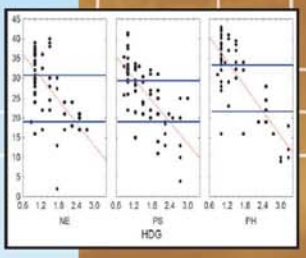
Development and Testing of Biomonitoring Tools for Macroinvertebrates in Florida Streams



DEPARTMENT OF ENVIRONMENTAL PROTECTION
FLORIDA

Nonpoint Source Bioassessment Program

DEVELOPMENT AND TESTING OF BIOMONITORING TOOLS FOR MACROINVERTEBRATES IN FLORIDA STREAMS (STREAM CONDITION INDEX AND BIORECON)

Final Report

Prepared for:

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Dedication

On behalf of the Florida Department of Environmental Protection, the Biocriteria Committee dedicates this document to Dr. James R. Karr.

We would like to thank Dr. Karr for his vision, guidance, and mentoring during the development of its monitoring, assessment, and water quality standards programs. We recognize and appreciate his lifelong dedication to the creation, improvement, and implementation of biological monitoring to protect water resources.

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FOREWARD

The original version of this document was completed in 2004. Subsequently, important observations made during the routine application of these bioassessment tools, as well as new EPA guidance for formulating biological criteria, prompted DEP to revise the document in two key areas.

First, the thresholds for interpreting the final Stream Condition Index values have been revised. This was partially due to responses from DEP biologists, who noted that many reference streams, formally evaluated as "excellent" using the pre-2004 SCI criteria, were now considered "fair", or sometimes "poor" using the SCI 2004 thresholds. Additionally, in 2006, EPA promoted using the Biological Condition Gradient approach as a nationally standardized and scientifically robust method for developing defensible biocriteria. Using this approach, and a second line of evidence concerning the variability of the reference condition, new SCI thresholds have been devised.

An additional revision to the document addressed measurement error of the SCI related to laboratory subsampling methods which were subsequently modified to improve the precision of the SCI without substantially increasing the cost. This involved increasing the number of individuals examined and averaging the results from two independent aliquots from a field sample.

Science is an iterative process: incremental improvements are made as new evidence and experience accumulate. Such was the motivation behind the revisions made in the original document and as a result of additional statistical analyses conducted during 2004–2006. This document represents the work and expertise of many people, all dedicated to applying the best science possible for the protection of Florida's stream resources.

ABSTRACT

The Florida Department of Environmental Protection assesses the chemical, physical, and biological condition of hundreds of stream sites each year. This study used measures of hydrologic condition, riparian and channel habitat condition, water chemistry, and intensity of human land use to define a gradient of human disturbance for stream sites. We evaluated the sensitivity and tolerance of over 1,000 stream macroinvertebrate taxa using the human disturbance gradient (HDG). We tested for correlation between the HDG and 36 biological measures of the stream macroinvertebrate assemblage (metrics), and selected the 10 most highly correlated metrics within 6 categories of biological organization: taxonomic richness (total number of taxa, number of Trichoptera taxa, and number of Ephemeroptera taxa); feeding group (percentage filterer individuals); voltinism (long-lived taxa richness); habit (clinger taxa richness); community structure (percentage dominance of the most abundant taxon and percentage Tanytarsini midges); and sensitivity and tolerance (sensitive taxa richness and percentage very tolerant individuals).

Metrics were combined into an overall Stream Condition Index (SCI) by transforming metric values into unitless scores and summing the scores. The SCI was highly correlated with the HDG for an independent dataset (Spearman's $r = -0.81$, $p < 0.001$). The SCI was independent of watershed size and geographic region (Florida panhandle, peninsula, and northeast). Across 10 years of sampling, the index showed a similar response to the HDG. The SCI was somewhat higher for winter versus summer samples (3.5%). To summarize the statistical precision of the SCI, we used confidence intervals to calculate that the SCI could distinguish 3.7 categories of biological condition. These results supported subsequent selection of three assessment categories for interpreting SCI values in a regulatory context. A large portion of the variability of the SCI was due to subsampling in the laboratory (49%). To reduce this variability, laboratory sorting procedures have subsequently been modified to target two aliquots of 150 individuals, with the resulting SCI values averaged.

We also tested biological metrics for a second stream macroinvertebrate sampling protocol (the BioReconnaissance Index, or BioRecon), which was based on the sorting of invertebrates in the field and taxonomic identification in the laboratory. Of the 10 SCI metrics, 6 taxa richness metrics were tested using BioRecon data; all were highly correlated with both the SCI and HDG. From these metrics, the BioRecon Index was calculated as the sum of scores for the 6 metrics. The statistical precision of the BioRecon index was sufficient to detect 2.5 categories of biological condition which supported the subsequent decision to select three assessment categories for regulatory purposes.

We primarily followed the EPA 2006 Biological Condition Gradient approach for translating index values into biocriteria for Florida streams. For this approach, a panel of 22 regional scientists examined data representing multiple tiers of aquatic life use for the ultimate purpose of defining biological criteria for state water quality standards. In addition, the experts recommended a threshold of biological condition at which impairment occurs as a result of human disturbance. A secondary, but parallel analysis based on departure from reference condition yielded similar index values for the impairment threshold. Based on these complementary results, three categories of aquatic life use are proposed for Florida streams and described as Category 1 ("exceptional"), Category 2 ("healthy"), and Category 3 ("impaired").

CHAPTER 1: INTRODUCTION

Florida, along with a handful of other states, leads the nation in the development and implementation of water protection policies based on biological criteria (McCarron and Frydenborg, 1997; U. S. Environmental Protection Agency [EPA], 2002). Under the federal Clean Water Act, states are required to define designated uses for specific waterbodies and develop criteria to protect them (Karr, 1991; Ransel, 1995). Over time, the emphasis has shifted from what was primarily the chemical monitoring of pollutants to include the direct measurement of the condition of the biological assemblage (Yoder and Rankin, 1998).

Florida classifies its surface waters according to their designated uses. The five categories are ranked in order of the level of protection that they require, as follows:

Class I–Drinking water;
Class II–Shellfish propagation or harvesting (primarily for coastal waters);
Class III–Recreation and propagation and maintenance of a healthy, well-balanced population of fish and wildlife;
Class IV–Agricultural water; and
Class V–Navigation and industrial use.

This document describes the development and testing of biological monitoring tools for macroinvertebrate stream samples for the assessment of freshwater streams that are applicable to Class I and III waters. FDEP has convened a Designated Uses Policy Advisory Committee (DUPAC) to explore an alternative system of designated use classification for Florida (see Chapter 4 below). We anticipate that FDEP will propose Aquatic Life Use (AL) categories, to which these biological monitoring tools for Class I and III streams would also apply.

The EPA has developed numerous guidance documents to support biological assessment (EPA, 1998; Barbour et al., 1999; Bowman et al., 2000; Jackson et al., 2000; EPA, 2001; EPA, 2002; EPA, 2003a, b, c; Fore, 2003). These documents typically emphasize multimetric indexes to measure the biological condition of surface waters (Karr, 1981; Karr et al., 1986; Barbour et al., 1999). The EPA's recent document, *Draft Report on the Environment 2003,* specifically recognizes multimetric indexes as an "approach that addresses the need to measure critical multiple variables" (EPA, 2003a).

Multimetric indexes are composed of biological measures, called metrics, that show a consistent response to human disturbance (Karr and Chu, 1999). Metrics are converted to unitless scores and summed to obtain a summary index. Multimetric indexes have been developed for fish, birds, invertebrates, and algae in a variety of ecological contexts in North America, Europe, and Asia (Thorne and Williams, 1997; Hughes et al., 1998; Hughes and Oberdorff, 1998; Karr and Chu, 1999; Bryce et al., 2002; Fore and Grafe, 2002; Klemm et al., 2003). Most states use multimetric indexes for biological assessment, although other techniques have been developed (Hawkins et al., 2000; EPA, 2003b, c).

Background

Barbour et al. (1996) developed multimetric indexes for Florida that have been used since the early 1990s. The authors tested metrics by comparing values at sites with minimal human influence (reference sites) and sites with known disturbance (test sites). They identified three unique geographic regions based on the presence of shared taxa and defined metric expectations for each region.

