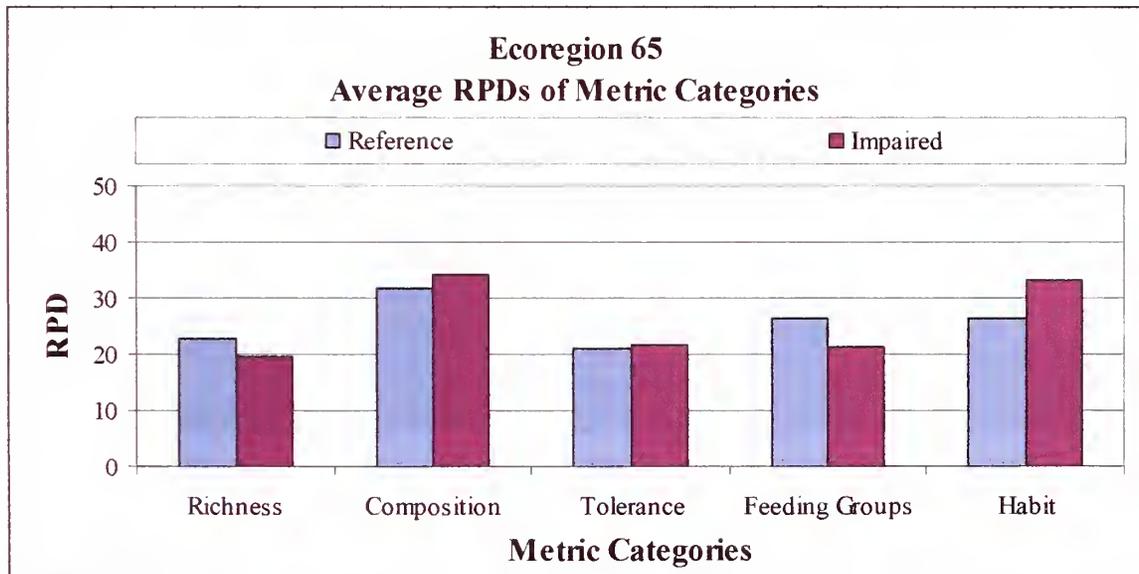
**Figure 17.** Comparison of Relative Percent Difference (RPD) values averaged for all raw metric values of the functional feeding group metrics per ecoregion designation and for stream class Quality Control (QC) samples. (No Reference QC samples were collected for Ecoregion 68.)



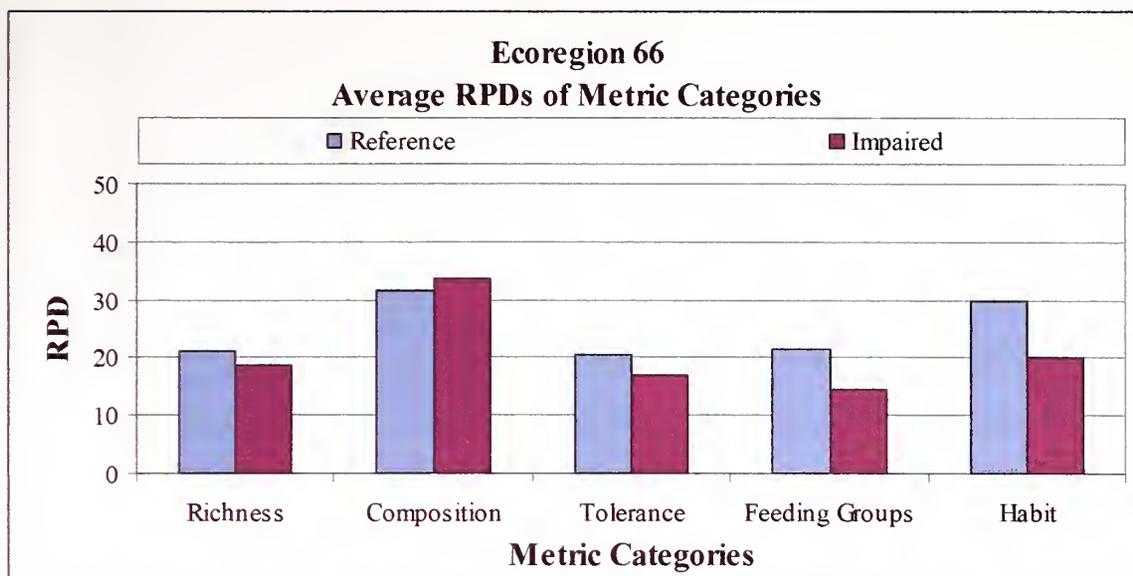
**Figure 18.** Comparison of Relative Percent Difference (RPD) values averaged for all raw metric values of the life habit metrics per ecoregion designation and for stream class Quality Control (QC) samples. (No Reference QC samples were collected for Ecoregion 68.)



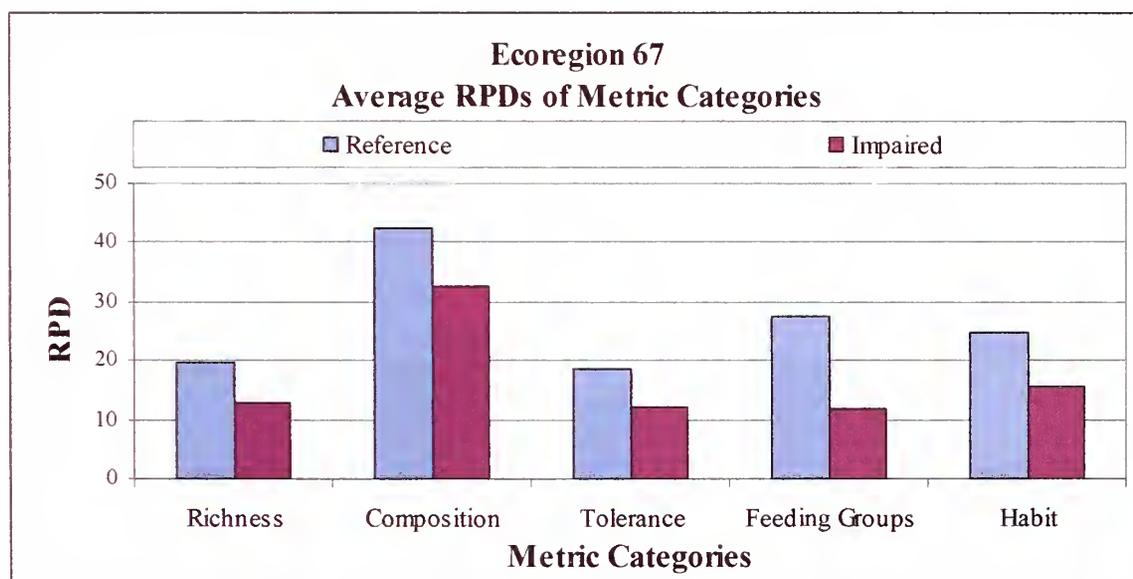
**Figure 19.** Comparison of Relative percent Difference (RPD) values averaged for all raw metric values by stream class designation per metric category for primary Ecoregion 45.



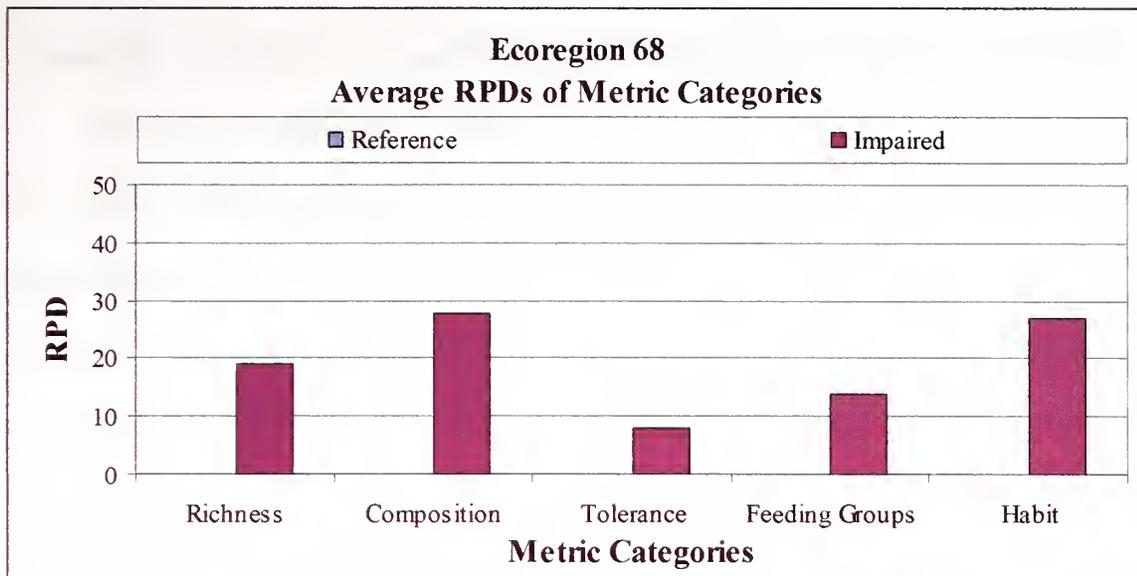
**Figure 20.** Comparison of Relative percent Difference (RPD) values averaged for all raw metric values by stream class designation per metric category for primary Ecoregion 65.



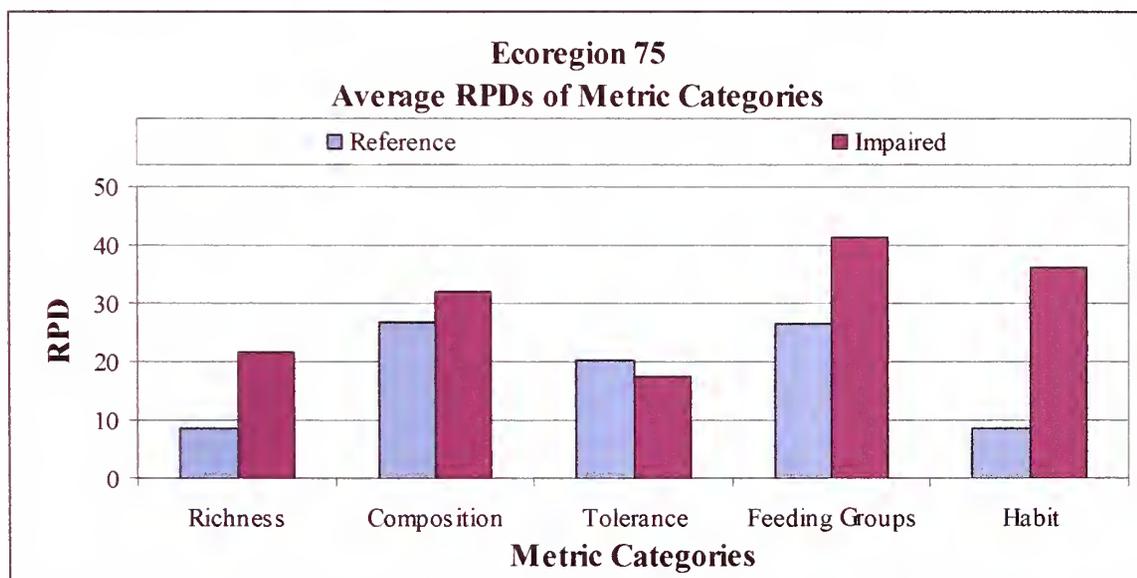
**Figure 21.** Comparison of Relative percent Difference (RPD) values averaged for all raw metric values by stream class designation per metric category for primary Ecoregion 66.



**Figure 22.** Comparison of Relative percent Difference (RPD) values averaged for all raw metric values by stream class designation per metric category for primary Ecoregion 67.



**Figure 23.** Comparison of Relative percent Difference (RPD) values averaged for all raw metric values by stream class designation per metric category for primary Ecoregion 68. (No Reference QC samples were collected for Ecoregion 68.)



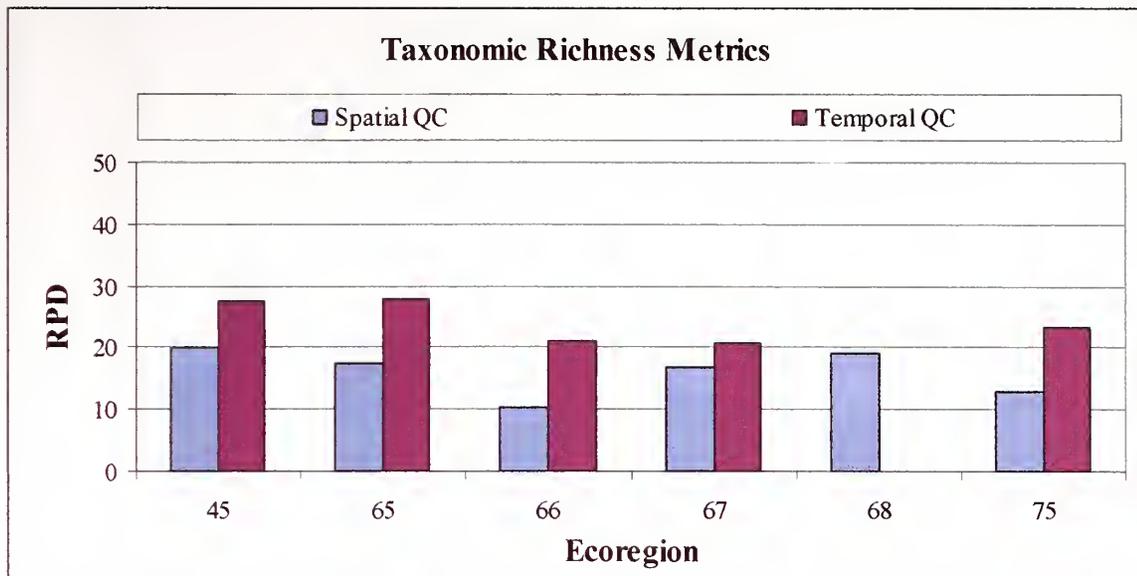
**Figure 24.** Comparison of Relative percent Difference (RPD) values averaged for all raw metric values by stream class designation per metric category for primary Ecoregion 75.

### Comparison of Relative Percent Difference for Spatial and Temporal QC Samples

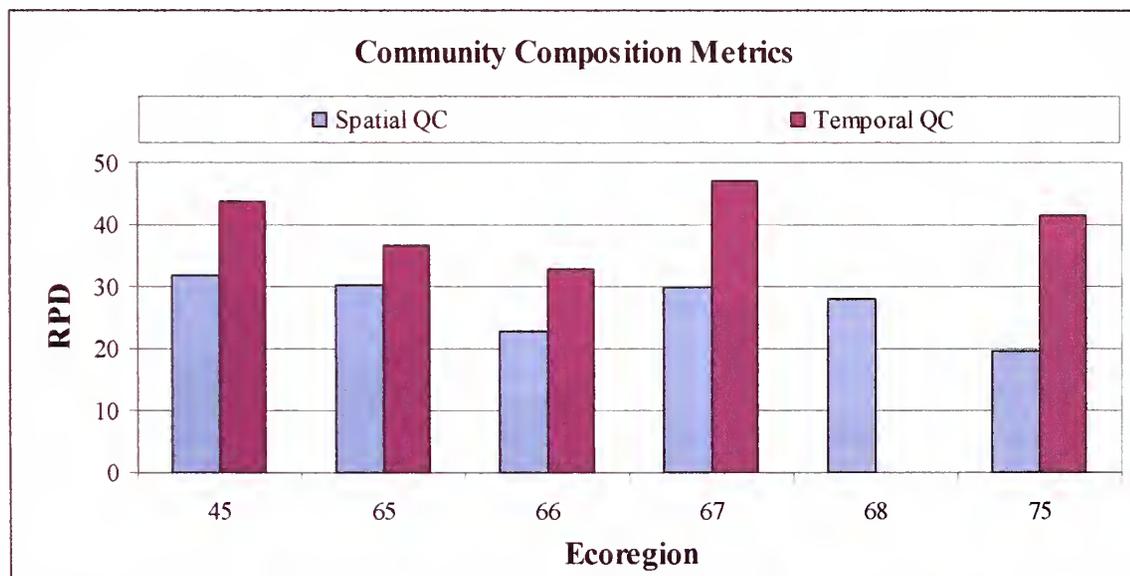
Similar to the analysis of average RPD of raw metric values for all metrics within each metric category and per stream class designation, differences in RPDs between spatial and temporal QC samples were also considered. RPD values are summarized in Table 67 and are also illustrated in corresponding Figures 25 to 35. Again, these values are illustrated in two manners: (1) each metric category is compared individually between ecoregional designations, and (2) each metric category is compared to each other per primary ecoregion designation. These illustrations demonstrate variability for each metric category in relation to other metric categories, as well as the variability of each metric category within each ecoregional designation. Metric specific calculations of RPDs for each stream QC designation at the ecoregional and subecoregional level are included on the CD-Rom in the pocket materials of this research paper.

**Table 67.** Average Relative Percent Difference (RPD) of Quality Control (QC) sites per QC type and per primary ecoregion. Values are averaged for all raw metric values within the metric group category. ("na" denotes no QC sample was collected for the QC type designation.)

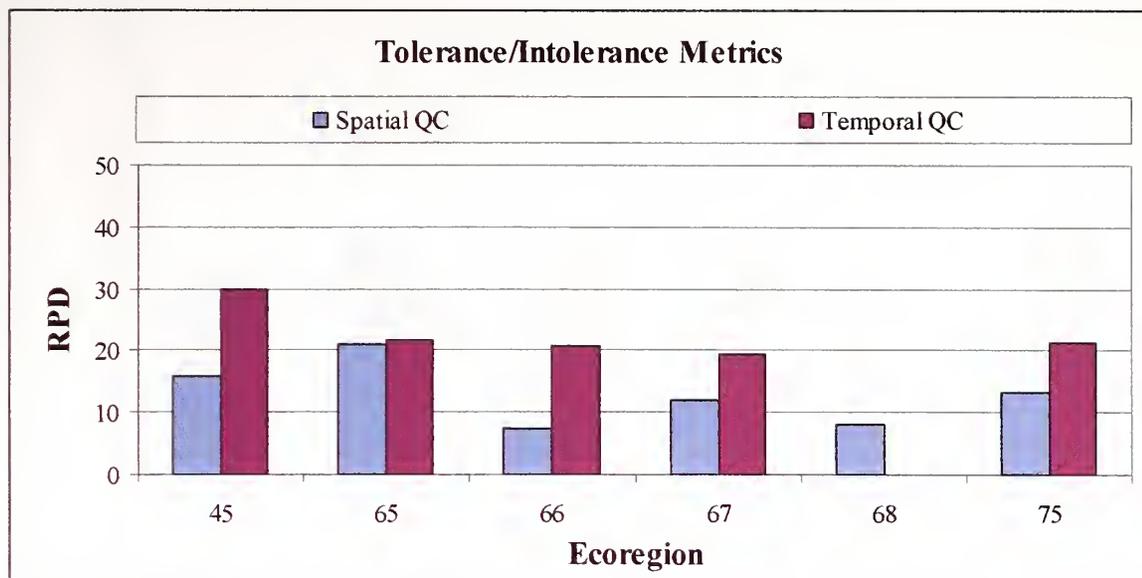
Metric Groups	QC Type	45	65	66	67	68	75
Taxonomic Richness	Spatial	20.1	17.7	10.3	16.9	19.2	13.0
	Temporal	27.4	27.9	21.1	20.6	na	23.5
Community Composition	Spatial	32.1	30.5	22.9	30.0	27.9	19.8
	Temporal	44.0	36.7	33.0	47.1	na	41.5
Tolerance/Intolerance	Spatial	15.9	21.2	7.4	11.9	8.0	13.5
	Temporal	29.9	21.6	20.6	19.4	na	21.3
Functional Feeding Group	Spatial	22.6	22.9	15.8	13.0	13.9	35.3
	Temporal	33.7	26.9	19.5	31.4	na	40.4
Life Habit	Spatial	32.1	36.4	27.2	19.5	32.1	20.2
	Temporal	32.7	30.8	35.1	34.5	na	39.7



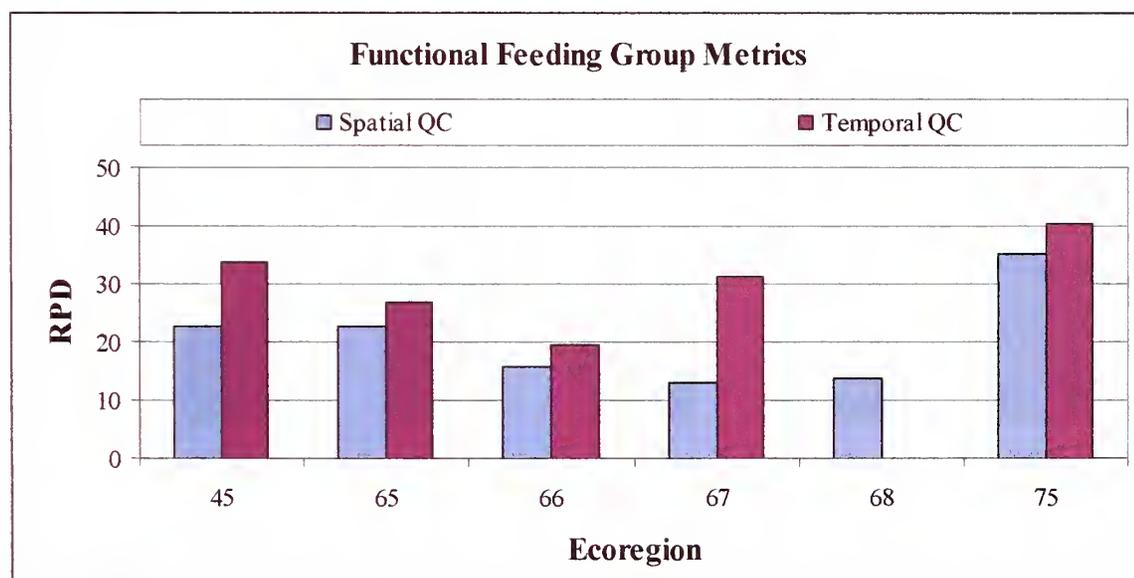
**Figure 25.** Comparison of Relative Percent Difference (RPD) values averaged for all raw metric values of the taxonomic richness metrics per ecoregion designation and by Quality Control (QC) sample designation. (No Temporal QC samples were collected for Ecoregion 68.)



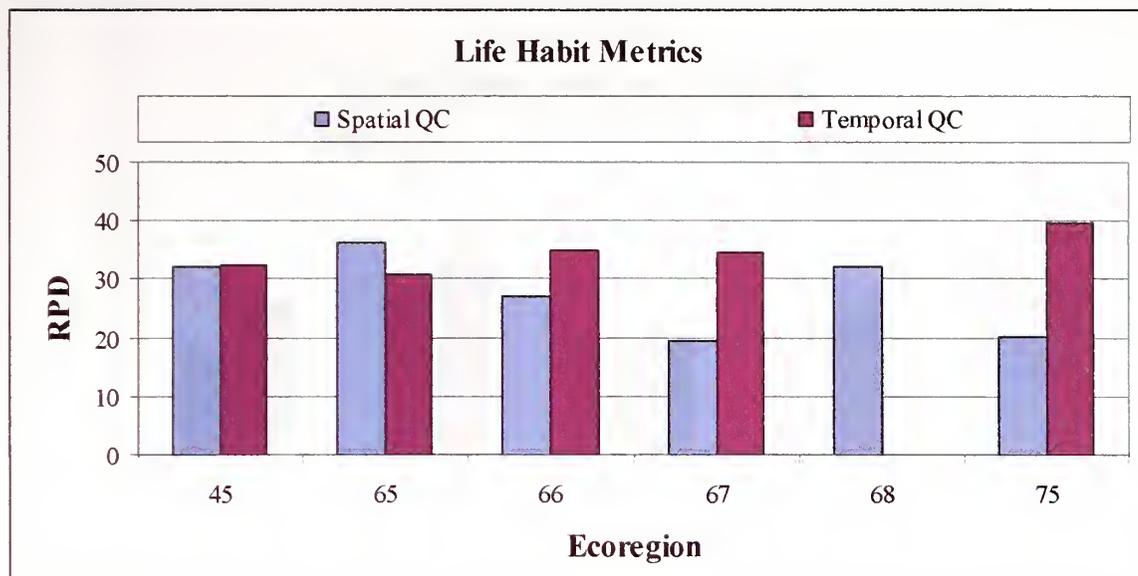
**Figure 26.** Comparison of Relative Percent Difference (RPD) values averaged for all raw metric values of the community composition metrics per ecoregion designation and by Quality Control (QC) sample designation. (No Temporal QC samples were collected for Ecoregion 68.)



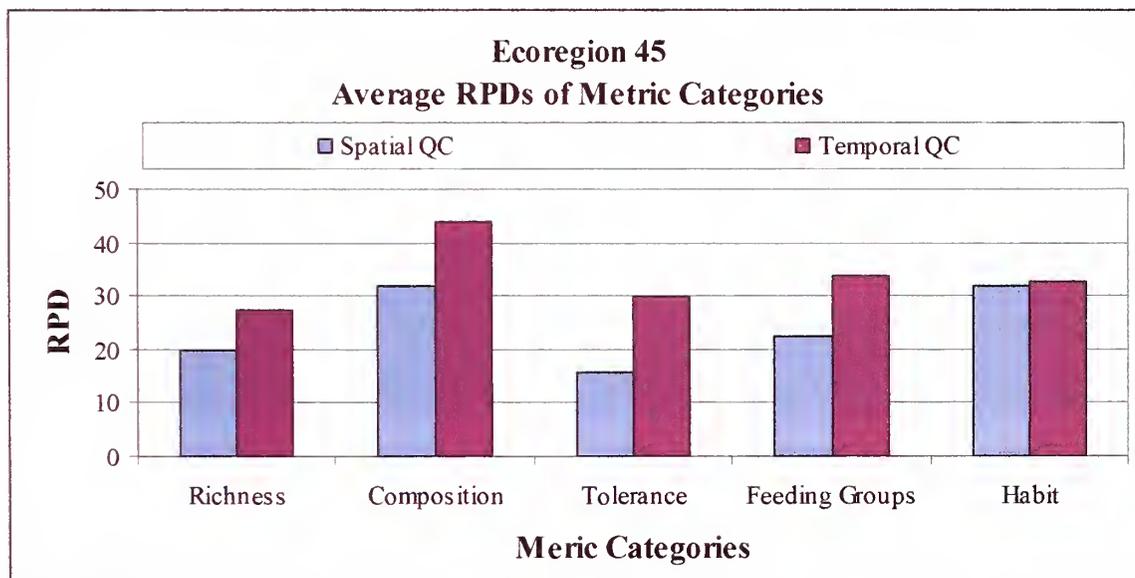
**Figure 27.** Comparison of Relative Percent Difference (RPD) values averaged for all raw metric values of the tolerant/intolerant individuals metrics per ecoregion designation and by Quality Control (QC) sample designation. (No Temporal QC samples were collected for Ecoregion 68.)



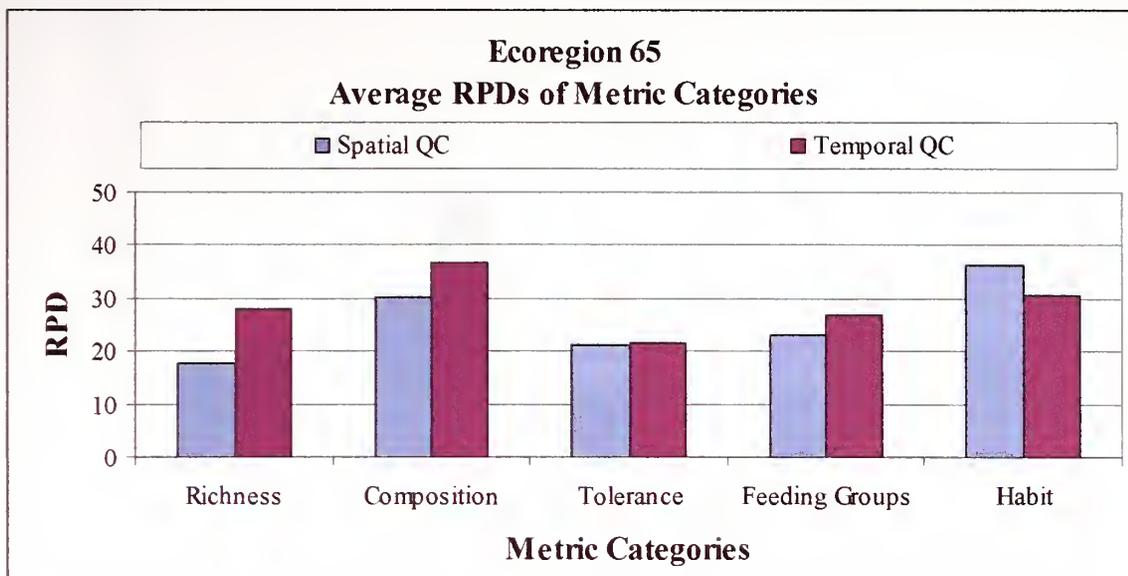
**Figure 28.** Comparison of Relative Percent Difference (RPD) values averaged for all raw metric values of the functional feeding group metrics per ecoregion designation and by Quality Control (QC) sample designation. (No Temporal QC samples were collected for Ecoregion 68.)



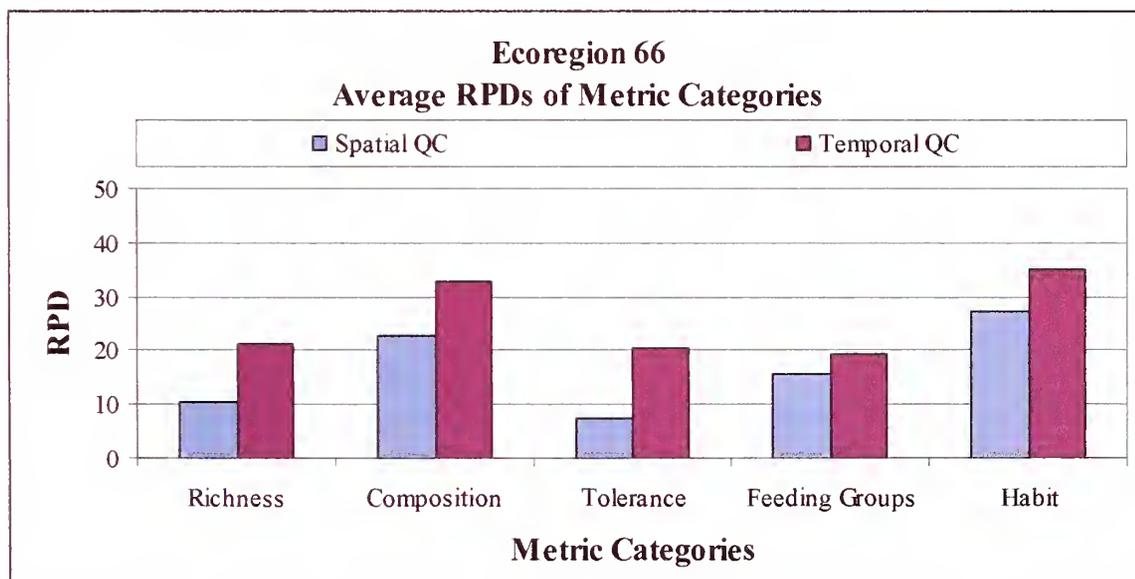
**Figure 29.** Comparison of Relative Percent Difference (RPD) values averaged for all raw metric values of the life habit metrics per ecoregion designation and by Quality Control (QC) sample designation. (No Temporal QC samples were collected for Ecoregion 68.)