In contrast, this study selected metrics on the basis of their correlation with a gradient of human disturbance (Bryce et al., 1999). The sensitivity and tolerance of over 1,000 taxa were also evaluated individually, using the human disturbance gradient (HDG). We used confidence intervals to estimate the

precision associated with the indexes. Working with a panel of regional experts, aquatic life use categories were derived to support management decision related to listing stream sites as impaired and prioritizing sites for additional evaluation as part of the TMDL (Total Maximum Daily Load) process (Karr and Yoder, 2004).

From the original analysis by Barbour et al. (1996), two multimetric indexes were developed for Florida streams based on different sampling and identification protocols. The Stream Condition Index (SCI) was derived from laboratory processing and the identification of samples, while the Biological Reconnaissance (BioRecon) Index was based on field sorting and laboratory identification. This study develops new bioassessment tools for both sampling protocols. For simplicity's sake, the names of the indexes have been retained for this new analysis, and the previous indexes are referred to as the old SCI and the old BioRecon Index within this document.

Methods

Study area

Portions of Florida have been repeatedly inundated by seawater in recent geologic history; as a consequence, elevation and organism recruitment from adjacent, noninundated areas play an important role in the present-day geographic distribution of macroinvertebrates. Florida can be divided geographically into the northern panhandle, the southern peninsula, and a transitional region known as the northeast. The middle and lower Suwannee Basin provides a natural demarcation between the panhandle and peninsula, with the northeast region straddling the upper Suwannee northeast of the Cody Escarpment (White, 1970).

In the Florida panhandle, rivers typically flow from north to south, and the elevation of headwater streams typically exceeds 200 to 250 feet. The recruitment of stream organisms (over geologic time) from upstream, higher elevation areas (Georgia and Alabama) makes the panhandle richer in freshwater stream taxa than other parts of the state (Florida Natural Areas Inventory [FNAI], 1990). Vegetation communities in the panhandle generally consist of mixed pine/oak/hickory forests (*Pinus* spp., *Quercus* spp., *Carya* spp.); longleaf pine forests (*Pinus palustris*); hardwood forests with a beech/magnolia climax community (*Fagus grandiflora*/*Magnolia grandiflora*); and swamp hardwood forests of cypress (*Taxodium* spp.) or tupelo (*Nyssa* spp.), interspersed with a mosaic of pine plantations, cropland (e.g., corn, soy beans, and peanuts), and pasture (Soil and Water Conservation Society (SWCS), 1989; Fernald and Purdum, 1992). The panhandle is less densely populated by humans than the other areas.

The peninsula has a sandy highland ridge extending down its center almost to Lake Okeechobee. The elevation of the central ridge is approximately 150 to 200 feet. Rivers west of the ridge flow into the Gulf of Mexico. Of the two large drainages east of the ridge, the Kissimmee River flows south into Lake Okeechobee, and the St. Johns River flows north to the Atlantic. Streams in the peninsula tend to have lower water velocity, flowing through wetlands and pine flatwoods (White, 1958). The peninsula has fewer freshwater invertebrate taxa due to the lack of upstream recruitment sources that did not experience marine inundation. Plecoptera, for example, are common in the panhandle but extremely rare in the peninsula, probably due to a combination of poor recruitment, warmer temperatures, and lower water velocities. Peninsular vegetation communities on the ridge consist of longleaf pine/turkey oak forests (*Pinus palustris/Quercus laevis*); on flat areas are slash pine (*Pinus eliottii*) or loblolly pine (*Pinus teada*) with a palmetto/gallberry understory (*Serenoa repens/Ilex glabra*); and in depressional areas are marsh/wet prairies (maidencane, pickerel weed) and hardwood wetlands of sweetbay (*Magnolia virginiana*), cypress (*Taxodium* spp.), and ash (*Fraxinus* spp.; SWCS, 1989). The dominant land use is pasture, cropland (e.g., watermelons, nursery products, and tomatoes), and urban areas (Fernald and Purdum, 1992). Dense population centers are located at Tampa and Orlando.

The northeast region includes portions of the Okeefenokee Swamp, parts of the upper Suwannee drainage, the Black Creek drainage, and the Sea Island flatwoods. From the perspective of stream invertebrates, this region tends to be intermediate between the panhandle and peninsula regions. With the exception of the Black Creek Basin, with headwaters approximately 200 feet above sea level, other streams in this area tend to be low-relief, wetland systems (Snell and Anderson, 1970). Plant communities consist of longleaf pine/turkey oak (*Pinus palustris/Quercus laevi)* on the sandy uplands; hardwood wetlands of cypress (*Taxodium* spp.), tupelo (*Nyssa* spp.), and loblolly bay (*Gordonia lasianthus*); pine flatwoods; and marsh (FNAI, 1990). Jacksonville is the region's only major population center.

Barbour et al. (1996) used macroinvertebrate stream samples collected from sites with minimal human influence to evaluate the influence of geographic locations on species assemblages. The authors found that the panhandle, peninsula, and northeast regions, which are primarily defined according to drainage patterns, were better predictors of species assemblages than were subecoregions described by Griffith et al*.* (1994). Regional differences translate into expectations of higher taxonomic diversity in the panhandle relative to the peninsula, with the northeast region representing a transitional area.

Site selection and datasets

The Florida Department of Environmental Protection (FDEP) has collected thousands of macroinvertebrate samples from stream sites throughout the state from 1991–2001. Many sites have been visited repeatedly. Sites and visits were selected from this large database using different criteria for different testing situations **(Table 1).** For example, when testing for metric correlation with disturbance, a range of site conditions was needed; to evaluate variability, sites with many repeat visits were needed; to evaluate laboratory subsampling, large samples were needed. Some datasets contained data from the same sites, and other sites were selected to provide an independent test. For some testing situations, data from site visits were averaged by sites—for example, when one variable in the analysis had only one value for each site, such as the HDG.

The relationship between human disturbance and biological condition was evaluated in several ways. To develop the HDG, FDEP biologists selected sites from their regions that represented the broadest possible range of human influence. First, to ensure that the relationships observed between human land use and biological metrics were consistent and not specific to a particular set of sites, 2 independent sets of sites were tested. These data were then combined to obtain 223 sites to test for metric correlation with the HDG. An additional, independent set of 23 sites was used to test the correlation between the SCI and HDG.

Additional datasets were compiled to estimate the variability of the biological metrics and indexes associated with repeat visits to a site, estimate the variability associated with laboratory subsampling, and evaluate the influence of field sorting versus laboratory sorting. To evaluate SCI variability, data from 62 sites with 2 to 8 visits were selected. About half the sites (32) had repeat visits on the same day. Most of the other repeat visits were during different years, and a few were within the same year. Thus, these data could not be used to estimate the influence of seasons on variability. Instead, additional data were compiled to test for seasonal (winter versus summer) differences in the SCI. To test the influence of subsampling during laboratory processing for the SCI protocol, 3 subsamples were selected from 59 samples collected from 54 sites (5 sites also had 2 duplicate samples each).

To test metrics and evaluate their variability for the BioRecon protocol, 2 additional datasets were used. The first dataset included 116 sites with macroinvertebrate samples collected according to both the BioRecon and SCI sampling protocols. We tested BioRecon metrics for correlation with the SCI and HDG. Most sites had at least 2 visits, and several had 3 to 6 visits. Of the 116 sites, 53 had values calculated for the HDG because they were used initially to develop the HDG. To evaluate the variability of the BioRecon metrics and index, 128 sites were selected, with 2 to 7 visits per site.

The dataset used to test the SCI and its component metrics against the HDG was large; therefore, simple scatter plots of data against the HDG would be difficult to interpret with the many data points and overstrikes. Consequently, we used box plots to display the range of values associated with different values of the HDG. In all figures, the box defined the 25th and 75th percentiles, the whiskers were the nonoutlier range, and the outliers were calculated as values greater (or less) than 1.5 times the length of the distance from the 25th to the 75th percentile value. During 1991–2001, laboratory protocols mandated a target number of 100 individuals; however, this target was often exceeded due to the laboratory protocols of the time. Several graphs include data from samples with 75 to 175 individuals in order to more closely match the target sample size. A more restrictive range (e.g., 75–125 individuals) would have resulted in too high a loss of sites for testing. Additional analysis (not shown) indicated that the ranges of metric values were similar for an upper limit of 125 and 175 individuals. In recent years (2004–2006), FDEP has modified laboratory protocols to more often match the target number of individuals **(see Appendix G)**. For the BioRecon testing, the number of individuals identified was not recorded; therefore, all site visits were used within a particular analysis, unless specifically stated otherwise.