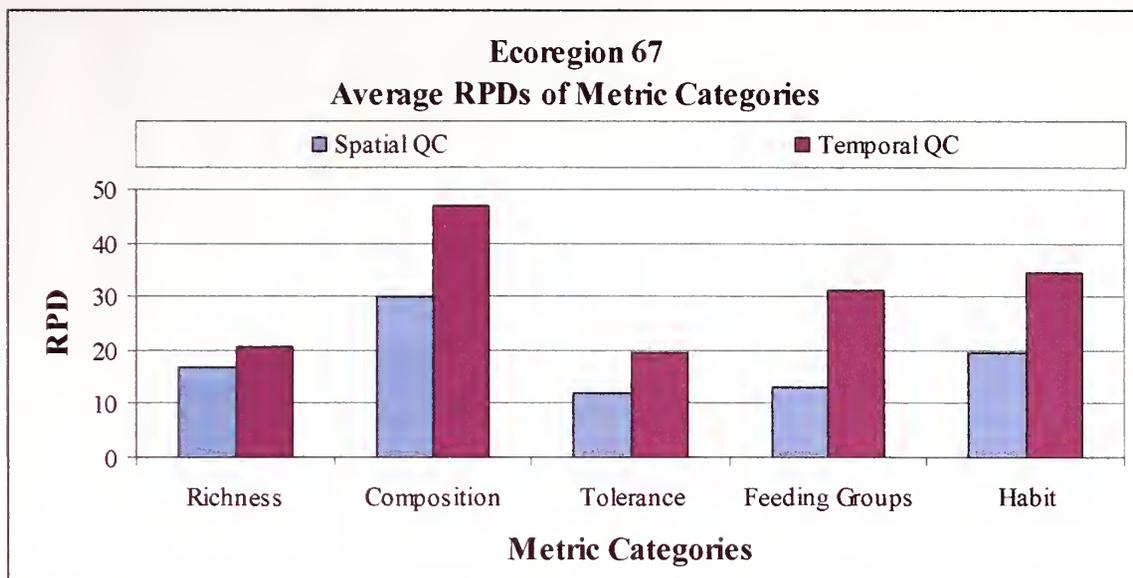
**Figure 30.** Comparison of Relative percent Difference (RPD) values averaged for all raw metric values by Quality Control (QC) sample designation per metric category for primary Ecoregion 45.



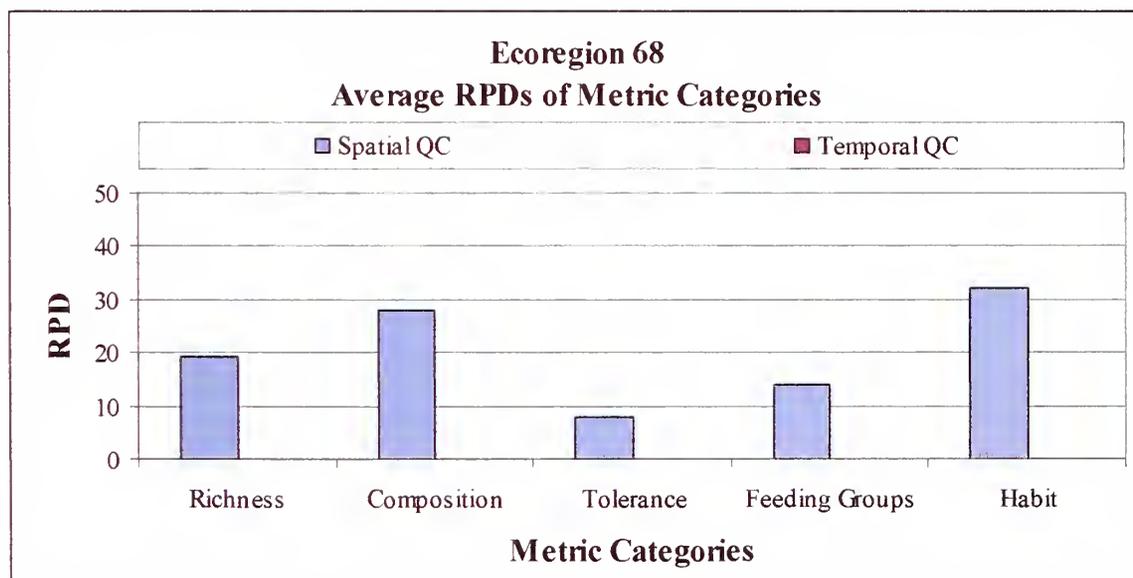
**Figure 31.** Comparison of Relative percent Difference (RPD) values averaged for all raw metric values by Quality Control (QC) sample designation per metric category for primary Ecoregion 65.



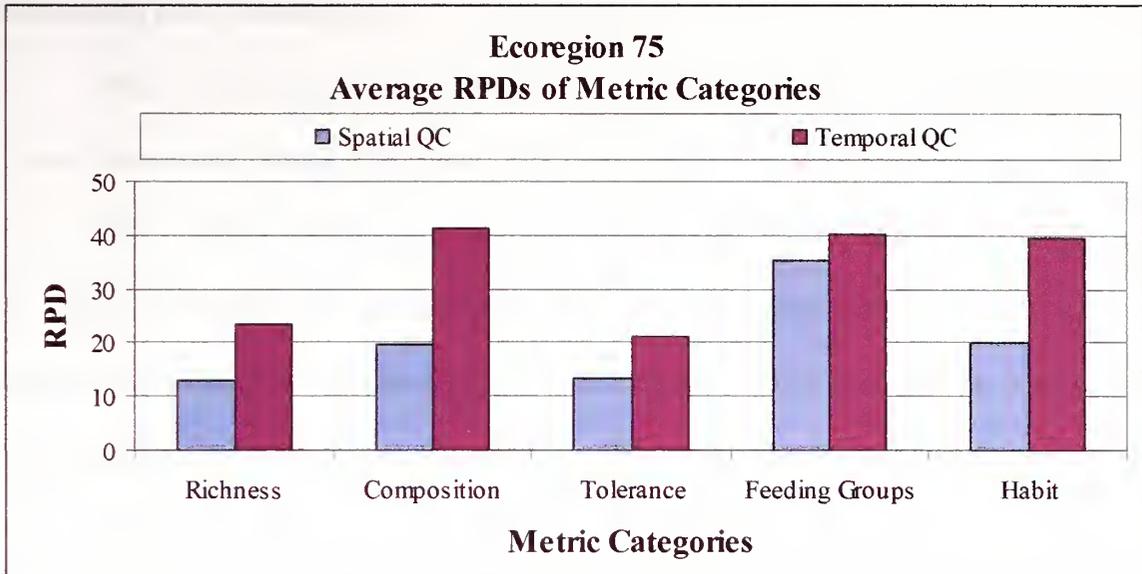
**Figure 32.** Comparison of Relative percent Difference (RPD) values averaged for all raw metric values by Quality Control (QC) sample designation per metric category for primary Ecoregion 66.



**Figure 33.** Comparison of Relative percent Difference (RPD) values averaged for all raw metric values by Quality Control (QC) sample designation per metric category for primary Ecoregion 67.



**Figure 34.** Comparison of Relative percent Difference (RPD) values averaged for all raw metric values by Quality Control (QC) sample designation per metric category for primary Ecoregion 68. (No Temporal QC samples were collected for Ecoregion 68.)



**Figure 35.** Comparison of Relative percent Difference (RPD) values averaged for all raw metric values by Quality Control (QC) sample designation per metric category for primary Ecoregion 67.

## Discussion and Conclusions

Rapid bioassessment is essentially a “biological shortcut” in comparison with impact assessment studies of the past, where the goal is to sample a wide range of aquatic biota with the fastest methodology (Metzeling and Miller 2001). The underlying premise of rapid bioassessment is the “minimal” effort needed to characterize macroinvertebrate communities that result in “maximum” information. At this point in the evolution of bioassessment protocols, minimal effort is reflected by the limited number of sample replicates and a limitation on the number of collected organisms to be used in metric calculations (Metzeling and Miller 2001). Although the stream conditions determined by biological assessments are relayed to the general public and water resource managers as narrative descriptions, (*i.e.* reference vs. impaired lotic systems, or rankings of “good/fair/poor”), the final determination of the biological condition is the result of quantitative, numerical indicators with decision thresholds.

Measurement errors in an ecoregional study, due to its complexity and high level of effort, can be compounded from out-dated land use data, as well as errors in field sampling, laboratory subsampling, taxonomic identification and enumeration, data entry, and final metric calculations. The accumulation of errors from these multiple sources results in uncertainty and overall variability (Diamond *et al.* 1996; Clark and Whitfield 1994). Calculations of variance within the biological parameters measured are necessary for identifying the effects of measurement errors and/or inherent differences between sampling sites in relation to the overall variance of a metric or index on an ecoregional and sub-ecoregional level (Karr and Chu 1997).

At first glance it is apparent that the majority of the average RPD values for the metric categories considered in this study, (for both raw metric scores and standardized metric scores), are above the precision thresholds of the measurement quality objectives dictated by the QAPP document {see Tables 4 (p. 27), 5 (p.29), 6 (p.30) and Figs. 2 and 3}. The raw metric values for taxonomic richness and tolerance/intolerance metric categories appear to have better precision overall compared to community composition and life habit measures {Fig. 2 (p. 30)}. For each ecoregion, the average RPD values for the taxonomic richness and tolerance/intolerance metric categories fall close to the QAPP prescribed MQO of 20 percent, while community composition and life habit measures are consistently above the 20 percent precision threshold.

After standardization of the raw metric values, the average RPDs of the metric scores, again, are above the precision thresholds established by the QAPP document {Fig. 3 (p. 31)}. While the average RPDs of the raw metric values for some metric categories were close to the prescribed precision threshold of twenty percent, the average RPDs for standardized metric scores for all of the metric categories are considerably higher than the precision criterion of five percent.

There appears to be some consistency among the averages of the metric RPDs from one ecoregion to another, as well as between the metric categories {Table 5 (p. 29)}. With the exception of RPD averages for metrics associated with measures of tolerance/intolerance and taxonomic richness which hovered at the 20 percent precision threshold, the remaining RPD averages were typically much greater than the prescribed MQO. This is better illustrated in Figure 2, where it is evident that metrics falling into

the richness and tolerance/intolerance categories generally have less variability and more precision than the other metric categories.

As stated before, RMSE levels were to be established as a result of this study. Ultimately, the ranges of RMSE considered to be acceptable for a bioassessment program will depend on the objectives of the water resource manager and the best professional judgment of the data analyst. The overall measures of error associated with biological data determine some level of data quality. Interpretations of data quality are important for the data user and decision makers to evaluate the degree of the reliance on technical and scientific information (Costanza *et al.* 1992). As RMSE values are estimates of the standard deviation, which is also considered as a measure of precision. The assumption is that the larger the RMSE value, the less precision, and/or greater variability within the measures.

Figure 4 ( p. 32) illustrated an interesting pattern of average RMSE values for the raw metric values associated with each metric category in each primary ecoregion. Each metric category seemed to exhibit a “proportional” trend in variability when compared to one another, with the greatest ranges of variability still associated with the community composition measures. After metric value standardization, the range in variability for all metric categories increased significantly when compared to the RMSE values for the raw metric values {see Figure 5 (p. 33)}. Additionally, the variability between each metric category generally became relatively uniform within each primary ecoregion.

For the precision measure of CV, when compared to the RMSE values, an opposite trend in variability ranges occurred between raw metric values and standardized

metric scores {see Figs. 6 (p. 34) and 7 (p. 35)}. This was most evident when comparing variability ranges of the community composition measures with CVs for the raw metric values which were consistently almost twice the value of CVs for the standardized metric scores for that metric category. CV values ranges for all other metric categories did decrease between raw metric values and standardized scores, but not as dramatically. Again, as with most other precision measures presented, community composition measures were still consistently much higher in their range of variability compared to the other metric categories.

Table 11 (p. 36) presented a generalized summary of the precision measures associated with the final bioassessment scores associated with each primary ecoregion. Upon comparing the range of variability of RPDs, RMSEs, and CVs, it should be noted that these average values were inclusive only of the standardized metrics that were included in the final biotic index. Consequently, these average precision measures were based on a much smaller group of metrics, (5 to 8), than the other values presented which were averaged for the entire suite of metrics within each metric category. Additionally, since the metrics chosen to represent the biotic index for an ecoregion was based on their standardized values, primarily those metric that provided the highest additive score, and exhibiting the best stress responses, were considered for the index. Although the variability between the metrics considered to quantify the final bioassessment score was most likely minimal, resulting in smaller ranges of variability, the MQO criterion of 5 percent for RPDs between bioassessment scores was still not met.

When considering most of the raw count metrics, (*i.e.* number of taxa per order, functional feeding groups, and habit), the values of RPDs are probably not as important as the evaluations of variance among the final bioassessment scores. Comparing the RPD values of the raw metric values and standardized metric scores {Tables 5 and 6 (pp. 29 and 30) with corresponding Figures 2 and 3}, to the RPD values for the final bioassessment scores {Table 11 and Figure 8 (p. 36)}, it is apparent that the range of overall variability decreased among the measures. Although the RPD values for the final bioassessment scores, are still higher than the precision thresholds dictated by the QAPP for bioassessment scores, the values, overall, are much closer to the prescribed five percent threshold, (as compared to the RPD values for raw metric values and standardized metric scores). As for the other precision measures of RMSE and CV, the range of values associated with the final bioassessment scores are also much narrower than the RMSEs and CVs calculated for raw metric values and standardized scores.

This initial examination of the analysis of data precision leads to two questions: (1) assuming that SOP protocols for field sampling of invertebrates were followed with minimal error, what are the factors that could possibly influence the range of variability between an established sample site and its QC sample?; and, (2) if we assume that the samples collected to determine the biological condition are a valid or characteristic representation of the ecosystem, then how should the data be interpreted in relationship to the predetermined threshold values for precision and data quality?

Considering the sampling methods implemented for bioassessment programs, there are many factors to consider after examining the RPD values for both raw metric

values and standardized metric scores. The first factor to consider is the methodology for sampling the invertebrate community. As mentioned before, some bioassessment studies have centered upon specific habitat types (*i.e.* riffles and/or runs) to determine the biological integrity of a lotic system. With the invertebrate sampling protocols used for this study, a multiple habitat sampling approach, while being more inclusive of the assortment of macroinvertebrates in freshwater systems, can also lend itself to greater variability because of the mixture of habitats sampled.

The “twenty-jab” method prescribed by the RBP protocol and the Georgia DNR (CSU 2000) was designed to sample of a variety of habitats with a relatively equal levels of effort in proportion to those habitats that typically occur in high- or low-gradient lotic systems {*see* Table 2 (p. 15)}. In instances where the designated one- hundred-meter sampling reach did not provide the required distribution of effort among the different habitat types, the level of effort was “reallocated” and distributed evenly among those habitats that were more dominant in the sampling reach. This may be more of a factor with spatial (200 meter) QC samples, if the variety of habitats designated for sampling did not occur equally from one sampling reach to the next. Therefore, some raw metric values may be distorted if “jabs” assigned to typically more productive habitats (*i.e.* riffles and snags) are replaced by “jabs” of less productive habitats (*i.e.* sand), and vice-versa. These changes in the distribution of effort among habitat types can cause large variations in the invertebrate assemblages collected that can ultimately affect the range of metric values between sites and within all of the metric categories. In turn, this can be one factor

possibly responsible for higher and/or lower values RMSEs and CVs than may be expected, as well as RPDs above the precision thresholds predetermined for this project.

Another factor in the inclusion and/or exclusion of certain taxa from the composite samples collected is the use of random subsampling. Caton (1991) developed a gridded screen technique to increase objectivity in the laboratory subsampling of benthic macroinvertebrates. For biomonitoring programs, subsampling has been recommended as a valid and cost-effective procedure where time and monetary resources are limited. The rationale behind the use of subsampling is twofold, where the level of effort expended on each sample collected is relatively equal and representative estimates of the invertebrate population sampled are selected or picked.

In some instances, it is possible for this sampling methodology to skew the average values of RPDs and RMSEs. In cases where there may be taxa that are rare, the occurrence of just one organism picked from either a primary or QC sample would cause an RPD value of "100" percent to be assigned to that metric if there were no occurrence of that same taxon in the corresponding QC or primary sample from the same stream. This could be misleading when considering precision thresholds between samples, as the samples are most likely more similar than the RPD value may indicate. In these instances it may be necessary to consider the raw taxonomic data and their effect on the final value of the metric before assuming that the quality of the data is substandard.

Also, it is important to consider the characteristics of the metrics themselves and to what ecoregion and/or subcoregion they are being applied. For instance, the varying geomorphology across the state is directly responsible for the variability of habitats and

water chemistry in the lotic systems being analyzed. This, in turn, will dictate the presence and/or absence of certain taxonomic groups and individual organisms based upon habitat requirements. Systems that are more dominated by high gradient, headwater streams (*e.g.* ecoregions 66 and 67) with allochthonous inputs will tend to have higher percentages of shredders and scrapers (*e.g.*, Plecoptera and Coleoptera), while systems with low gradient streams (*e.g.* ecoregions 65 and 75) will tend to have higher numbers of filterers and collectors (*e.g.* Trichoptera) (Vannote *et al.* 1980). Therefore, some metric calculations and corresponding determinations of the average RPD, RMSE, and CV values for an ecoregion and/or subcoregion may not be truly indicative of the range of variability and/or quality of the data collected. Again, the data analyst may have to examine the individual sites within each subcoregion to assess the validity of the metric values for the region that was sampled. Fortunately, those candidate metrics that may not be significant or indicative of the invertebrate assemblages in an ecoregion and/or subcoregion are filtered out through DE calculations and box-and-whisker plot examination during the development of the biotic index.