Table 1. Description of various datasets, number of sites sampled, total number of visits to all sites (varied by site), and total number of subsamples taken in the laboratory for all visits

Some sites were included in more than 1 dataset, except for the 23 independent sites used to verify the correlation between the SCI and HDG.

Purpose	Sites	Visits	Lab Subsamples
Test HDG consistent for 2 rounds of data			
Round 1	154	469	0
Round 2	69	160	0
Test metrics vs. HDG	223	629	0
Score metrics (same data as above, but only samples with 75 to 175 individuals included)	176	420	0
Test sensitive and tolerant taxa versus HDG	226	632	0
Verify SCI with independent data	23	23	0
Determine SCI variability	62	220	0
Determine seasonal variation of SCI	78	578	0
Evaluate laboratory subsampling variability for SCI	54	59	177 (3 each)
Calibrate BioRecon metrics with SCI	116	165	0
Test BioRecon metrics versus HDG	53		0
Evaluate BioRecon variability	128	293	0

Quantifying human disturbance

Karr and colleagues describe five factors to summarize the ways in which humans alter and degrade rivers and streams (Karr et al., 1986; Karr et al., 2000): flow regime, physical habitat structure, water quality, energy source, and biological interactions. First, the natural flow regime of a stream may be altered by dams, water removal, or water return. In extreme cases, changes in water volume or flow timing can result in flooding or dry channels. Second, stream macroinvertebrates depend on a variety of physical habitat types for food, shelter, and reproduction. A greater diversity of substrate size, types of vegetation, and bank architecture translates into a greater variety of organisms. Third, water quality may

be evaluated in terms of turbidity, conductivity, nutrient concentrations, or the presence of other chemicals or metals. Fourth, energy source describes the seasonal availability, type of organic material, and size and shape of material available as food. Fifth, the introduction of exotic species or disease and the harvest of fish and shellfish for sport, commercial, and subsistence uses can alter biological interactions such as competition or predation within the natural assemblage.

Data were available to measure four of the five factors. FDEP biologists developed a hydrologic scoring system based on their knowledge of water removal, patterns of drought, and hydrographs for the sites **(Table 2).** To measure habitat condition, FDEP biologists routinely calculate a habitat condition index whenever stream macroinvertebrate samples are collected (FDEP, 2004). The index evaluates substrate condition and availability, water velocity, habitat smothering (e.g., by sand and silt), channelization, bank stability, and the width and condition of riparian vegetation. During the period of data collection, the habitat index was modified to include additional measures that raised the total number of possible points. To make habitat condition comparable across years, the total habitat score was converted to a percentage by dividing the score by the total possible points.

Table 2. Hydrologic condition of stream site, scoring range for hydrologic index, and description of human influences

The high or low values within each condition class were assigned at the discretion of the biologist based on the extent of disturbance. These changes were associated with human disturbances, not natural events such as hurricanes or extreme droughts.

Condition	Score	Description
Excellent	1–2	Flow regime as it naturally occurs (slow and fairly continual release of water after rains), few impervious surfaces; high connectivity with ground water and surface features delivering water (e.g., sandhills, wetlands; no ditches or berms)
Good	3–4	Flow regime minimally changed; some water withdrawals; some wetland drainage, some impervious surfaces, some ditching
Moderate	5–6	Flow regime moderately altered; hydrograph moderately flashy (scouring after rain events with subsequent reductions in flow), ground water pumping evident; much wetland drainage, topographic alterations reduce natural water input; more impervious surfaces, dams/control structures change normal water delivery schedule
Poor	7–8	Flow regime highly altered; hydrograph very flashy (scouring after rain events with subsequent reductions in flow, leading to stagnant or dry conditions related to large amounts of impervious surfaces and/or ditching); water withdrawals and impoundments or control structures severely alter flows; large areas of impervious surfaces
Very poor	9–10	Flow regime entirely human controlled; hydrograph very flashy (scouring after rain events with subsequent reductions in flow, leading to stagnant or dry conditions related to impervious surfaces and ditching); intensity of water withdrawals and impoundments fundamentally alter the nature of the ecosystem

FDEP biologists also routinely collect measurements of turbidity, temperature, dissolved oxygen, conductivity, and nutrient concentrations. Total phosphate (TP), ammonia (NH_3), total Kjeldahl nitrogen (TKN), and nitrites/nitrates (NO_x) were measured to summarize nutrients. We selected NH_3 to summarize water quality because it was the most consistently associated with the other water quality measures and had the most complete record of data. TP had similar high correlations and complete data, but may be

more closely associated with fertilizer and farming practices, while NH_3 may be a more general indicator of both urbanization and agriculture.

A measure of the type and quantity of energy available to organisms living within the stream was not available. However, Brown and colleagues have developed an index to estimate the intensity of human land use based on nonrenewable energy flow (Brown et al., 1998; Brown and Vivas, 2005). The Landscape Development Intensity (LDI) Index was calculated as the percentage area within the catchment of particular types of land use multiplied by the coefficient of energy use associated with that land use, summed over all land use types in the catchment **(Table 3).**

$$LDI = \sum \left(LDI_i * \%LU_i \right).$$

Where:

LDIi = the nonrenewable energy land use for land use i, and
%LUi = the percentage of land area in the catchment with land use i.

Brown and colleagues derived the coefficients for each land use type from actual billing records and published literature, translated reported energy use into standardized units, and then averaged the values by land use type and standardized to a per-unit area. The calculations used only nonrenewable energies, which included electricity, fuels, fertilizers, pesticides, and water (both public water supply and irrigation).

Land use was derived from aerial photos manipulated as layers in a geographic information system (GIS) computer program. The LDI was calculated in 2 ways. First, a buffer area of 100 meters on each side of the stream and 10 kilometers upstream of the sampling point was used (LDI_BF). Because the definition of the buffer region was somewhat arbitrary, the LDI was also calculated for the entire upstream catchment (LDI_WS) in order to determine which spatial scale was a better predictor of site condition. We selected LDI_BF to include in the HDG because it was slightly more highly correlated with NH_3, the hydrologic index, and the habitat index **(Table 4).**

To define the HDG, we converted the 4 measures of human disturbance to unitless scores and summed the scores to create HDG values for each stream site **(Appendix A)**. Three of the measures had values of 0, 1, or 2, indicating low, moderate, or high levels of human influence. One measure, hydrologic condition, had an additional value of 4 available to indicate extreme levels of disturbance **(Table 5).** Thus, values for the HDG ranged from 0 (indicating minimal human disturbance) to 9 (indicating extreme disturbance). The scores for the HDG were derived from a graphical analysis of EPT taxa richness plotted against each measure of disturbance. We identified values for each disturbance measure for which the number of EPT taxa was consistently lower. For NH_3, 9 sites had no information. Most of these sites had values for other measures indicating minimal human disturbance. We assumed for these sites that NH_3 was less than 0.1 milligram per liter (mg/L).

Table 3. Description of land use and the coefficient value used to calculate the LDI

Higher values indicate greater intensity of human land use (Brown and Vivas, 2005).

Land Use	LDI Value
Natural Open Water	1.00
Natural System (no human activity)	1.00
Pine Plantation	1.58
Woodland Pasture	2.02
Pasture	2.77
Recreational/Open Space (low-intensity)	2.77
Low-Intensity Pasture (with livestock)	3.41
Citrus	3.68
High-Intensity Pasture (with livestock)	3.74
Row crops	4.54
Single-Family Residential (low-density)	6.79
Recreational/Open Space (high-intensity)	6.92
High-Intensity Agriculture	7.00
Single-Family Residential (medium-density)	7.47
Single-Family Residential (high-density)	7.55
Low-Intensity Highway	7.81
Low-Intensity Commercial	8.00
Institutional	8.07
High-Intensity Highway	8.28
Industrial	8.32
Low-Intensity Multifamily Residential	8.66
High-Intensity Commercial	9.18
High-Intensity Multi-family Residential	9.19
Low-Intensity Central Business District	9.42
High-Intensity Central Business District	10.00

Table 4. Correlation table of NH_3 (mg/L), hydrologic index, habitat index, LDI for the buffer, LDI for the upstream watershed, natural log of watershed size, HDG, current SCI, and old SCI for 223 stream sites (except NH_3, for which n = 214)

Repeat visits to individual sites were averaged for each site. All values shown were significant (Spearman's r, p < 0.01).