In the ecoregional appendices, subcoregional and site-specific calculations of RPDs for raw metric values and standardized metric scores are provided for each subcoregion. The initial interpretation of RPDs for these sites must be considered cautiously. In instances where the RPD between two samples was calculated to be “zero”, there are two scenarios to consider: an RPD value of “zero” is the result of the raw numbers of the metric for the established sampling site and its QC to be either (1) equal in value, or (2) for there to be no occurrence of the organisms that define the value

of the metric in either sample. When examining the MQOs established for this project, a value of “zero” would appear to indicate that the original sample was a representative sample and/or there was minimal, or no error in performing the sampling (*i.e.* high data precision). However, the metrics themselves must be considered for their ecological significance to the target ecoregion. Since they are rare in that ecoregion, an RPD of “zero” for the number of Plecoptera in samples collected in the coastal plains (ecoregion75) is not as significant as RPD values for the abundant non-insect and oligochaete taxa. The inclusion of certain metrics with minimal biological importance to an ecoregion or subecoregion can skew the overall average RPD values for those regions, as well as affect the ranges of RMSE and CV values.

For RPD values that were calculated to be “100”, indicating absolute difference between QC and primary samples, some scrutiny is deserved, as well. An RPD value of 100 is essentially the result of a “presence/absence” scenario, where one sample may have as little as one individual, but the corresponding sample will have no occurrence of the same organism. Again, this value could be misinterpreted, as the presence of possibly “rare” individuals from one sample compared to a corresponding QC and/or primary sample that may not have the same organism would not be as significant as the presence of fifty individuals where the corresponding sample may have none. The data analyst may need to examine RPD values for individual sites to determine if the presence or absence of certain organisms is significant in relation to the ecoregion being considered.

After standardization of raw metric values to produce metric scores, RPDs and RMSEs are, again, calculated to determine precision estimates of the collected data.

Similarly, these values must also be examined with some scrutiny as the metrics are now ranked on a similar scale, (*i.e.* values are expressed on a 0 to 100 scale), where some calculated values may be negative and others may be above the upper limit values of “100”. Those calculated metric scores which fell into either one of these categories were changed to values of “zero” or “100” respectively.

As outlined before, there are many steps in determining the appropriate suite of metrics that should be used to characterize and monitor the biological condition of an ecoregion. The metrics chosen are not only indicative of the aquatic assemblages of a certain region, but are also the most sensitive to anthropogenic stresses in the ecosystem. Tables 11 through 22, (*starting on p. 36*), display the metrics that comprised the primary ecoregion biotic indices and their related precision measures for each ecoregional designation, as well as their DE values. The RPD, RMSE, and CV values presented are based upon the standardized metric scores for those metrics included in the index.

With the exception of a few metrics, the majority of the RPD values for the standardized metric scores of the metrics comprising each index are relatively high. Considering the precision thresholds dictated by the QAPP, the RPD values for standardized metric scores should ideally be less than or equal to five percent, but in relation to the overall trends that RPDs have exhibited for raw metric values and standardized scores, these results are consistent. Conversely, there appears to be no definitive correlation between the range and/or variability of the precision measures and the DE values associated with each metric chosen. All DEs for the metrics that formulated the final indices for the primary ecoregions did meet the minimal criteria of

fifty percent, but higher DE values did not correspond with lower variability in the RPD, RMSE, or CV values for those metrics.

It has been noted in previous Georgia Ecoregion Project reports Gore *et al.* (2004 and 2005) that DE values at the subecoregional level improved, on average, when compared to the DE values at the primary ecoregional level. This indicated that the metrics used to characterize the biological condition at the subecoregional level were more indicative of the differences between the reference and impaired condition at a smaller scale. Although DE values at the subecoregional level improved, there was no corresponding trend in the improvement (*i.e.* reduction in the range of variability) of the precision measures of RPD, RMSE, and/or CV for those metrics that constitute the biotic index.

When considering the ranges of variability between the metric categories and ecoregions and examining the average RPD, RMSE, and CV values for the metric categories, it must be noted that the number of QC sites among the ecoregions and subecoregions were not distributed evenly. This was primarily due to the fact that QC sites and those sites classified as reference or impaired (both spatially and temporally) were randomly selected. Therefore, final averages of the precision measurements for raw metric values and standardized metric scores may not have been weighted evenly. One example of this occurs for ecoregion 68, which is comprised of only one subecoregion and had only one QC sampled for the impaired stream class. Upon examining the overall trends between the ecoregions, and for the majority of the precision measures applied, ecoregion 68 consistently exhibited a lower overall average RPD, RMSE, and CV when

compared to other ecoregions. In instances where there was a minimal number of QC samples per ecoregion or subecoregion, the RPD, RMSE, and CV values associated with data precision and variability should be considered with caution, as the number of replicated samples may not be sufficient to illustrate ranges of variability within a certain ecoregion or subecoregion.

Similarly, the number of QC sites designated as reference/impaired and spatial/temporal was not distributed evenly among the stream class nor the QC sample type. Although not required by the QAPP for analysis, additional precision measures were considered to examine possible influences of variability between reference and impaired sites, as well as spatial and temporal variability by metric category across each primary ecoregion. The values presented in Table 66 (p. 60) consisted of overall averages of all metrics within each metric group per stream class and primary ecoregional designation. Upon examination of Figures 14 to 24, (*starting on p. 60*), there does not appear to be any discernable overall pattern between metric category variability within each ecoregion (*i.e.* there is no consistency as to the range of variability between reference QCs when compared to impaired QCs).

There are only a few exceptions to this, more specifically repeatable differences between stream classes in relation to metric categories and ecoregional designation. One pattern that emerged was the difference in reference and impaired RPDs for the metric categories of taxonomic richness, functional feeding groups, and life habit from ecoregion 75. These metric groups within ecoregion 75 have the widest range of variability between the two stream classes, with the reference RPD values consistently

being lower. Additionally, the range of variability for reference sites associated with ecoregion 67 are, for all metric categories, is greater than the range for the impaired sites. In some respects, this particular pattern cannot be considered significant as the average impaired values was derived from only one sample.

Comparisons of RPD values for stream QC types were also considered at the primary ecoregional level by metric categories. It was apparent from the series of illustrations {Figures 25 to 35 (*starting on p. 67*)} derived from Table 67 (p. 66), that there was consistency between the RPD values for spatial and temporal QC samples at both the ecoregional and metric category designation. With minimal exceptions, temporal QC sites had higher ranges of variability when compared to spatial QC sites. In general, this might be an expected conclusion if the sites that were originally chosen for sampling were indeed indicative of a “typical” reach of the catchment being analyzed. Also, considering that spatial QC samples were essentially collected at the same time, overall variability should be minimized.

Looking at the average RPDs for the temporal QC samples, it was evident that at both the ecoregional level and by metric category designation that variability is much greater. Similarly, this might also be an expected result as there are many factors that can influence biological communities over time (*i.e.* rainfall patterns, temperature, *etc.*). In trying to minimize the effects of temporal influences, a sampling “index period”, as mentioned before, was utilized for all samples collected in this project. For sites that were designated for temporal QC sampling, field crews attempted to sample the phase QC stream at approximately the same time of year as previously done.

Unfortunately, a precise determination of temporal effects on variability may not be identifiable from one sampling period to the next. One factor to consider is the number of “degree-days” from year to year that cue the life stages of freshwater macroinvertebrates. Depending on daily temperature patterns between years, a sample collected one year may have third or fourth instar nymphs which would, typically, be easier to identify to a lower taxonomic level (*i.e.* genus and/or species versus family and/or order). Other corresponding temporal QC samples may not have had the same number of degree-days before sampling that could have resulted in earlier instar nymphs that may not be identifiable to the same taxonomic resolution as a previous sample. Ultimately, this will affect values of metrics that require lower taxonomic resolution to be quantified.

More importantly, another temporal factor to consider is changes in land use patterns across the state of Georgia. For some ecoregions, (*for example* ecoregion 45), that have highly urbanized areas, (*i.e.* Atlanta), land use can change on a weekly basis. Considering that most land use data used to identify reference and impaired catchments are not updated on yearly, the variability of the biological community from one year to the next could be extreme. This can also be exemplified in areas that have no urban influence at all, more specifically, areas with large amounts of acreage devoted to silviculture. Although less extreme in the rates of changes as compared to urbanized areas, from one year to the next catchments may be either mature stands or clearcut. Even with the required buffer strips emplaced to protect streams in these areas, there still

exists the possibility of wide variances in the biological community from one sampling event to the next.

Apart from the nuances of the statistical analysis of this data, there are many biological factors that must also be considered when interpreting data precision and variability. As mentioned previously, some consideration must be given to individual metrics and their applicability to the ecoregion in question. In some instances, the initial analysis of raw metric values may have not provided DE values at fifty percent or greater, which indicated the lack of differentiation between the biological character of a reference and impaired stream. One such region that exemplified this was ecoregion 75 (Coastal Plains). The metrics that comprise the biotic index for ecoregion 75 {Table 21 (p. 40)} do not encompass measures from each metric category (*i.e.* taxonomic richness, functional feeding groups, *etc.*), but are limited to the measures of community composition and tolerant individuals. This is a direct reflection of the invertebrate assemblages that are most characteristic of the ecosystem in that region and are dictated, in part, by habitat features, (*i.e.* predominance of sand and silt substrates, presence of woody debris, *etc.*). The majority of the organisms in the coastal plain ecoregion is non-insect (*e.g.* amphipoda, isopoda, gastropoda, oligochaeta, *etc.*), being poorly described or quantified by traditional richness metrics (Gore *et al.* 2004).

Additionally, there were some metrics associated with the “Life Habit” category that were not considered in development of the biotic indices for all the ecoregions. Specifically, the metrics for the life habit category that were excluded for use in the biotic indices included percentages of burrowers, climbers, sprawlers, and swimmers in the lotic

community. Although the EDAS database did provide calculations for these metrics in question, there exists no definitive scientific literature to support what type of stress response would be demonstrated by the organisms included in those groups, but rather their responses are inferred from other “lifestyle” characteristics (Barbour *et al.* 1999, Fore *et al.* 1996, and DeShon 1995). For example, those benthic macroinvertebrates whose characteristic life habit are classified as sprawlers, burrowers, etc., are also categorized by other attributes such as a taxonomic order (*i.e.* Ephemeroptera, Plecoptera, *etc.*), and/or a feeding mechanisms (*i.e.* predators, shredders, collectors, *etc.*). Therefore, stress responses that have been established at an order, tolerance, and/or feeding level have been correlated to a similar stress response for the organism in accord with its life habit characteristics.

In conjunction with the physical character of a habitat, the chemical character of the lotic ecosystem must be considered as a possible source of variability in macroinvertebrate communities. Many ecoregions and subecoregions contained both “clearwater” and “blackwater” streams within. In contrast to clearwater streams, blackwater stream systems are typified by high tannin inputs (from terrestrial organic material), more acidic pH levels, and lower concentrations of dissolved oxygen. As a result, the benthic communities that dominate these systems can be significantly different from clearwater streams. The physical habitat of blackwater streams is characterized by sandy substrates and fine particulate organic matter, which serves as an ideal environment for oligochaetes, dipteran taxa, and mollusks, whereas clearwater streams in the same region would be dominated more by Trichoptera taxa and acid-intolerant

Chironomid taxa (Meyer 1990). There was no separation of designated blackwater and clearwater streams in the analysis and it has been suggested that clearwater and blackwater streams may need to be categorized separately when developing biotic indices for a specific region, as these distinctive invertebrate communities may respond to anthropogenic stresses very differently (Gore *et al.* 2004).

Similarly, there has been discussion of establishing different suites of metrics for streams in ecoregion 75 that empty into the Atlantic Ocean. In the initial selection process for selecting reference streams, there was no determination of the influences of tidal effects within coastal catchments. It became evident, after taxonomic identifications, that some samples contained invertebrate communities indicative of brackish water influences. Although these sites were not initially considered to be affected by estuarine influxes, the presence of salt tolerant, marine species, such as polychaetes and crabs, presented some problems in defining the biotic indices for streams influenced by tidal cycles. The primary problem is that the EDAS program does not account for metrics of marine species, which creates difficulty in developing biotic indices that are truly characteristic of the integrity of brackish water systems. To remedy this issue, Gore *et al.* (2004) suggested identification of reference condition for both so-called "inland" streams and "tidal-coastal" streams when there is an occurrence of both systems in an ecoregion or subecoregion.

One major environmental factor that affected the majority of the southeastern portion of the state (*i.e.*, below the "fall line"), was the occurrence of a sustained drought over most of the project's sampling phases from 1999 to 2003. Many perennial streams

in this region of the state were estimated to be dry for two years or more. This situation created two problems: (1) finding enough designated reference sites to satisfy the project requirements for number of sites to characterize the biological condition, and (2) when designated reference sites did have water, in most cases, there was no initial indication that the sample collected would be representative of an “unstressed,” typical biological community. Although Gore and Milner (1990) have demonstrated that disturbed lotic systems can be recolonized by macroinvertebrates in as little as fourteen to twenty-one days, there was no sure way for field teams to verify that normal stream functions and invertebrate communities had returned to their typical character. In instances where lotic systems have had some form of sustained stress, additional sampling should be performed so that the reference and/or impaired conditions can be adequately defined.

With all of the possible influences of the biological variables discussed, ecological responses to varying levels and types of stressors can be complex and difficult to accurately measure with a high degree of reliability (Murtaugh 1996). In some cases, the use of benthic macroinvertebrates for bioassessment may not provide a clear response to anthropogenic influences. Floods and droughts inevitably will affect aquatic ecosystems over the course of time. Where there are instances of “pulse” events in an ecosystem, the induced stress may not be significant enough to permanently alter the composition of the aquatic community, especially invertebrates with their ability to recolonize (Gore and Milner 1990), fecundity, and dispersal ability (Patrick 1975). Pulse events can be either naturally occurring (*i.e.* droughts and floods), or human induced (*i.e.* industrial discharges).

In bioassessment programs, it is important to identify reference and impaired sites that encompass natural variability within and between watersheds, as well as the variability of the influences of possible anthropogenic influences. This is crucial to the subsequent calculated metrics and biotic indices that water resource managers will utilize in their decision-making process. Evaluation of stream ecosystem health can be hindered by the cost and time constraints posed by large scale quantitative biomonitoring sampling protocols (Rosenberg and Resh 1993). The goal of quality control protocols is to measure the quality of a procedure so that it meets the needs of the user, while aiming to produce data that is dependable, adequate, and economical (USEPA 1995).

The concept of Measurements Quality Objectives (MQOs), (also referred to as Data Quality Objectives), in bioassessment programs is a useful tool in evaluating the consistency of data and limiting variability and potential sources of measurement error (Diamond *et al.* 1996). When comparing two samples to determine a level of precision, acceptable differences are typically predetermined by MQOs. These requirements for data quality should ideally be based on prior knowledge of sampling procedures and measurement variables that are specific to the region and/or ecosystem being studied (USEPA 1989). Since there were no initial, widespread biological characterizations of the lotic invertebrate communities in the state of Georgia prior to this ecoregional study, the precision values of the MQOs stated for this project {refer to Table 4 (p. 27)} may have been unrealistic and unattainable for the ranges of the natural biological condition.

Considering the precision thresholds established for this study, there may be some questions and/or concerns over the validity, repeatability, and quality of the data used to

determine the biotic indices for the ecoregions of Georgia. It is obvious that the majority of RPDs of the measurements parameters of metric values and standardized metric scores are above an established threshold which is presumed to be indicative of some level of acceptable data quality. It is also evident that values for RMSE and CV are highly variable. The initial interpretation of these results may lead water resource managers to believe that the data are not very precise. In reality, these precision thresholds may have to be reevaluated and reestablished by additional sampling.

Because lotic invertebrate communities can vary significantly between geographic regions, it can be difficult, at first, to determine what should typify the reference and/or impaired biological condition of an ecoregion. In some uses of biomonitoring protocols, particularly the Index of Biotic Integrity (IBI) for fish communities, there is some expectation of what a fish community should exhibit under a reference (or least impacted) condition (Karr *et al.* 1986; USEPA 1990). It is these reference-expectations that can hinder their application to other geographic regions (Simon and Lyons, 1995). Considering the invertebrate data produced by the Georgia Ecoregions Project, the consistency of all metric categories having average RPDs above the precision thresholds for both raw metric values and standardized metric scores may demonstrate that the lotic systems across the state of Georgia naturally have high variability from year-to-year and spatially within the catchment. This in turn may indicate that the established precision thresholds may not be indicative of the data quality for this specific project.

One possible way to determine if the variability within ecoregions and subecoregions is valid, or at least more indicative of the ecosystem, may be to standardize

the number of replicate samples so that there is some equality in the level of effort expended for each subecoregion. For this study, a random subset of sites was chosen based on the total number of sites sampled throughout Georgia. As per the requirements of the QAPP, ten percent of the primary sampling sites were chosen for QC sampling. There was no equal distribution of QC samples between or within subecoregions or ecoregions. In many scientific sampling protocols, the premise behind the use of an equal level of effort is to reduce bias and to improve consistency and repeatability (USEPA 1995; Plafkin *et al.* 1989).

If the MQOs for this project, or any other bioassessment program, are not to be changed from the USEPA (1995) guidelines, then there must be some evaluation of the rapid bioassessment protocols used and how it may be altered to achieve some established criteria for data quality. In a related research project, differences in subsample sizes were analyzed to determine if the prescribed two hundred organisms to develop the biotic indices for the state of Georgia were adequate to characterize the necessary biological criteria. Rai (2005) found that in most cases, (for the ecoregions analyzed), that a subsample of three hundred organisms improved discrimination efficiencies, and better characterized between the reference and impaired condition. With that preliminary research suggesting that the RBP methods used for Georgia may need to be altered, then it may be necessary to alter the bioassessment protocol further to achieve an acceptable level of data precision as generally mandated by the USEPA.

To begin with, with all of the biological and physical factors and variables previously discussed, more QC samples may need to be collected to better illustrate

ranges of variability between raw metrics and final bioassessment scores. Especially in light of the comparisons between spatial and temporal QC samples, and in conjunction with the possible influences of a 3 year drought, temporal variability may need to be addressed in more detail. As mentioned before, blind randomization of QC samples may not fully describe the ranges of variability, especially on a temporal scale. A more systematic QC sampling protocol should be employed for both reference and impaired stream classes, as well as from year to year.

Another factor to consider in relation to the variability among the temporal QC sites is climate patterns, primarily degree days from one ecoregion to the next. From a geographic and geomorphological context, ecoregions 68, 67, and 66, (because of latitude and elevation), will typically have less degree days than ecoregion 75 with a milder climate. As mentioned before, life stages could vary according to temperatures throughout the year, which, in turn could affect the emergence patterns of some macroinvertebrates. In colder climates, one index period may be sufficient to characterize the biological community, while in warmer regions, multiple index periods may be needed to ensure the collection of multi-voltine invertebrate species.

The initial results from the four phases of this ecoregion project have been used to develop biocriteria specific for the ecoregions and sub-ecoregions across the state of Georgia. The biocriteria used to develop the biotic indices must be reviewed through additional sampling in the future. Given the variability of hydrologic cycles over time, as well as changing land use patterns as a result of urbanization or agricultural practices, biocriteria themselves will not remain static. It is important to identify spatial and

temporal variability in these aquatic systems so that biocriteria can be used wisely in water management decisions.

The specific objective of biomonitoring and bioassessment projects is to obtain the information needed to accomplish the project goals and uses. The ultimate goal is to characterize the biological condition of lotic ecosystems and determine which metrics adequately discriminate between levels of impairment, whether the impairment is minimal or severe. Biological metrics and biotic indices are used as a gauge of the biological condition, as well as being indicative of some type of response to anthropogenic stress. These measures of biological integrity are subject to change over the course of a prescribed study and/or continued monitoring. Many metrics may ultimately be revised and/or reevaluated for their effectiveness and applicability to the ecoregional character and the expectations of the water quality program in question.

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**Appendix A. Land Use Characteristics and Statistics of the Ecoregions of Georgia.**

Ecoregion / Sub-ecoregion	# of Catchments	Catchment Area (km <sup>2</sup> )			Agriculture		Barren		Urban	
		Mean	Minimum	Maximum	Maximum %	Minimum %	Maximum %	Minimum %	Maximum %	Minimum %
<b>Piedmont – Ecoregion 45</b>										
45a	91	63	18	142	47.4	1.4	9.7	0.0	52.3	0.1
45b	408	52	13	130	60.1	0.0	23.5	0.0	67.9	0.0
45c	21	37	11	105	38.1	1.6	15.5	0.1	1.3	0.0
45d	23	26	8	62	16.5	0.6	32.9	0.0	7.1	0.0
45h	16	29	8	77	21.1	0.7	10.4	0.0	8.4	0.0
<b>45</b>	<b>559</b>	<b>41</b>	<b>8</b>	<b>142</b>	<b>60.1</b>	<b>0.0</b>	<b>32.9</b>	<b>0.0</b>	<b>67.9</b>	<b>0.0</b>
<b>Southeastern Plains – Ecoregion 65</b>										
65c	92	41	11	120	62.9	0.3	32.7	0.0	57.8	0.0
65e	39	42	12	101	31.2	0.3	16.3	0.0	72.3	0.0
65g	137	32	9	90	85.9	11.7	12.4	0.9	15.2	0.0
65h	211	29	10	102	75.1	5.4	31.8	0.8	74.5	0.0
65k	143	31	8	80	86.2	0.2	42.6	0.0	22.0	0.0
65l	409	30	10	96	85.2	4.1	40.6	0.3	20.5	0.0
65o	28	33	10	78	38.6	1.6	21.9	5.2	16.4	0.0
<b>65</b>	<b>1059</b>	<b>34</b>	<b>8</b>	<b>120</b>	<b>86.2</b>	<b>0.2</b>	<b>42.6</b>	<b>0.0</b>	<b>74.5</b>	<b>0.0</b>
<b>Blueridge – Ecoregion 66</b>										
66d	32	51	17	119	7.5	0.0	1.2	0.0	1.4	0.0
66g	68	41	12	113	21.6	0.0	18.5	0.0	5.8	0.0
66j	12	37	20	84	13.7	2.8	2.6	0.0	1.2	0.0
<b>66</b>	<b>112</b>	<b>43</b>	<b>12</b>	<b>119</b>	<b>21.6</b>	<b>0.0</b>	<b>18.5</b>	<b>0.0</b>	<b>5.8</b>	<b>0.0</b>
<b>Ridge &amp; Valley And Cumberland Plateau – Ecoregions 67 &amp; 68</b>										
67f&l	42	46	12	125	51.3	6.1	11.0	0.0	38.1	0.0
67g	24	30	7	64	27.5	0.8	14.5	0.0	4.9	0.0
67h	9	10	4	26	14.9	0.1	13.7	0.0	0.0	0.0
68c&d	10	41	18	75	25.1	2.6	4.0	0.0	2.2	0.1
<b>67 &amp; 68</b>	<b>85</b>	<b>32</b>	<b>4</b>	<b>125</b>	<b>51.3</b>	<b>0.1</b>	<b>14.5</b>	<b>0.0</b>	<b>38.1</b>	<b>0.0</b>
<b>Coastal Plains – Ecoregion 75</b>										
75e	80	38	10	101	53.6	0.0	30.2	0.4	2.8	0.0
75f	147	38	10	107	45.1	0.0	31.6	1.4	62.8	0.0
75h	73	37	11	108	60.3	1.0	17.7	1.7	45.5	0.0
75j	43	10	4	33	23.8	0.0	17.2	0.0	61.5	0.0
<b>75</b>	<b>343</b>	<b>31</b>	<b>4</b>	<b>108</b>	<b>60.3</b>	<b>0.0</b>	<b>31.6</b>	<b>0.0</b>	<b>62.8</b>	<b>0.0</b>
<b>Georgia</b>	<b>2158</b>	<b>36</b>	<b>4</b>	<b>142</b>	<b>86.2</b>	<b>0.0</b>	<b>42.6</b>	<b>0.0</b>	<b>74.5</b>	<b>0.0</b>

From Gore *et al.* 2004.

**Appendix B.** Water Chemistry Parameters Analyzed for the Georgia Ecoregions Project.

<b>Parameter Measured</b>	<b>Type of Sample Taken</b>	<b>Method / Instrumentation Used</b>	<b>Range of Detection</b>
Ammonia (mg/l as N)	Grab Sample	EPA Method #350.3	0.03 to 1400 NH <sub>3</sub> -N/L
Nitrite (mg/l as N)	Grab Sample	EPA Method #354.1	0.01 to 1.0mg NO <sub>2</sub> -N/L
Nitrate (as N)	Grab Sample	EPA Method #353.3	0.01 to 1.0mg NO <sub>3</sub> -N/L
Total Phosphorus (mg/l as P)	Grab Sample	EPA Method #365.3	0.01 to 1.2 mg P/L
Copper (mg/l)	Grab Sample	EPA Method #220.1	low detection limit is 0.1ppm
Iron (mg/l)	Grab Sample	EPA Method #236.1	low detection limit is 0.1ppm
Manganese (mg/l)	Grab Sample	EPA Method #243.1	low detection limit is 0.1ppm
Zinc (mg/l)	Grab Sample	EPA Method #289.1	low detection limit is 0.1ppm
Conductivity (mS/cm)	In situ Measurement	HydroLab H-20 probe	1 to 100 mS/cm
Dissolved Oxygen (%)	In situ Measurement	HydroLab H-20 probe	0 to 100 %
Dissolved Oxygen (mg/l)	In situ Measurement	HydroLab H-20 probe	0.2 to 18.8 mg/L
PH	In situ Measurement	HydroLab H-20 probe	0 to 14 units
Turbidity (NTU)	In situ Measurement	HydroLab H-20 probe	5 to 1000 NTU
Water Temperature (°C)	In situ Measurement	HydroLab H-20 probe	-5 to 50°C
Alkalinity (mg/l as CaCO <sub>3</sub> )	Grab Sample	EPA Method #310.1	All concentration ranges of alkalinity
Hardness (mg/l as CaCO <sub>3</sub> )	Grab Sample	EPA Method #130.2	All concentration ranges of hardness

From Gore *et al.* 2004.

Appendix C. Physical Characterization/Water Quality Field Data Sheet.

**PHYSICAL CHARACTERIZATION/WATER QUALITY FIELD DATA SHEET (FRONT)**

STREAM NAME		LOCATION	
STATION # _____ RIVER MILE		STREAM CLASS	
LAT _____ LONG		RIVER BASIN	
STORET #		AGENCY	
INVESTIGATORS			
FORM COMPLETED BY		DATE _____ AM PM	REASON FOR SURVEY

SITE LOCATION/MAP	Draw a map of the site and indicate the areas sampled		
STREAM CHARACTERIZATION	<b>Subsystem Classification</b> <input type="checkbox"/> Perennial <input type="checkbox"/> Intermittent		<b>Stream Type</b> <input type="checkbox"/> Tidal <input type="checkbox"/> Coldwater <input type="checkbox"/> Warmwater
WEATHER CONDITIONS	<b>Now</b> <input type="checkbox"/> storm (heavy rain) <input type="checkbox"/> rain (steady rain) <input type="checkbox"/> showers (intermittent) <input type="checkbox"/> _____% cloud cover <input type="checkbox"/> clear/sunny	<b>Past 24 hours</b> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> _____% cloud cover <input type="checkbox"/> clear/sunny	<b>Has there been a heavy rain in the last 7 days?</b> <input type="checkbox"/> Yes <input type="checkbox"/> No  <b>Air Temperature</b> _____ °C  <b>Other</b> _____

Appendix C. Physical Characterization/Water Quality Field Data Sheet. (cont.)

**PHYSICAL CHARACTERIZATION/WATER QUALITY FIELD DATA SHEET (BACK)**

RIPARIAN ZONE/ INSTREAM FEATURES	<b>Predominant Surrounding Landuse</b> <input type="checkbox"/> Forest <input type="checkbox"/> Commercial <input type="checkbox"/> Field/Pasture <input type="checkbox"/> Industrial <input type="checkbox"/> Agricultural <input type="checkbox"/> Other _____ <input type="checkbox"/> Residential		<b>Local Water Erosion</b> <input type="checkbox"/> None <input type="checkbox"/> Moderate <input type="checkbox"/> Heavy		
			<b>Estimated Stream Width</b> _____ m		
	<b>Local Watershed NPS Pollution</b> <input type="checkbox"/> No evidence <input type="checkbox"/> Some potential sources <input type="checkbox"/> Obvious sources		<b>Estimated Stream Depth</b> <input type="checkbox"/> Riffle _____ m <input type="checkbox"/> Run _____ m <input type="checkbox"/> Pool _____ m		
			<b>Velocity</b> _____ m/sec		
	<b>Canopy Cover</b> <input type="checkbox"/> Partly open <input type="checkbox"/> Partly shaded <input type="checkbox"/> Shaded		<b>Estimated Reach Length</b> _____ m		
	<b>High Water Mark</b> _____ m		<b>Channelized</b> <input type="checkbox"/> Yes <input type="checkbox"/> No		
			<b>Dam Present</b> <input type="checkbox"/> Yes <input type="checkbox"/> No		
RIPARIAN VEGETATION (18 meter buffer)	<b>Indicate the dominant type and record the dominant species present</b> <input type="checkbox"/> Trees <input type="checkbox"/> Shrubs <input type="checkbox"/> Grasses <input type="checkbox"/> Herbaceous  dominant species present _____				
AQUATIC VEGETATION	<b>Indicate the dominant type and record the dominant species present</b> <input type="checkbox"/> Rooted emergent <input type="checkbox"/> Rooted submergent <input type="checkbox"/> Rooted floating <input type="checkbox"/> Free Floating <input type="checkbox"/> Floating Algae <input type="checkbox"/> Attached Algae  dominant species present _____				
	<b>Portion of the reach with vegetative cover</b> _____ %				
SEDIMENT/ SUBSTRATE	<b>Odors</b> <input type="checkbox"/> Normal <input type="checkbox"/> Sewage <input type="checkbox"/> Petroleum <input type="checkbox"/> Chemical <input type="checkbox"/> Anaerobic <input type="checkbox"/> None <input type="checkbox"/> Other _____		<b>Deposits</b> <input type="checkbox"/> Sludge <input type="checkbox"/> Sawdust <input type="checkbox"/> Paper fiber <input type="checkbox"/> Sand <input type="checkbox"/> Relict shells <input type="checkbox"/> Other _____		
	<b>Oils</b> <input type="checkbox"/> Absent <input type="checkbox"/> Slight <input type="checkbox"/> Moderate <input type="checkbox"/> Profuse		<b>Looking at stones which are not deeply embedded, are the undersides black in color?</b> <input type="checkbox"/> Yes <input type="checkbox"/> No		
WATER QUALITY	<b>Temperature</b> _____ °C		<b>Water Odors</b> <input type="checkbox"/> Normal/None <input type="checkbox"/> Sewage <input type="checkbox"/> Petroleum <input type="checkbox"/> Chemical <input type="checkbox"/> Fishy <input type="checkbox"/> Other _____		
	<b>Specific Conductance</b> _____		<b>Water Surface Oils</b> <input type="checkbox"/> Slick <input type="checkbox"/> Sheen <input type="checkbox"/> Globs <input type="checkbox"/> Flecks <input type="checkbox"/> None <input type="checkbox"/> Other _____		
	<b>Dissolved Oxygen</b> _____		<b>Turbidity (if not measured)</b> <input type="checkbox"/> Clear <input type="checkbox"/> Slightly turbid <input type="checkbox"/> Turbid <input type="checkbox"/> Opaque <input type="checkbox"/> Water color <input type="checkbox"/> Other _____		
	<b>pH</b> _____				
	<b>Turbidity</b> _____				
	<b>WQ Instrument Used</b> _____				
<b>INORGANIC SUBSTRATE COMPONENTS</b> (should add up to 100%)			<b>ORGANIC SUBSTRATE COMPONENTS</b> (does not necessarily add up to 100%)		
Substrate Type	Diameter	% Composition in Sampling Reach	Substrate Type	Characteristic	% Composition in Sampling Area
Bedrock			Detritus	sticks, wood, coarse plant materials (CPOM)	
Boulder	> 256 mm (10")				
Cobble	64-256 mm (2.5"-10")		Muck-Mud	black, very fine organic (FPOM)	
Gravel	2-64 mm (0.1"-2.5")				
Sand	0.06-2mm (gritty)		Marl	grey, shell fragments	
Silt	0.004-0.06 mm				
Clay	< 0.004 mm (slick)				

From Columbus State University's Quality Assurance Project Plan (QAPP 2000).