	NH_3	Hydro	Habitat	LDI_BF	Ln (Size)	HDG	SCI
NH_3	-	0.43	–0.45	0.39	–0.20	0.50	–0.56
Hydro	0.43	-	–0.66	0.61	–0.36	0.84	–0.71
Habitat	–0.45	–0.66	-	–0.64	0.41	–0.78	0.66
LDI_BF	0.39	0.61	–0.64	-	–0.37	0.79	–0.60
LDI_WS	0.34	0.56	–0.55	0.74	–0.28	0.70	–0.59
Ln (Size)	–0.20	–0.36	0.41	–0.37	-	–0.41	0.36
HDG	0.50	0.84	–0.78	0.79	–0.41	-	–0.76
SCI	–0.56	–0.71	0.66	–0.60	0.36	–0.76	-
Old SCI	–0.43	–0.59	0.56	–0.54	0.29	–0.69	0.86

Table 5. Scoring rules for measures used to calculate the HDG

The HDG is the sum of the scores for each site.

Measure	0	1	2	3
NH_3 (mg/L)	<0.1	0.1–2	>2	
Habitat index (% of total possible points)	>65	50–65	<50	
Hydrologic index	<6	6–7	8–9	10
LDI	<2	2-3.5	>3.5	

Stream macroinvertebrate sampling

FDEP uses two protocols to collect invertebrate samples from streams. The protocols differ in the number of dip net sweeps collected, the location of sample sorting (field versus laboratory), and the final index used for assessment. The BioRecon sampling protocol was designed to be a quick field assessment that could yield a same-day measure of stream site condition. The SCI sampling protocol was meant to be more precise. Both protocols use a D-frame dip net with a 0.25-meter opening and a 600-micrometer mesh net. Both protocols distribute the sweeps among the best available substrates on an approximately equal basis. Florida streams typically have a sandy substrate that supports very few invertebrates. Therefore, the habitat sampled for macroinvertebrates includes logs, roots, and undercut banks; partially decayed leaf packs; aquatic vegetation; and rocks or large cobble. During SCI sampling, if 4 productive substrates are present (snags, roots, vegetation, and leaf packs), each substrate would be sampled 4 times, with sand being sampled 4 times, for a total of 20 sweeps.

In the laboratory, the SCI sample is divided by spreading it onto gridded trays. Squares are randomly selected, and all the material within a square is sorted. Macroinvertebrates are identified to the lowest practical taxonomic level. For the 2004 to 2006 time period, squares were selected until the target sample size of 100 individuals was reached. To avoid bias, the final square selected is always finished,

even if it contains more than 100 individuals. The current method targets two separate aliquots of 150 individuals. The number of taxa and the relative abundance of each taxon are derived from these data.

BioRecon data are collected using four dip net sweeps from a stream site. Samples are sorted for live macroinvertebrates in the field. The sample is spread in a pan, and the biologist searches through all the material for every unique taxon present. Each taxon is preliminarily identified in the field, and representative specimens of each taxon are preserved and returned to the laboratory for final identification under a dissecting microscope. From this field-sort protocol, the number of taxa present at a site can be measured, but their relative abundance (% composition) cannot.

Metric development and testing

Several authors have described relevant candidate metrics for stream macroinvertebrate assemblages (Barbour et al., 1996 and 1999; Kerans and Karr, 1994; and Klemm et al., 2002). For this study, we evaluated 36 candidate metrics related to taxonomic richness, feeding group, voltinism, habit, community structure, and tolerance or sensitivity for their association with the HDG **(Table 6).** Within each category, we selected metrics with the highest correlation with the HDG (Spearman's *r*). We used one-sided tests to calculate *p*-values because if a metric indicated better biological condition in response to increased human disturbance, that relationship would not be *biologically* significant even if it were statistically significant. All metrics were also evaluated graphically to ensure that they were reliable indicators of disturbance. We tolerated variability in metric values for minimally disturbed sites (low HDG) because not all sources of disturbance were known. For example, water chemistry data were unavailable for metals, pesticides, or other toxics. In contrast, we considered an inconsistent response at more highly disturbed sites to be unacceptable.

Taxonomic richness

Richness was calculated as the number of unique taxa found within a particular group, such as the Ephemeroptera, Plecoptera, or Trichoptera, or their combination (EPT).

Feeding group

Trophic designations followed Merritt and Cummins (1996); FDEP biologists and others modified some designations to reflect natural histories in Florida (Merritt et al., 1996). All feeding group metrics were calculated as the number of individuals of a particular group, divided by the total number of individuals in the sample times 100 (percentage).

Voltinism

Most stream insects spend the majority of their lives as larvae underwater and emerge as adults to mate. The length of time between generations, which is called voltinism, may be less than a year, approximately one year, or greater than one year. We used published sources to identify taxa that require more than one year to complete their life cycles (**Appendix B**; Brigham et al., 1982; Thompson, 1984; Thorp and Covich, 1991; Pescador et al., 1995; Corbet, 1999; Pescador et al., 2000; Smith, 2001; Rasmussen and Pescador, 2002). These taxa require consistent flows and other favorable conditions throughout the year to complete their life cycles. Data on generation length were not available for all Florida taxa; therefore, the current list may not include some long-lived taxa.

Table 6. Candidate metric, correlation with the HDG, and whether the metric was included in the final SCI, BioRecon, or original SCI

Correlation was tested for 223 sites, with repeat visits to each site averaged before statistical testing. Hyperabundance was tested without averaging, n = 577. All correlations shown were significant at p < 0.01 (Spearman's r, one-sided test).

Metric	HDG	SCI	BioRecon	Old SCI
Taxonomic richness				
EPT	–0.73			yes
Trichoptera	–0.66	yes	yes	
Ephemeroptera	–0.66	yes	yes	
Total taxa	–0.52	yes	yes	yes
Diptera	–0.44			
Chironomidae	–0.40			yes
Plecoptera	–0.37			
Oligochaeta	0.32			
Noninsect	-			
Feeding group				
% Filterer	–0.46	yes		yes
% Scavenger	–0.38			
% Browser/grazer	–0.30			
% Collector/gatherer	0.29			
% Predator	–0.21			
% Plant piercer	–0.18			
% Parasite	-			
% Scraper	-			
% Shredder	-			
Voltinism				
Long-lived taxa	–0.41	yes	yes	
% Long-lived	–0.35			
Habit				
Clinger taxa	–0.71	yes	yes	
% Clinger	–0.61			
Community structure				
% Dominance	0.47	yes		yes
Hyperabundance	-			
% Ephemeroptera	–0.58			
% Trichoptera	–0.55			
% Tanytarsini (Chironomidae)	–0.45	yes		
% Plecoptera	–0.37			
% Oligochaeta	0.32			
% Non-insect	0.32			
% Chironomidae	-			
% Diptera	-			yes
Sensitivity/tolerance				
Sensitive taxa	–0.75	yes	yes	
Florida index (sensitive)	–0.71			yes
% Very tolerant	0.70	yes		
Very tolerant taxa	0.61			

The list included all taxa in the family Cordulegastridae and selected taxa in the families Aeshnidae, Gomphidae, and Libellulidae (Odonata); the family Leuctridae and selected genera in the families Perlidae and Pteronarcidae (Plecoptera); the family Corydalidae (Megaloptera); and the genera *Ceraclea, Pycnopsyche, Molanna,* and *Rhyacophila* (Trichoptera). Several Coleoptera taxa (beetles) also require more than a year to complete their life cycles, but were not included because the FDEP database does not currently distinguish between larvae (which cannot travel to find water) and adults (which can). Noninsects included in the long-lived list were all taxa in the family Decapoda, the genera *Fossaria* and *Pomacea* (Gastropoda), and the families Unionidae and Corbiculidae (Pelecypoda).

Habit

Other studies have noted a strong correlation between the taxonomic richness of clingers and stream condition (Fore et al., 2001; Karr and Morishita Rossano, 2001; Mebane, 2002). Clingers have morphological and behavioral adaptations that allow them to cling to substrate (Merritt and Cummins, 1996). Channel dredging and the removal of riparian vegetation eliminate the habitat preferred by clingers.