**Appendix D. Habitat Assessment Field Data Sheets. [From Columbus State University's Quality Assurance Project Plan (QAPP 200).]**

**HABITAT ASSESSMENT FIELD DATA SHEET -- HIGH GRADIENT STREAMS (FRONT)**

STREAM NAME _____		LOCATION _____	
STATION # _____		LAT _____	LONG _____
INVESTIGATORS _____			
FORM COMPLETED BY _____		DATE _____	REASON FOR SURVEY _____

Habitat Parameter	Condition Category			
	Optimal	Suboptimal	Marginal	Poor
<b>1. Epifaunal Substrate/ Available Cover</b>	Greater than 70% of substrate favorable for epifaunal colonization and fish cover; mix of snags, submerged logs, undercut banks, cobble or other stable habitat and at stage to allow full colonization potential (i.e., logs/snags that are <u>not</u> new fall and <u>not</u> transient).	40-70% mix of stable habitat; well-suited for full colonization potential; adequate habitat for maintenance of populations; presence of additional substrate in the form of new fall, but not yet prepared for colonization (may rate at high end of scale).	20-40% mix of stable habitat; habitat availability less than desirable; substrate frequently disturbed or removed.	Less than 20% stable habitat; lack of habitat is obvious; substrate unstable or lacking.
<b>SCORE</b>	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
<b>2. Embeddedness</b>	Gravel, cobble, and boulder particles are 0-25% surrounded by fine sediment. Layering of cobble provides diversity of niche space.	Gravel, cobble, and boulder particles are 25-50% surrounded by fine sediment.	Gravel, cobble, and boulder particles are 50-75% surrounded by fine sediment.	Gravel, cobble, and boulder particles are more than 75% surrounded by fine sediment.
<b>SCORE</b>	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
<b>3. Velocity/Depth Regime</b>	All four velocity/depth regimes present (slow-deep, slow-shallow, fast-deep, fast-shallow). (Slow is < 0.3 m/s, deep is > 0.5 m.)	Only 3 of the 4 regimes present (if fast-shallow is missing, score lower than if missing other regimes).	Only 2 of the 4 habitat regimes present (if fast-shallow or slow-shallow are missing, score low).	Dominated by 1 velocity/depth regime (usually slow-deep).
<b>SCORE</b>	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
<b>4. Sediment Deposition</b>	Little or no enlargement of islands or point bars and less than 5% of the bottom affected by sediment deposition.	Some new increase in bar formation, mostly from gravel, sand or fine sediment; 5-30% of the bottom affected; slight deposition in pools.	Moderate deposition of new gravel, sand or fine sediment on old and new bars; 30-50% of the bottom affected; sediment deposits at obstructions, constrictions, and bends; moderate deposition of pools prevalent.	Heavy deposits of fine material, increased bar development; more than 50% of the bottom changing frequently; pools almost absent due to substantial sediment deposition.
<b>SCORE</b>	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
<b>5. Channel Flow Status</b>	Water reaches base of both lower banks, and minimal amount of channel substrate is exposed.	Water fills >75% of the available channel; or <25% of channel substrate is exposed.	Water fills 25-75% of the available channel, and/or riffle substrates are mostly exposed.	Very little water in channel and mostly present as standing pools.
<b>SCORE</b>	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0

Appendix D. Habitat Assessment Field Data Sheets. (cont.)

HABITAT ASSESSMENT FIELD DATA SHEET -- HIGH GRADIENT STREAMS (BACK)

Habitat Parameter	Condition Category			
	Optimal	Suboptimal	Marginal	Poor
<b>6. Channel Alteration</b>	Channelization or dredging absent or minimal; stream with normal pattern.	Some channelization present, usually in areas of bridge abutments; evidence of past channelization, i.e., dredging, (greater than past 20 yr) may be present, but recent channelization is not present.	Channelization may be extensive; embankments or shoring structures present on both banks; and 40 to 80% of stream reach channelized and disrupted.	Banks shored with gabion or cement; over 80% of the stream reach channelized and disrupted. Instream habitat greatly altered or removed entirely.
SCORE	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
<b>7. Frequency of Riffles (or bends)</b>	Occurrence of riffles relatively frequent; ratio of distance between riffles divided by width of the stream <7:1 (generally 5 to 7); variety of habitat is key. In streams where riffles are continuous, placement of boulders or other large, natural obstruction is important.	Occurrence of riffles infrequent; distance between riffles divided by the width of the stream is between 7 to 15.	Occasional riffle or bend; bottom contours provide some habitat; distance between riffles divided by the width of the stream is between 15 to 25.	Generally all flat water or shallow riffles; poor habitat; distance between riffles divided by the width of the stream is a ratio of >25.
SCORE	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
<b>8. Bank Stability (score each bank)</b>	Banks stable; evidence of erosion or bank failure absent or minimal; little potential for future problems. <5% of bank affected.	Moderately stable; infrequent, small areas of erosion mostly healed over. 5-30% of bank in reach has areas of erosion.	Moderately unstable; 30-60% of bank in reach has areas of erosion; high erosion potential during floods.	Unstable; many eroded areas; "raw" areas frequent along straight sections and bends; obvious bank sloughing; 60-100% of bank has erosional scars.
Note: determine left or right side by facing downstream.				
SCORE (LB)	Left Bank 10 9	8 7 6	5 4 3	2 1 0
SCORE (RB)	Right Bank 10 9	8 7 6	5 4 3	2 1 0
<b>9. Vegetative Protection (score each bank)</b>	More than 90% of the streambank surfaces and immediate riparian zone covered by native vegetation, including trees, understory shrubs, or nonwoody macrophytes; vegetative disruption through grazing or mowing minimal or not evident; almost all plants allowed to grow naturally.	70-90% of the streambank surfaces covered by native vegetation, but one class of plants is not well-represented; disruption evident but not affecting full plant growth potential to any great extent; more than one-half of the potential plant stubble height remaining.	50-70% of the streambank surfaces covered by vegetation; disruption obvious; patches of bare soil or closely cropped vegetation common; less than one-half of the potential plant stubble height remaining.	Less than 50% of the streambank surfaces covered by vegetation; disruption of streambank vegetation is very high; vegetation has been removed to 5 centimeters or less in average stubble height.
SCORE (LB)	Left Bank 10 9	8 7 6	5 4 3	2 1 0
SCORE (RB)	Right Bank 10 9	8 7 6	5 4 3	2 1 0
<b>10. Riparian Vegetative Zone Width (score each bank riparian zone)</b>	Width of riparian zone >18 meters; human activities (i.e., parking lots, roadbeds, clear-cuts, lawns, or crops) have not impacted zone.	Width of riparian zone 12-18 meters; human activities have impacted zone only minimally.	Width of riparian zone 6-12 meters; human activities have impacted zone a great deal.	Width of riparian zone <6 meters; little or no riparian vegetation due to human activities.
SCORE (LB)	Left Bank 10 9	8 7 6	5 4 3	2 1 0
SCORE (RB)	Right Bank 10 9	8 7 6	5 4 3	2 1 0

Total Score \_\_\_\_\_

## Appendix D. Habitat Assessment Field Data Sheets. (cont.)

## HABITAT ASSESSMENT FIELD DATA SHEET -- LOW GRADIENT STREAMS (FRONT)

STREAM NAME _____		LOCATION _____	
STATION # _____		LAT _____	LONG _____
INVESTIGATORS _____			
FORM COMPLETED BY _____		DATE _____ TIME _____ AM PM	REASON FOR SURVEY _____

Habitat Parameter	Condition Category			
	Optimal	Suboptimal	Marginal	Poor
<b>1. Epifaunal Substrate/ Available Cover</b>	Greater than 50% of substrate favorable for epifaunal colonization and fish cover; mix of snags, submerged logs, undercut banks, cobble or other stable habitat and at stage to allow full colonization potential (i.e., logs/snags that are <u>not</u> new fall and <u>not</u> transient).	30-50% mix of stable habitat; well-suited for full colonization potential; adequate habitat for maintenance of populations; presence of additional substrate in the form of new fall, but not yet prepared for colonization (may rate at high end of scale).	10-30% mix of stable habitat; habitat availability less than desirable; substrate frequently disturbed or removed.	Less than 10% stable habitat; lack of habitat is obvious; substrate unstable or lacking.
SCORE	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
<b>2. Pool Substrate Characterization</b>	Mixture of substrate materials, with gravel and firm sand prevalent; root mats and submerged vegetation common.	Mixture of soft sand, mud, or clay; mud may be dominant; some root mats and submerged vegetation present.	All mud or clay or sand bottom; little or no root mat; no submerged vegetation.	Hard-pan clay or bedrock; no root mat or vegetation.
SCORE	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
<b>3. Pool Variability</b>	Even mix of large-shallow, large-deep, small-shallow, small-deep pools present.	Majority of pools large-deep; very few shallow.	Shallow pools much more prevalent than deep pools.	Majority of pools small-shallow or pools absent.
SCORE	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
<b>4. Sediment Deposition</b>	Little or no enlargement of islands or point bars and less than <20% of the bottom affected by sediment deposition.	Some new increase in bar formation, mostly from gravel, sand or fine sediment; 20-50% of the bottom affected; slight deposition in pools.	Moderate deposition of new gravel, sand or fine sediment on old and new bars; 50-80% of the bottom affected; sediment deposits at obstructions, constrictions, and bends; moderate deposition of pools prevalent.	Heavy deposits of fine material, increased bar development; more than 80% of the bottom changing frequently; pools almost absent due to substantial sediment deposition.
SCORE	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
<b>5. Channel Flow Status</b>	Water reaches base of both lower banks, and minimal amount of channel substrate is exposed.	Water fills >75% of the available channel; or <25% of channel substrate is exposed.	Water fills 25-75% of the available channel, and/or riffle substrates are mostly exposed.	Very little water in channel and mostly present as standing pools.
SCORE	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0

## Appendix D. Habitat Assessment Field Data Sheets. (cont.)

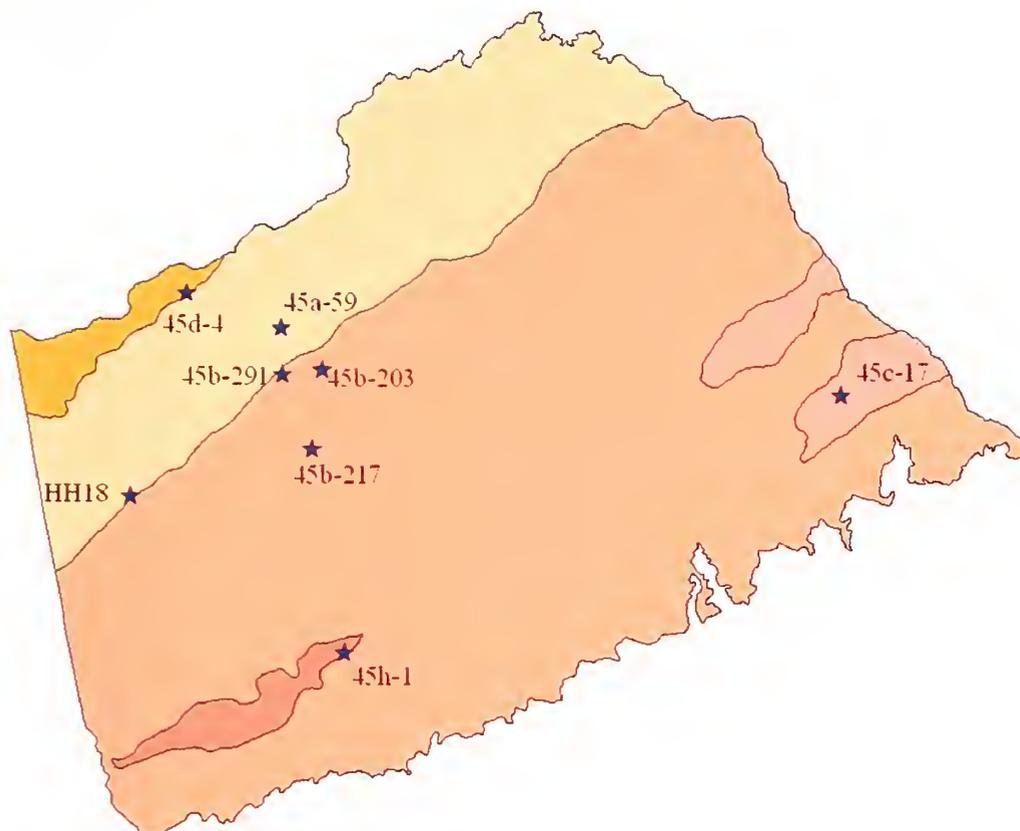
## HABITAT ASSESSMENT FIELD DATA SHEET -- LOW GRADIENT STREAMS (BACK)

Habitat Parameter	Condition Category			
	Optimal	Suboptimal	Marginal	Poor
<b>6. Channel Alteration</b>	Channelization or dredging absent or minimal; stream with normal pattern.	Some channelization present, usually in areas of bridge abutments; evidence of past channelization, i.e., dredging, (greater than past 20 yr) may be present, but recent channelization is not present.	Channelization may be extensive; embankments or shoring structures present on both banks; and 40 to 80% of stream reach channelized and disrupted.	Banks shored with gabion or cement; over 80% of the stream reach channelized and disrupted. Instream habitat greatly altered or removed entirely.
SCORE	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
<b>7. Channel Sinuosity</b>	The bends in the stream increase the stream length 3 to 4 times longer than if it was in a straight line. (Note - channel braiding is considered normal in coastal plains and other low-lying areas. This parameter is not easily rated in these areas.)	The bends in the stream increase the stream length 2 to 3 times longer than if it was in a straight line.	The bends in the stream increase the stream length 1 to 2 times longer than if it was in a straight line.	Channel straight; waterway has been channelized for a long distance.
SCORE	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
<b>8. Bank Stability (score each bank)</b>	Banks stable; evidence of erosion or bank failure absent or minimal; little potential for future problems. <5% of bank affected.	Moderately stable; infrequent, small areas of erosion mostly healed over. 5-30% of bank in reach has areas of erosion.	Moderately unstable; 30-60% of bank in reach has areas of erosion; high erosion potential during floods.	Unstable; many eroded areas; "raw" areas frequent along straight sections and bends; obvious bank sloughing; 60-100% of bank has erosional scars.
SCORE (LB)	Left Bank 10 9	8 7 6	5 4 3	2 1 0
SCORE (RB)	Right Bank 10 9	8 7 6	5 4 3	2 1 0
<b>9. Vegetative Protection (score each bank)</b>	More than 90% of the streambank surfaces and immediate riparian zone covered by native vegetation, including trees, understory shrubs, or nonwoody macrophytes; vegetative disruption through grazing or mowing minimal or not evident; almost all plants allowed to grow naturally.	70-90% of the streambank surfaces covered by native vegetation, but one class of plants is not well-represented; disruption evident but not affecting full plant growth potential to any great extent; more than one-half of the potential plant stubble height remaining.	50-70% of the streambank surfaces covered by vegetation; disruption obvious; patches of bare soil or closely cropped vegetation common; less than one-half of the potential plant stubble height remaining.	Less than 50% of the streambank surfaces covered by vegetation; disruption of streambank vegetation is very high; vegetation has been removed to 5 centimeters or less in average stubble height.
SCORE (LB)	Left Bank 10 9	8 7 6	5 4 3	2 1 0
SCORE (RB)	Right Bank 10 9	8 7 6	5 4 3	2 1 0
<b>10. Riparian Vegetative Zone Width (score each bank riparian zone)</b>	Width of riparian zone >18 meters; human activities (i.e., parking lots, roadbeds, clear-cuts, lawns, or crops) have not impacted zone.	Width of riparian zone 12-18 meters; human activities have impacted zone only minimally.	Width of riparian zone 6-12 meters; human activities have impacted zone a great deal.	Width of riparian zone <6 meters; little or no riparian vegetation due to human activities.
SCORE (LB)	Left Bank 10 9	8 7 6	5 4 3	2 1 0
SCORE (RB)	Right Bank 10 9	8 7 6	5 4 3	2 1 0

**Total Score** \_\_\_\_\_

**Appendix E. Geographic Maps of Quality Control Sampling Sites of the Georgia Ecoregions Project.**

**Ecoregion 45 – Piedmont  
Spatial Quality Control Sampling Sites**



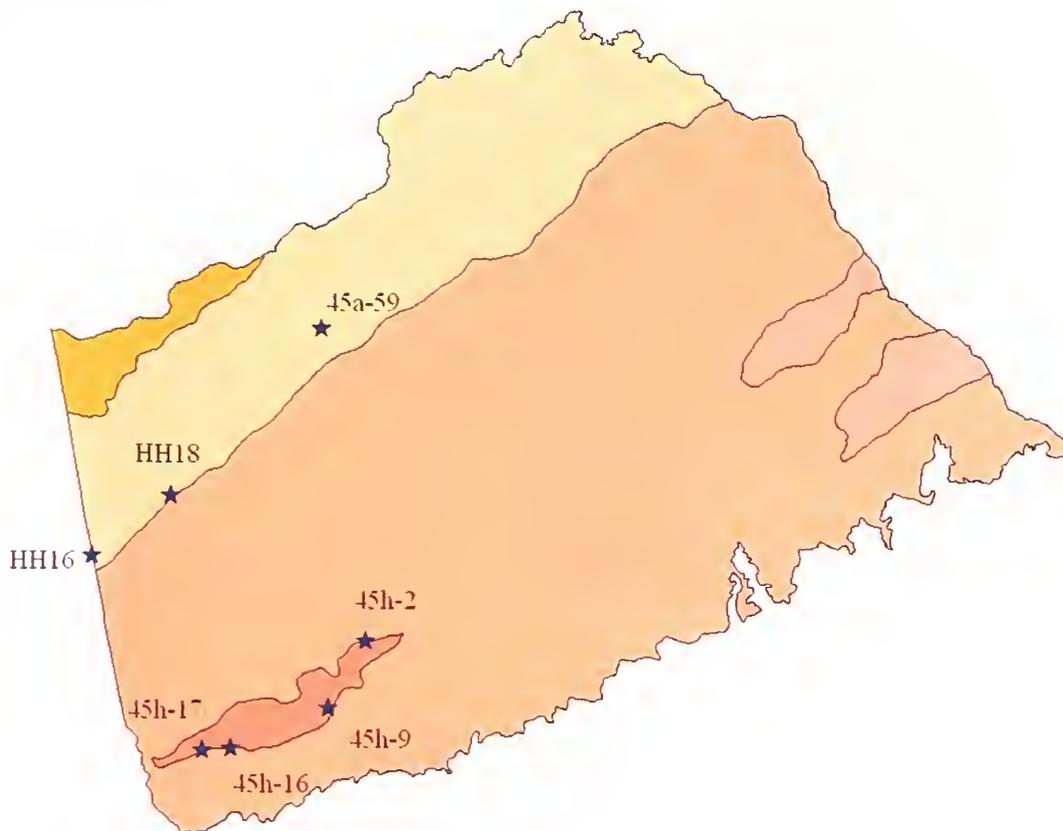
**Figure 36.** Spatial Quality Control Sampling Sites for Ecoregion 45.

**Ecoregion 45 – Spatial Quality Control Sampling Site List**

<u>Ecoregion</u>	<u>Subcoregion</u>	<u>Site ID</u>	<u>Site Name</u>	<u>Impairment Status</u>
45	45a	HH - 18	Whooping Creek	Reference
		45a - 59	Rottenwood Creek	Impaired
	45b	45b - 203	South Fork	Impaired
		45b - 217	Flint River	Impaired
		45b - 291	Proctor Creek	Impaired
	45c	45c - 17	Upton Creek	Impaired
	45d	45d - 4	West Fork Pumpkinvine Creek	Reference-GIS
	45h	45h - 1	Three Mile Creek	Impaired

**Appendix E. Geographic Maps of Quality Control Sampling Sites of the Georgia Ecoregions Project. (cont.)**

**Ecoregion 45 – Piedmont  
Temporal Quality Control Sampling Sites**



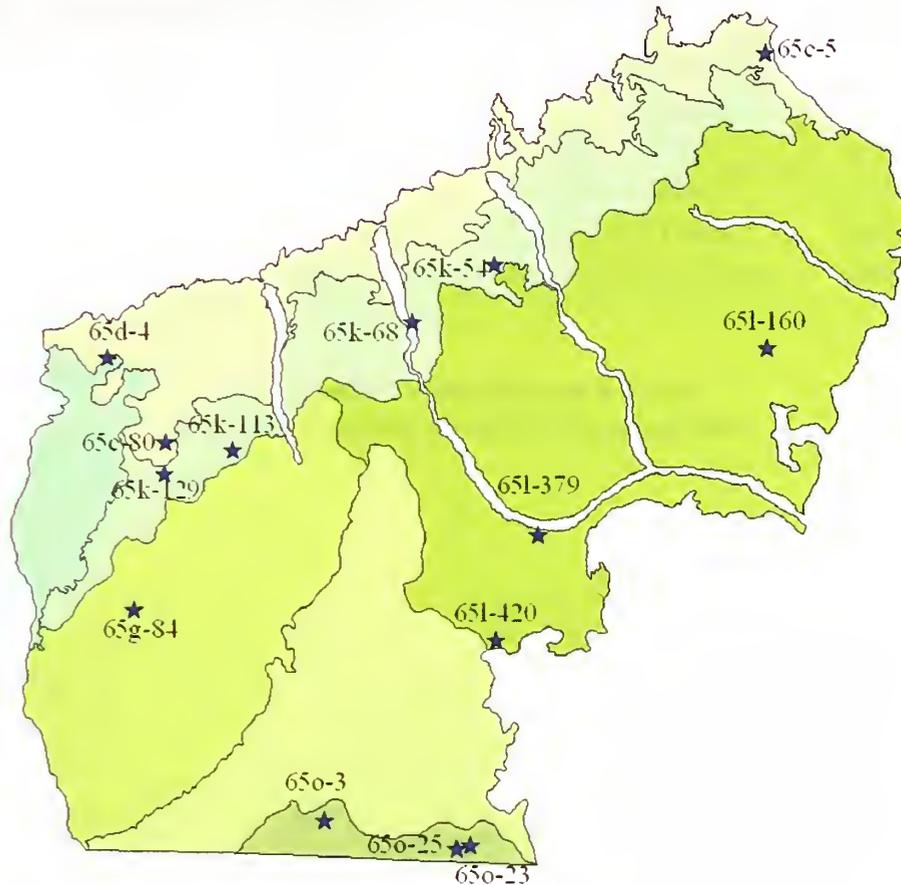
**Figure 37.** Temporal Quality Control Sampling Sites for Ecoregion 45.

**Ecoregion 45 – Temporal Quality Control Sampling Site List**

<u>Ecoregion</u>	<u>Subcoregion</u>	<u>Site ID</u>	<u>Site Name</u>	<u>Impairment Status</u>
45	45a	HH - 16	Town Creek	Impaired
		HH - 18	Whooping Creek	Reference
		45a - 59	Rottenwood Creek	Reference
	45h	45h - 2	Powder Creek	Reference
		45h - 9	Mud Creek	Reference
		45h - 16	Williams Creek	Reference-GIS
		45h - 17	Barnes Creek	Reference

**Appendix E. Geographic Maps of Quality Control Sampling Sites of the Georgia Ecoregions Project. (cont.)**

**Ecoregion 65 – Southeastern Plains  
Spatial Quality Control Sampling Sites**



**Figure 38.** Spatial Quality Control Sampling Sites for Ecoregion 65.

**Ecoregion 65 – Spatial Quality Control Sampling Site List**

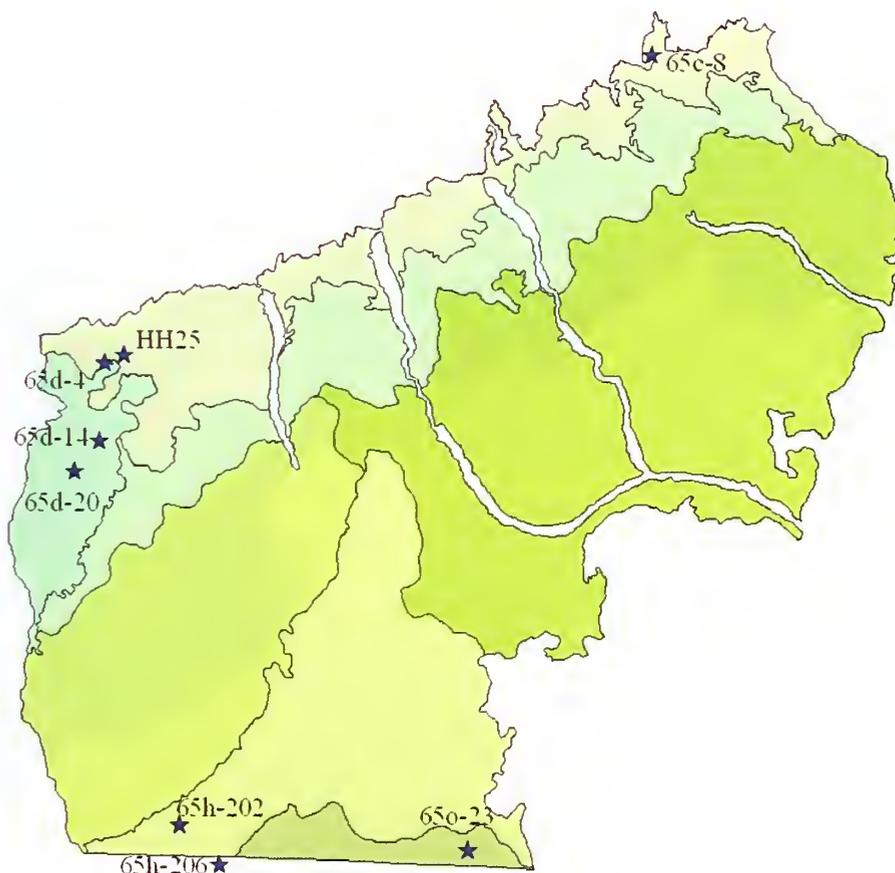
<u>Ecoregion</u>	<u>Subcoregion</u>	<u>Site ID</u>	<u>Site Name</u>	<u>Impairment Status</u>
65	65c	65c - 5	Butler Creek	Impaired
		65c - 80	Lanahassee Creek	Reference-GIS
	65d	65d - 4	Sally Branch	Reference-GIS
	65g	65g - 84	Trib. to Pachitla Creek	Impaired
	65k	65k - 54	Maiden Creek	Reference-GIS
		65k - 68	Crooked Creek	Reference-GIS
		65k - 113	Town Creek	Impaired
		65k - 129	Trib. to KinchafooneeCreek	Impaired
	65o	65o-3		
	65o	65o-25		
65o	65o-23			

**Appendix E. Geographic Maps of Quality Control Sampling Sites of the Georgia Ecoregions Project. (cont.)**

**Ecoregion 65 – Spatial Quality Control Sampling Site List (cont.)**

<u>Ecoregion</u>	<u>Subcoregion</u>	<u>Site ID</u>	<u>Site Name</u>	<u>Impairment Status</u>
65	651	651 - 160	Trib. to Canoochee River	Impaired
		651 - 379	Red Bluff Creek	Reference
		651 - 420	Mill Branch	Impaired
	65o	65o - 3	Olive Creek	Impaired
		65o - 23	Clyatt Mill Creek	Reference-GIS
		65o - 25	Tributary to New River	Reference-GIS

**Ecoregion 65 – Southeastern Plains  
Temporal Quality Control Sampling Sites**



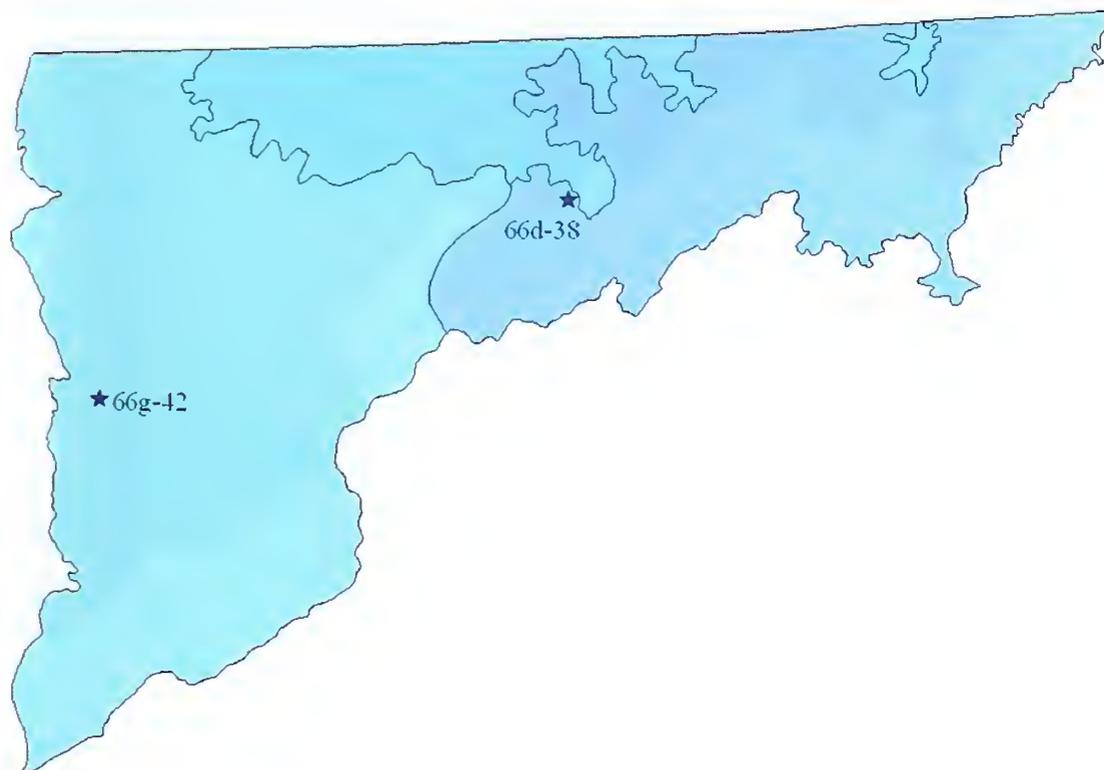
**Figure 39. Temporal Quality Control Sampling Sites for Ecoregion 65.**

**Appendix E. Geographic Maps of Quality Control Sampling Sites of the Georgia Ecoregions Project. (cont.)**

**Ecoregion 65 – Temporal Quality Control Sampling Site List**

<u>Ecoregion</u>	<u>Subcoregion</u>	<u>Site ID</u>	<u>Site Name</u>	<u>Impairment Status</u>
65	65c	HH - 25	Pine Knot Creek	Reference
		65c - 8	Sweetwater Creek	Impaired
	65d	65d - 4	Sally Branch	Reference-GIS
		65d - 14	Hannahatchee Creek	Reference-GIS
		65d - 20	Day Creek	Impaired
	65h	65h - 202	Callahan Branch	Reference-GIS
		65h - 206	Shaw Creek	Reference-GIS
	65o	65o - 23	Clyatt Mill Creek	Reference-GIS

**Ecoregion 66 – Blue Ridge  
Spatial Quality Control Sampling Sites**



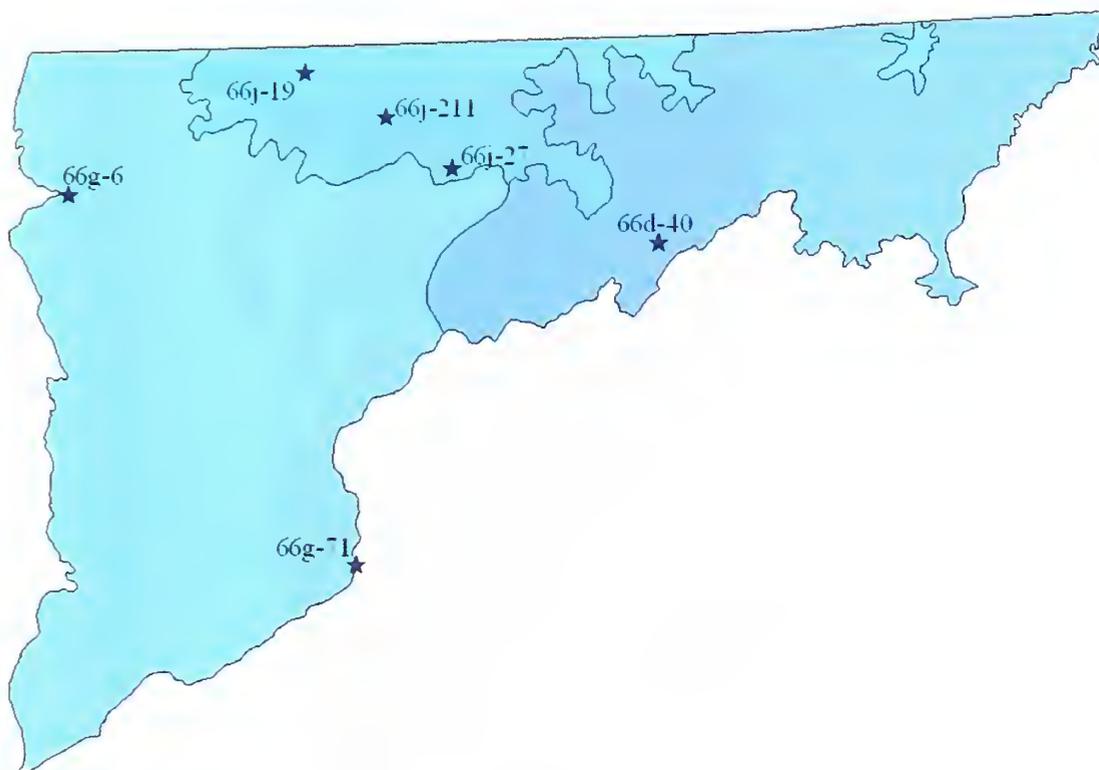
**Figure 40.** Spatial Quality Control Sampling Sites for Ecoregion 66.