Community structure

Percentage dominance was calculated as the number of individuals in the most abundant taxon, divided by the total number of individuals in the sample. ”Hyperabundance” represented a measure of the density of stream macroinvertebrates present at a site. Sites with high organic enrichment (e.g., sewage effluent) may have a high density of invertebrates due to the increased source of food for many species. The actual number of individuals present in the total sample was not recorded; however, the number of grids in the sample tray that were searched to best approximate the target number of 100 individuals was recorded. We used the number of grid cells searched as a measure of the density of invertebrates present, or hyperabundance. Other measures of community structure were based on the number of individuals within specific taxonomic groups divided by the total number of individuals.

Sensitivity and tolerance

Beck (1954) developed a list of sensitive taxa for Florida that was used to calculate a biotic index, which was later modified to be the Florida Index. To calculate the Florida Index (which is more accurately termed a metric), very sensitive taxa are given a score of 2 and sensitive taxa a score of 1. The scores are summed to calculate this metric.

We used the HDG to evaluate the sensitivity and tolerance of 1,195 taxa names. The taxa names included taxa that were not unique because the database included synonyms for the same taxa, as well as specimens identified to genus and species for the same genus. Because many sites had multiple visits and because less-disturbed (reference) sites tended to have a greater number of visits, we summarized data for each taxon *by site* to avoid bias associated with greater sampling effort at less-disturbed sites. We counted a taxon as present at a site if it was found for >= 50% of the site visits. We divided the 226 sites into 2 groups and, for simplicity’s sake, refer to these groups as “good” and “poor” for statistical testing. Good sites were defined as those with moderate levels of disturbance for no more than 2 measures (e.g., the LDI or NH_3) or high disturbance for no more than 1 measure (e.g., habitat condition). This translated into HDG <= 2 and included 141 sites. The remaining 85 sites were classified as poor. Thus, the 2 groups might be more accurately labeled better and worse, rather than good and poor. The relatively high number of sites selected to represent good condition reflects the large number of reference sites in the dataset.

We used contingency table analysis to determine what percentage of taxon occurrences in the good-versus-poor range of HDG values represented a statistically significant difference (chi-square test, Yates continuity correction). As for any test, the number of sampling points increases the power of the

test, such that less extreme difference will be statistically significant. For example, if a taxon occurred at 10 sites, all of the occurrences (100%) would have to be in the good range of the HDG to be statistically significant ($p < 0.05$). In contrast, if a taxon occurred at 38 sites, it would only need to occur at 30 of the good sites (79%) and would still be statistically significant.

For this reason, we chose not to use a strictly statistical criterion for selecting sensitive taxa. Instead, for more common taxa we only selected those with 87% or more of their occurrences in good sites. Using this criterion of 87%, if a taxon occurred at 30 sites, 27 or more of the sites would have to be in the good range of the HDG. The *p*-value associated with this would be $p < 0.002$. A strictly statistical criterion would have selected taxa that *prefer* good conditions, rather than taxa that *require* good conditions. Thus, we used a statistical rule to define the minimum number of occurrences needed for statistical significance but made the standards for selection more strict ($p << 0.05$) for taxa that were more common.

Defining a statistical rule for the selection of tolerant taxa was not possible, because tolerant taxa can also "tolerate" good conditions. Instead, very tolerant taxa were defined as those found in at least 10 sites and for which >= 50% of their occurrences were in the poor range of the HDG. In some cases, multiple species within a genus failed to meet the criteria for inclusion as sensitive (or very tolerant) because they were not present at enough sites. If several species within a genus (or family) were consistently found at good (or poor) sites, we combined the species into the parent genus (or family) and evaluated the genus (or family).

For the BioRecon data, we only tested metrics used in the SCI in order to make the indexes as similar as possible. Relative abundance metrics could not be calculated for these data; therefore, only taxa richness metrics were evaluated. BioRecon metrics were tested for correlation with the SCI and HDG.

Index development and testing

We defined 2 indexes by selecting metrics that showed the most consistent response to the HDG. One index was developed for the SCI protocol (20 dip net samples and laboratory sorting of macroinvertebrates) and another for the BioRecon protocol (4 dip net samples and field sorting).

For the SCI, we transformed metrics into unitless scores by determining the 95th percentile values for each metric and then dividing each metric value by its range and multiplying by 10. Metrics that declined with disturbance were scored from 0 to 10, and metrics that increased with disturbance were scored from 10 to 0, so that the best biological condition was consistently scored as a 10. For skewed metrics, the natural log was calculated before dividing by the metric range. This process was done separately for the northeast, peninsula, and panhandle regions. We tested for differences in metric expectation associated with different regions by comparing the regression lines for each metric against the HDG and adjusted metric scoring as necessary to ensure similar responses for all metrics in the 3 regions.

For the BioRecon Index, we scored metrics to match the SCI metric scores as closely as possible. Using histograms to evaluate the overlap of the BioRecon and SCI metrics measured at the same sites, we adjusted metric scoring for the BioRecon metrics as needed. In order to distinguish between the BioRecon and SCI, BioRecon metrics were scored from 0 to 1 (rather than 0 to 10). In this way, the index values provide a quick clue as to which index is being reported.

After correlation with disturbance was confirmed for both indexes, we evaluated different sources of variability, either due to natural sources (e.g., watershed size or season) or the sampling method (e.g., laboratory subsampling). We used correlation to test for dependence of the SCI on watershed size. To test the influence of season on the SCI, 78 sites with summer and winter samples were compared using a paired *t*-test. Summer was defined as May through October and winter as November through April. Within each season, data for repeat visits to each site were averaged.

To evaluate other sources of variance, such as site differences, year differences, and repeat visits, we used an ANOVA model to estimate variance components. For the SCI and BioRecon, we estimated variance associated with site, year, site x year interaction, and error. Error was defined as variance associated with repeat visits to the same site within a year. For the SCI data, most of the repeat visits within a year occurred on the same day, rather than at different times during the year. For the BioRecon, most of the repeat visits were on different days within the same or a different year; very few were same day.

Error estimates from ANOVA (mean squared error) were used to calculate the 90% confidence interval for both indexes and the number of categories of biological condition that each index can reliably detect (Zar, 1984). Confidence limits were calculated as:

$$\text{SCI} \pm \left(\sqrt{\frac{s^2}{n}} * 1.645 \right),$$

Where:

s^2 = error variance estimated from ANOVA (mean squared error), and
n = number of samples taken from the site.

To calculate the number of categories each index could detect, we divided its range (0 to 100 for the SCI and 0 to 10 for the BioRecon) by the confidence interval. Other statistical approaches could be used to calculate the number of categories of biological condition that the indexes can reliably detect. For example, a 90% confidence interval could also be constructed based on the *difference* in SCI values for 2 sites. This approach would yield a smaller confidence interval: instead of *n* = 1 in the equation above, *n* would equal 2 because 2 SCI values would be involved in calculating the difference. Currently there are no standards for which statistical model to use; therefore, we used confidence intervals around the SCI for a single site because they are conceptually simple and somewhat conservative from a statistical point of view. In other words, this approach is less likely to be controversial (Johnson, 1999; Hoenig and Heisey, 2001). The number of categories of biological condition that an index could reliably detect were only used to summarize the precision of the indexes. The assessment categories used to define biocriteria for water quality standards were derived from an entirely different process (see Chapter 4: *Application of Biomonitoring Tools* below).

We also used an ANOVA model to estimate variability associated with laboratory subsampling. For this model, data from 59 sites with 3 subsamples each were used to estimate site variance and error variance. For this model, error was defined as the differences associated with subsamples from the same site visit.

Statistical model assumptions

The method described here for calculating confidence intervals assumes that the multimetric indexes are normally distributed. Other studies have evaluated the statistical properties of multimetric indexes and found that multimetric indexes for fish and invertebrates are unimodal and symmetric (Fore et al., 1994; Doberstein et al., 2000; Blocksom, 2003); thus, an underlying normal distribution is an appropriate assumption for multimetric indexes. A separate and additional consideration is whether to use *z*-values from the normal distribution to calculate 90% confidence limits or values from Student's *t* distribution. For small sample sizes, the *t*-distribution should be used for calculating confidence limits

because variance may be underestimated; for large sample sizes (df > 30), however, the two distributions converge. We used data from 61 sites to estimate variance; therefore, *z*-values were appropriate.

The 2 datasets used to estimate variance for the SCI and BioRecon Index were unbalanced—that is, the number of visits to each site varied for each site. In contrast, the dataset used to evaluate laboratory subsampling was balanced with 3 subsamples for each site visit. ANOVA assumes a balanced design but is fairly robust to violations of this assumption (Milliken and Johnson, 1992). The primary consequence of an unbalanced design is related to the significance testing of model effects against the error term, which was not the purpose of this analysis. Rather, the goal for these ANOVAs was to estimate an error to use in the confidence interval calculations and to evaluate the relative contributions of year and error to the metrics and indexes. For estimation of the error term, the large sample sizes mitigate any potential inaccuracy associated with unbalanced cells. Sample sizes greater than about 30 are considered large, and this analysis had 61 sites for the SCI and 128 for the BioRecon.

CHAPTER 2: RESULTS

Human disturbance gradient

The human disturbance gradient (HDG) was a consistent predictor of biological condition for two independent rounds of testing. When metrics were regressed against HDG for the two rounds of data, the slopes and intercepts of the regression lines were nearly identical, indicating that measures included in the HDG yielded a reliable measure of human disturbance **(Figure 1).**

Correlations among the four measures of human disturbance used to define the HDG were high, indicating good agreement among the different measures **(see Table 4).** All the individual measures, however, were more highly correlated with the HDG, suggesting that the HDG was a better overall measure of general human disturbance. The SCI showed a similar pattern and was more highly correlated with the HDG than the individual measures of human disturbance.

Metric selection

On average, 30 insect taxa were found in each sample for the 629 site visits used to test metrics. Diptera dominated the samples, with 23 taxa counted on average; of these taxa, about half were in the family Chironomidae. Of the remaining groups, 3 oligochaete, 2 Trichoptera, 1 Ephemeroptera, and 1 Plecoptera taxa were found, on average.

For many metrics, the change in values from least to most disturbed sites was dramatic. We compared the 20 best sites (HDG = 0 and the lowest values for LDI_WS) with the 18 worst sites (HDG > 6) and found that total taxa richness went from 33 to 19, on average. The number of Ephemeroptera taxa declined from 2 to 0, Trichoptera taxa from 4 to 0, clinger taxa from 8 to 0, and sensitive taxa from 9 to 0. The relative abundance of filterers declined from 22% to 3%, while the relative abundance of tolerant individuals increased from 5% to 69%.

In general, we selected at least one metric from each of the categories below according to which metric was most highly correlated with the HDG. If two metrics were calculated from the same set of taxa (redundant metrics), we only included one metric.

Taxonomic richness

EPT taxa richness was most highly correlated with the HDG, followed by Trichoptera and Ephemeroptera taxa richness **(see Table 6).** Both taxonomic groups disappeared from samples with high levels of disturbance **(Figure 2).** Correlation between the HDG and Plecoptera taxa richness was somewhat lower, primarily because very few plecopterans were found in samples from the peninsula region. We selected Ephemeroptera and Trichoptera taxa richness as separate metrics because they have the potential to respond to different types of disturbance. We did not select Plecoptera taxa richness as a metric because it could only be applied in two of the three regions. Total taxa richness had the next highest correlation and was selected as an indicator of the variety of taxa that a stream site could support.

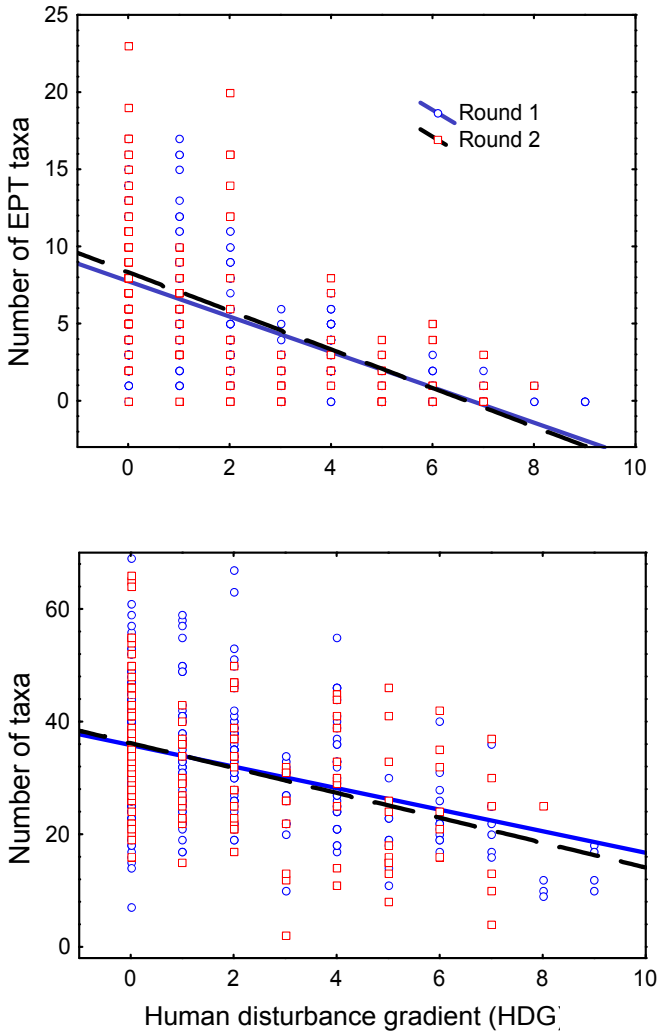

Figure 1. Number of EPT taxa and total number of taxa declined as human disturbance increased. Nearly identical regression lines for two independent sets of sites (rounds 1 and 2) indicated a consistent relationship between HDG and biological condition.