**Appendix E. Geographic Maps of Quality Control Sampling Sites of the Georgia Ecoregions Project. (cont.)**

**Ecoregion 66 – Spatial Quality Control Sampling Site List**

<u>Ecoregion</u>	<u>Subecoregion</u>	<u>Site ID</u>	<u>Site Name</u>	<u>Impairment Status</u>
66	66d	66d - 38	West Fork Wolf Creek	Impaired
	66g	66g - 42	Trib. to Talking Rock Creek	Impaired

**Ecoregion 66 – Blue Ridge  
Temporal Quality Control Sampling Sites**



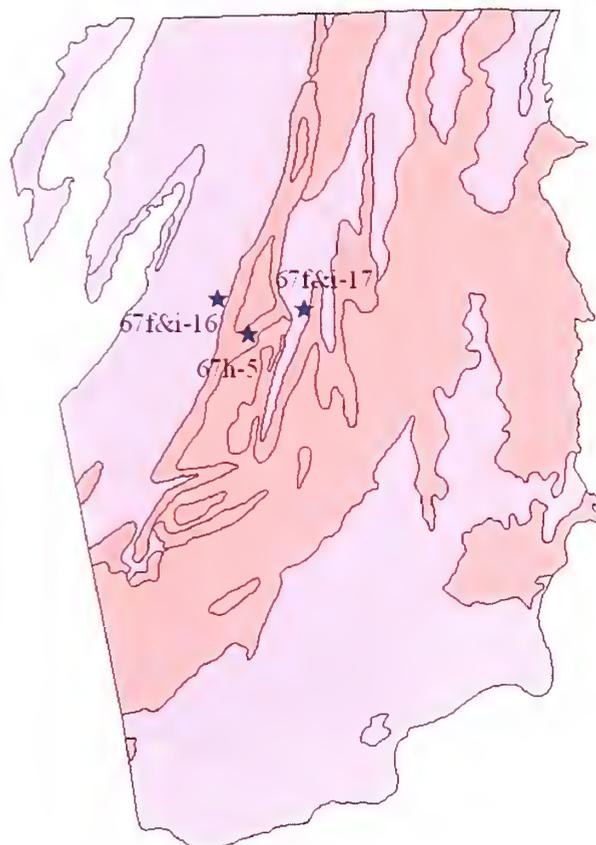
**Figure 41.** Temporal Quality Control Sampling Sites for Ecoregion 66.

**Appendix E. Geographic Maps of Quality Control Sampling Sites of the Georgia Ecoregions Project. (cont.)**

**Ecoregion 66 – Temporal Quality Control Sampling Site List**

<u>Ecoregion</u>	<u>Subcoregion</u>	<u>Site ID</u>	<u>Site Name</u>	<u>Impairment Status</u>
66	66d	66d - 40	Chattahoochee River	Reference-GIS
	66g	66g - 6	Holly Creek	Reference-GIS
		66g - 71	Yellow Creek	Impaired
	66j	66j - 19	Hothouse Creek	Reference
		66j - 27	Young Cane Creek	Impaired
		66j - 211	Bryan Creek	Reference-GIS

**Ecoregion 67 – Ridge and Valley  
Spatial Quality Control Sampling Sites**



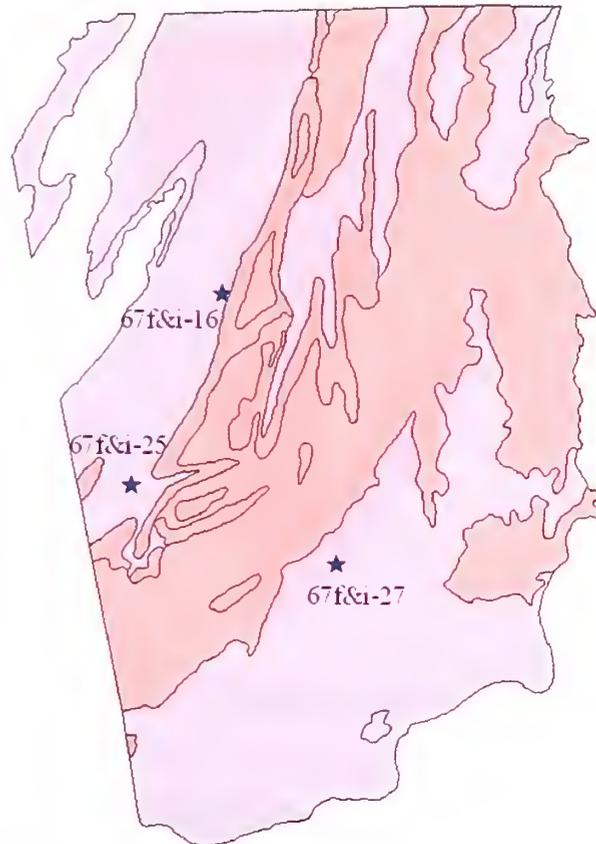
**Figure 42.** Spatial Quality Control Sampling Sites for Ecoregion 67.

**Appendix E. Geographic Maps of Quality Control Sampling Sites of the Georgia Ecoregions Project. (cont.)**

**Ecoregion 67 – Spatial Quality Control Sampling Site List**

<u>Ecoregion</u>	<u>Subcoregion</u>	<u>Site ID</u>	<u>Site Name</u>	<u>Impairment Status</u>
67	67f&i	67f&i - 16	Cane Creek	Reference-GIS
		67f&i - 17	Armuchee Creek	Reference-GIS
	67h	67h - 5	Trib. to Ruff Creek	Impaired

**Ecoregion 67 – Ridge and Valley  
Temporal Quality Control Sampling Sites**



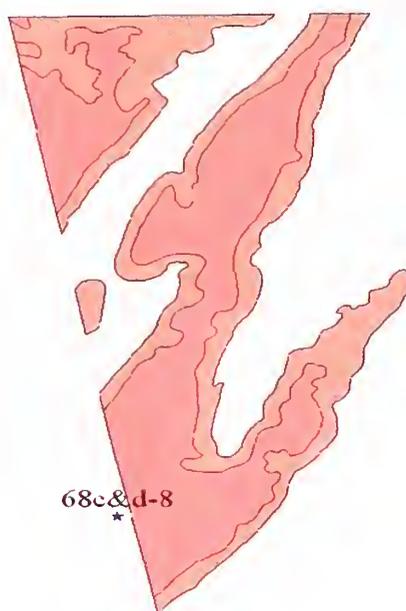
**Figure 43.** Temporal Quality Control Sampling Sites for Ecoregion 67.

**Appendix E. Geographic Maps of Quality Control Sampling Sites of the Georgia Ecoregions Project. (cont.)**

**Ecoregion 67 – Temporal Quality Control Sampling Site List**

<u>Ecoregion</u>	<u>Subcoregion</u>	<u>Site ID</u>	<u>Site Name</u>	<u>Impairment Status</u>
67	67f&i	67f&i - 16	Cane Creek	Reference-GIS
		67f&i - 25	Clarks Creek	Reference-GIS
		67f&i - 27	Dykes Creek	Reference-GIS

**Ecoregion 68 – Southwestern Appalachians  
Spatial Quality Control Sampling Sites**



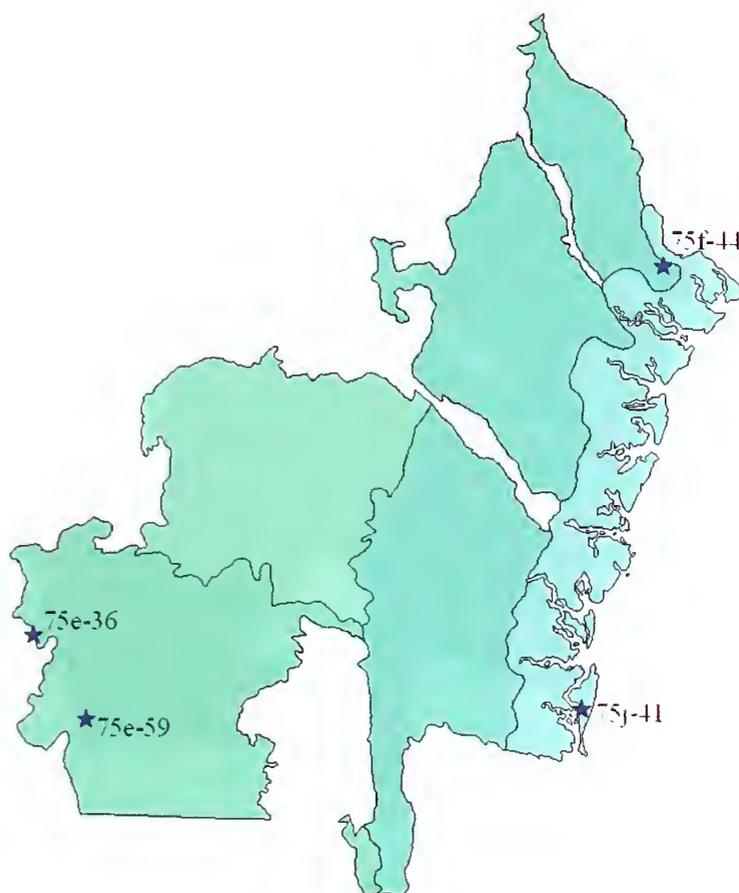
**Figure 44.** Spatial Quality Control Sampling Sites for Ecoregion 68.

**Ecoregion 68 – Spatial Quality Control Sampling Site List**

<u>Ecoregion</u>	<u>Subcoregion</u>	<u>Site ID</u>	<u>Site Name</u>	<u>Stream Class</u>
68	68c&d	68c&d-8	Tributary to Middle Fork Little River	Impaired

**Appendix E.** Geographic Maps of Quality Control Sampling Sites of the Georgia Ecoregions Project. (*cont.*)

**Ecoregion 75 – Southern Coastal Plains  
Spatial Quality Control Sampling Sites**



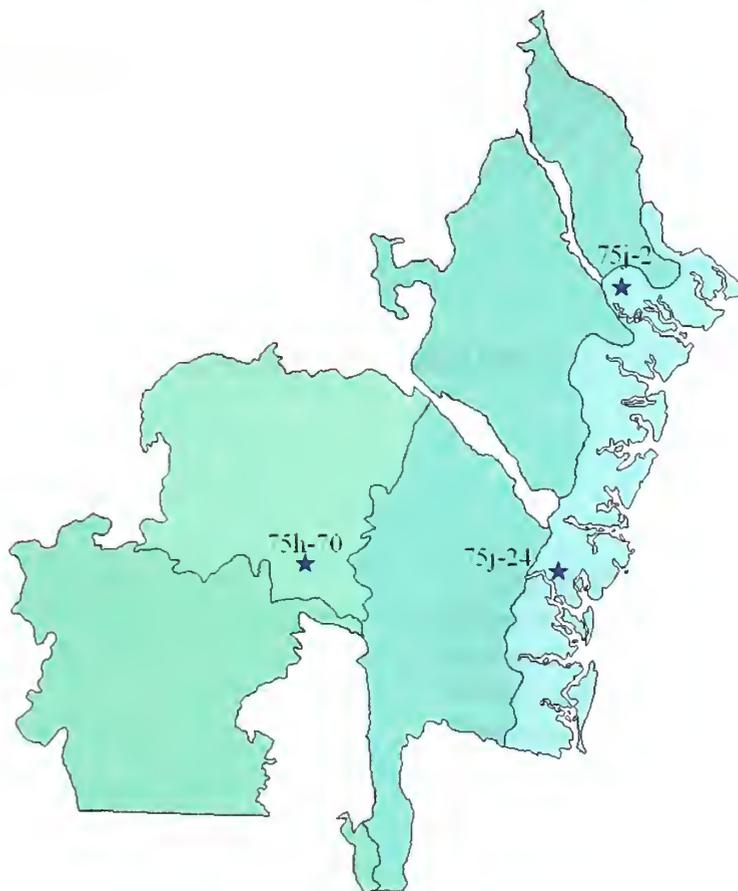
**Figure 45.** Spatial Quality Control Sampling Sites for Ecoregion 75.

**Ecoregion 75 – Spatial Quality Control Sampling Site List**

<u>Ecoregion</u>	<u>Subcoregion</u>	<u>Site ID</u>	<u>Site Name</u>	<u>Impairment Status</u>
75	75e	75e - 36	Swain Creek	Impaired
		75e - 59	Ray Branch	Reference-GIS
	75f	75f - 44	Springfield Canal	Impaired
	75j	75j - 41	White Branch	Reference

**Appendix E. Geographic Maps of Quality Control Sampling Sites of the Georgia Ecoregions Project. (cont.)**

**Ecoregion 75 – Southern Coastal Plains  
Temporal Quality Control Sampling Sites**



**Figure 46.** Temporal Quality Control Sampling Sites for Ecoregion 75.

**Ecoregion 75 – Temporal Quality Control Sampling Site List**

<u>Ecoregion</u>	<u>Subcoregion</u>	<u>Site ID</u>	<u>Site Name</u>	<u>Impairment Status</u>
75	75h	75h - 70	Pond Fork	Impaired
	75j	75j - 2	Trib. to Little Ogeechee River	Impaired
		75j - 24	Yellow Bluff Creek	Impaired

I have submitted this thesis in partial fulfillment of the requirements for the degree of Master of Science.

December 9, 2005  
Date

Tracy J. Ferring  
Tracy J. Ferring

We approve the thesis of Tracy J. Ferring as presented here.

9 Dec 2005  
Date

James A. Gore  
James A. Gore  
Professor of Environmental Science,  
Policy, and Geography  
Thesis Advisor

9 Dec 2005  
Date

George E. Stanton  
George E. Stanton  
Dean of Science  
Professor of Biology

12/9/05  
Date

Harlan J. Hendricks  
Harlan J. Hendricks  
Associate Professor of Biology

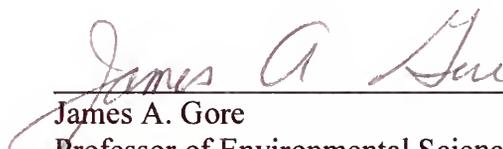
I have submitted this thesis in partial fulfillment of the requirements for the degree of Master of Science.

December 9, 2005  
Date

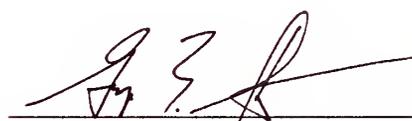
  
Tracy J. Ferring

We approve the thesis of Tracy J. Ferring as presented here.

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Professor of Environmental Science,  
Policy, and Geography  
Thesis Advisor

9 Dec 2005  
Date

  
George E. Stanton  
Dean of Science  
Professor of Biology

12/9/05  
Date

  
Harlan J. Hendricks  
Associate Professor of Biology

Analysis of QA/QC Protocols and Value of Data  
to the Development of  
Reference Criteria in the Georgia Ecoregions Project

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APPENDIX F - APPENDIX O  
Columbus State University  
Masters Thesis