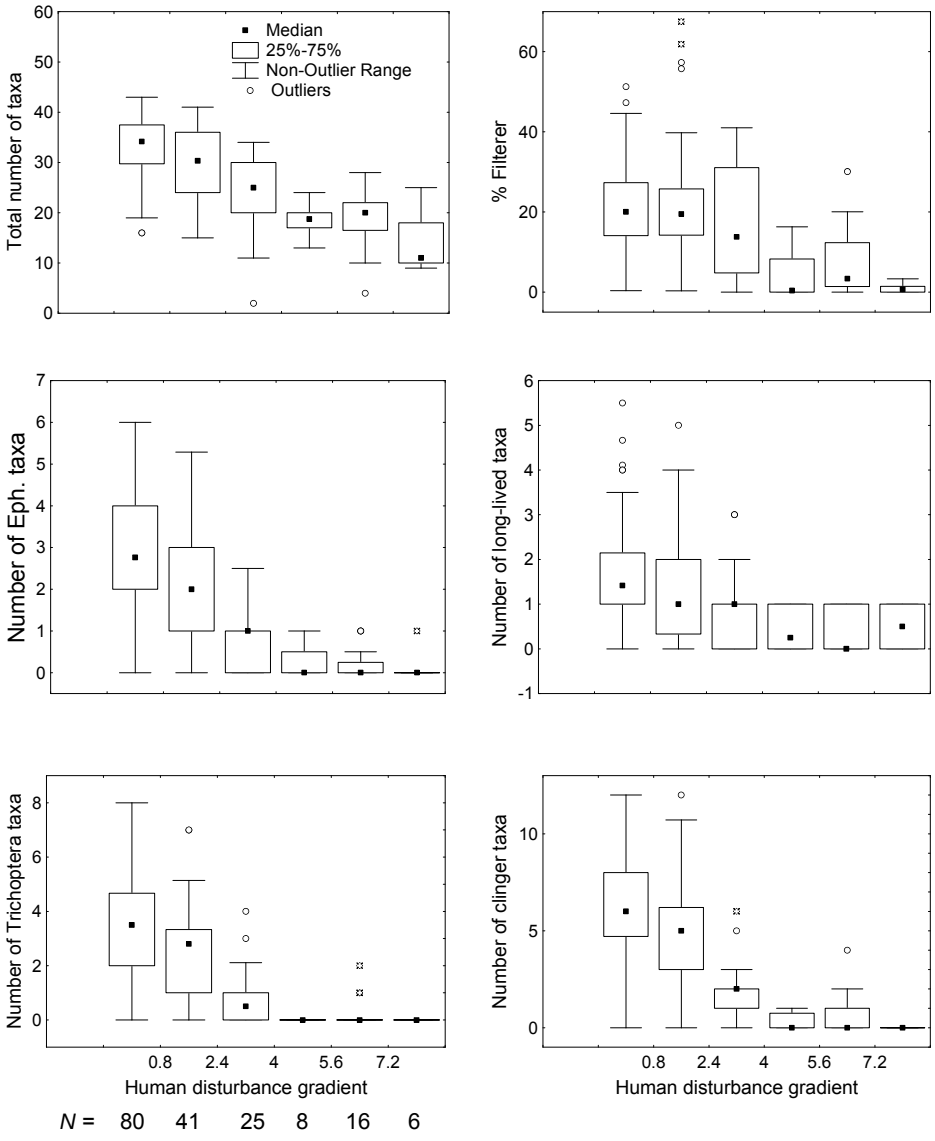

Figure 2. Six of the ten metrics included in the SCI plotted against the human disturbance gradient. All metrics declined with an increase in human disturbance. Metric values were averaged for each of the 176 sites. Samples with greater than 175 or less than 75 individuals were excluded before averaging. Number of sites within each box are shown (*N*). Plotted values are less than or equal to values on x-axis.

Feeding group

The relative abundance of filterers (percentage filterer individuals) had the highest correlation with the HDG and the most consistent relationship with the HDG when graphed. Other feeding groups failed to show the consistent decline with disturbance that percentage filterer did. We also tested this metric without *Cheumatopsyche* (a somewhat tolerant genus of Trichoptera), but the changes in correlation were very small, and so all taxa were retained (Spearman's r = –0.46 with *Cheumatopsyche* and –0.47 without).

Voltinism

Correct information about generation times was not easily obtained for all Florida taxa; therefore, we only tested long-lived taxa richness and percentage long-lived individuals. Long-lived taxa included semivoltine insects and noninsects that require more than one year to complete their life cycles. Long-lived taxa richness was more highly correlated with the HDG than was relative abundance of long-lived individuals; consequently, long-lived taxa richness was included in the SCI.

Habit

Clinger metrics measured either as taxa richness or as percentage of individuals were among the most highly correlated metrics with the HDG. We selected clinger taxa richness for inclusion in the SCI.

Community structure

Percentage dominance increased with the HDG and was included in the SCI **(Figure 3)**; hyperabundance failed to correlate with the HDG. Although percentage Ephemeroptera and Trichoptera individuals (relative abundance) were both strongly correlated with the HDG, they were calculated from the same information used to calculate the taxa richness of those two groups. Because the taxa richness metrics were more highly correlated with the HDG, the relative abundance of Ephemeroptera and Trichoptera was not included in the index. Correlation for the relative abundance of Tanytarsini midges was not quite as high as the other composition metrics (–0.45), but was included in the SCI as the best available measure of the chironomid assemblage.

Sensitivity and tolerance

The number of taxa selected as sensitive equaled ~12% of the taxa tested, and the number of very tolerant taxa was ~10% of the taxa tested. Many sensitive taxa belonged to the Ephemeroptera or Trichoptera; several chironomids were also included **(Appendix C).** All the Plecoptera were included, though most of the genera and species in this group were too rare to test. Considered as a group, out of 191 occurrences of Plecoptera taxa, only one was found in the poor section of the HDG. All Plecoptera taxa showed a strong tendency to be found in the best of the good (minimally disturbed) sites. Many of the sensitive taxa selected by this study were previously noted by Beck (1954) and used in the Florida Index, but several taxa were new to the list. Many of the taxa included on Beck's list were too uncommon to test with this dataset.

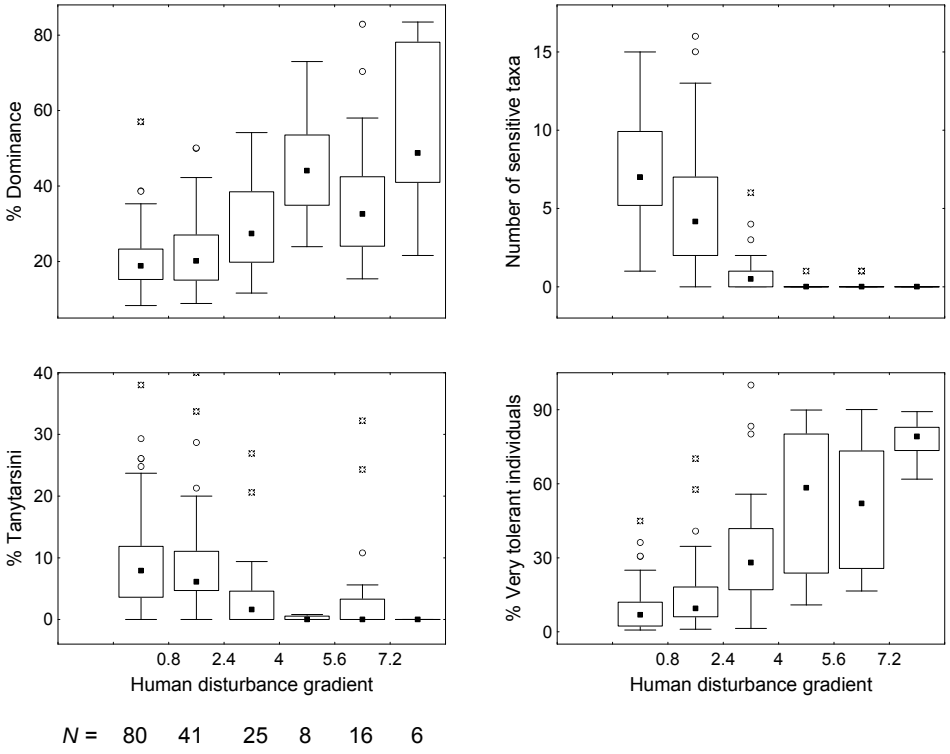

Figure 3. Four of the ten metrics included in the SCI plotted against the human disturbance gradient. Percent dominance and very tolerant individuals increased with disturbance; sensitive and Tanytarsini midge taxa declined. Metric values were averaged for each of the 176 sites. Samples with greater than 175 or less than 75 individuals were excluded before averaging. Number of sites within each box are shown (*N*).

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The very tolerant list included one odonate (*Argia sedula*) and several chironomid taxa (*Larsia spp.*, *Cricotopus bicinctus,* and *Polypedilum illinoiense* grp.) previously noted as sensitive and included in the Florida Index **(Appendix D).** The very tolerant list was dominated by noninsects and chironomids, along with several odonate taxa. One species of tubificid worm, *Limnodrilus hoffmeisteri*, was included as very tolerant, although it was present at 47% of the poor sites that failed to meet the criterion of > = 50% of poor sites. *L. hoffmeisteri* was included as very tolerant because it was very common at all sites. It occurred at 70 out of 85 poor sites, and tended to increase in relative abundance with disturbance. Other studies have also reported tolerance of *L. hoffmeisteri* to a variety of disturbance types (Chapman, 2001; Chapman et al., 1982)

The total number of taxa with 10 or more occurrences was 168. When a large number of tests are conducted, questions arise regarding the probability of Type I error. The detection of a statistically significant result will occur with the approximate frequency of *p*-value. For a *p*-value of 0.05 and 168 tests, approximately 8 taxa may be selected by chance alone (0.05 * 168 = 8.4). The number of sensitive taxa falsely selected from this analysis is likely much lower because the criterion for selection was more strict than statistical significance. In other words, the *p*-values associated with 87% occurrence of more common taxa, e.g., taxa present at 20 or more sites, were much smaller.

BioRecon metrics

The six taxa richness metrics calculated from BioRecon data were significantly correlated with the HDG and highly correlated with the SCI (*r* ranged from 0.60–0.73; **Table 7**). The four SCI metrics calculated as percentages could not be tested because relative abundance was not recorded for BioRecon data. For this reason, we tested the very tolerant metric in terms of *taxa richness* rather than as a percentage. Its correlation was poor with the HDG and nonsignificant with the SCI, and therefore it was not included in the BioRecon Index.

Multimetric index construction and evaluation

Ten metrics were selected for inclusion in the SCI, and six were selected for the BioRecon Index. Most of the SCI metrics required regional scoring (**Table 8; Appendix E;** FDEP, 2007)**.** In general, taxa richness tended to be higher in the panhandle region than in the peninsula or northeast.

For the SCI, the highest correlation between metrics occurred between Trichoptera and clinger taxa (Spearman's *r* = 0.77) and clinger and sensitive taxa (*r* = 0.84). To determine whether these metrics were redundant, we evaluated the taxa included in each metric. Of the clinger taxa, approximately 41% were also sensitive taxa, and 33% were also Trichoptera taxa. Thus, we concluded that the statistical correlation was due to an underlying association with human disturbance, rather than redundant information.

Two metrics were somewhat skewed, that is, their distributions had long "tails" with sparse values at one end of their distribution. For these metrics, we calculated the natural log of metric values before dividing by the range of the metric values. In this way, metric scores were more evenly spread between 0 through 10. Two SCI metrics increased with disturbance (% dominance and % very tolerant individuals); all others declined with disturbance.

BioRecon metric scoring rules differed somewhat from SCI metric scoring. Taxa richness tended to be lower in two regions, while Ephemeroptera taxa richness was much higher in the panhandle **(Table 9).** More long-lived taxa tended to be found in all three regions for BioRecon samples.

Table 7. Correlation of BioRecon metrics and the old and new BioRecon Indexes with the HDG and SCI

Metrics included in the BioRecon Index are noted (*). All correlations shown were statistically significant (Spearman's r, p < 0.01, one-sided test, n = 116 for SCI, n = 53 for HDG). Data from multiple visits were averaged for each site.

Metric/Index	HDG	SCI
BioRecon Index	-0.58	0.75
Total taxa*	-0.36	0.53
Ephemeroptera taxa*	-0.47	0.62
Trichoptera taxa*	-0.65	0.68
Long-lived taxa*	-0.46	0.65
Clinger taxa*	-0.48	0.67
Sensitive taxa*	-0.63	0.69
Very tolerant taxa	NS	NS
Old BioRecon Index	-0.41	0.58
EPT	-0.61	0.71
Florida Index (metric)	-0.53	0.69

Table 8. SCI metric name and range of metric values used to assign a score of 0 (indicating degraded condition) to 10 (indicating minimal disturbance) by region

Metric values higher or lower than the listed range were assigned a score of 0 or 10 as appropriate. Metric ranges listed in reverse order indicate metrics that increased (rather than declined) with disturbance. For two marked metrics (*), the natural log was calculated for the range of values before scoring.

SCI Metric	Northeast	Panhandle	Peninsula
Total taxa	16–42	16–49	16–41
Ephemeroptera taxa	0–3.5	0–6	0–5
Trichoptera taxa	0–6.5	0–7	0–7
% Filterer	1–42	1–45	1–40
Long-lived taxa	0–3	0–5	0–4
Clinger taxa	0–9	0–15.5	0–8
% Dominance	54–10	43–10	54–10
% Tanytarsini *	0–26	0–26	0–26
Sensitive taxa	0–11	0–19	0–9
% Very tolerant *	78–0	36–0	59–0

Table 9. BioRecon metric name and range of metric values used to assign a score of 0 (indicating degraded condition) to 1 (indicating minimal disturbance) by region

Metric values higher or lower than the listed range were assigned a score of 0 or 10 as appropriate. Underlined text indicates metric ranges that differed from those for the SCI.

BioRecon Metric	Northeast	Panhandle	Peninsula
Total taxa	**14–37**	16–49	**11–36**
Ephemeroptera taxa	0–3.5	**0–12**	0–5
Trichoptera taxa	0–6.5	0–7	0–7
Long-lived taxa	**0–6**	**0–10**	**0–7**
Clinger taxa	**0–7**	0–15.5	0–8
Sensitive taxa	0–11	0–19	0–9

The SCI was highly correlated with the HDG, with minimal overlap between values for extremely disturbed and minimally disturbed sites **(Figure 4).** The BioRecon Index was also highly correlated with the HDG and was more responsive across the range of human disturbance than the original BioRecon Index **(Figure 5).**

Regional scoring at the metric level resulted in an SCI that showed a similar response to human disturbance within each region. The regression lines for the SCI versus HDG for the northeast and peninsula regions were nearly identical; the line for the panhandle region diverged somewhat from the other two regions for more degraded sites **(Figure 6; Appendix F).** The divergence was associated with two sites in the panhandle with SCI values that were higher than expected. We did not alter the metric scoring rules, because several other panhandle sites did obtain the lowest SCI values for high levels of disturbance.

We calculated the natural log of the area upstream of the sampling site to test for correlation between the SCI and watershed size. Watershed size had an underlying correlation with the HDG **(see Table 4)** because the most disturbed sites tended to have small watersheds. To avoid this confounding association, we divided the sites into 3 groups (low, medium, and high disturbance) before testing for correlation between the SCI and watershed size. The low disturbance group of sites had HDG values equal to 0. We defined medium disturbance as HDG values from 2 to 5 and high disturbance as HDG values > 5. The SCI was not significantly associated with watershed size for any of the 3 levels of disturbance (**Figure 7;** Spearman's $r = –0.01, –0.005, 0.12$, $p > 0.5$).

The relationship between the SCI and HDG was consistent across years as well. When site visits were divided into 2-year segments for 10 years of sampling (1992–2001) and the SCI regressed against the HDG, the 5 regression lines were nearly identical **(Figure 8).** This result indicates that the pattern of association between the SCI and HDG was independent of year.

To verify that the SCI was correlated with the HDG, we used an independent set of 23 sites not used in any of the previous analyses. Correlation was high (Spearman's $r = –0.81$, $p < 0.001$; **Figure 9**). Two sites with > 300 individuals in the samples had somewhat higher SCI values than predicted by the HDG, possibly due to the inflation of taxa richness measures associated with large samples.

Statistical precision of indexes

During the 2004-2006 period, the target number of individuals for laboratory identification for the SCI was 95 to 115. For the metric testing dataset, only 27% of the 629 site visits met this target. Most samples had well over 100 individuals. For these data, 32% of the samples had > 175 individuals, with some samples ranging up to hundreds of individuals. At the other end of the scale, only 36 samples (6%) had fewer than 90 individuals in the entire sample.

When we compared small sample sizes with standard (or large) sample sizes from the same sites, samples with < 75 individuals contributed a disproportionately large amount of variability to the repeat visits **(Figure 10).** SCI values were typically much lower for these small samples. For this reason, we excluded these 17 samples from the analysis of variability, which reduced the number of sites from 62 to 61.

Repeat visits to the same sites were used to evaluate the sources of variability for the SCI and BioRecon. The sources of variance included differences associated with sites, years, visits within years, season (summer versus winter), and laboratory subsampling.

From the ANOVA analysis, the variance associated with sites was greatest (304.9, 71%), the variance associated with year was relatively small (7.7, 2%), and the variance components associated with site x year interaction and error were similar (54.6, 12%, and 66.6, 15%; **Figure 11; Appendix F**). For this model, error was defined as repeat visits within years, about half of which were on the same day.

We used the estimate of error variance from the ANOVA to estimate the 90% confidence limits around the SCI as +/– 13.4 points, or a 27-point spread. Graphic representation of this range around the observed values for repeat visits captured all visits to the least disturbed sites (SCI values > 70), but failed to include all the visits to sites with lower SCI values **(Figure 10).** These sites graphically illustrate the site x year variance, that is, certain site-year combinations that changed in unique ways. The fact that undisturbed sites did not show this pattern, while disturbed sites did, suggests that the SCI was measuring real changes in biological condition, rather than “noise” associated with natural variability. Using the length of the 90% confidence interval (27 points) to divide the range of the SCI (0 to 100) yielded 3.7 categories of biological condition that SCI could reliably detect based on a single site visit.

Variance components calculated for the SCI component metrics showed similar patterns to the SCI. Site differences and site x year variance typically contributed the largest percentages to the overall variance **(Figure 12).** The exception was long-lived taxa richness, which had a large error component (with error defined as variance associated with repeat visits within years, as above). The least variable metrics were Ephemeroptera, clinger and sensitive taxa richness, and % very tolerant. Overall, the SCI was less variable than most of its component metrics.

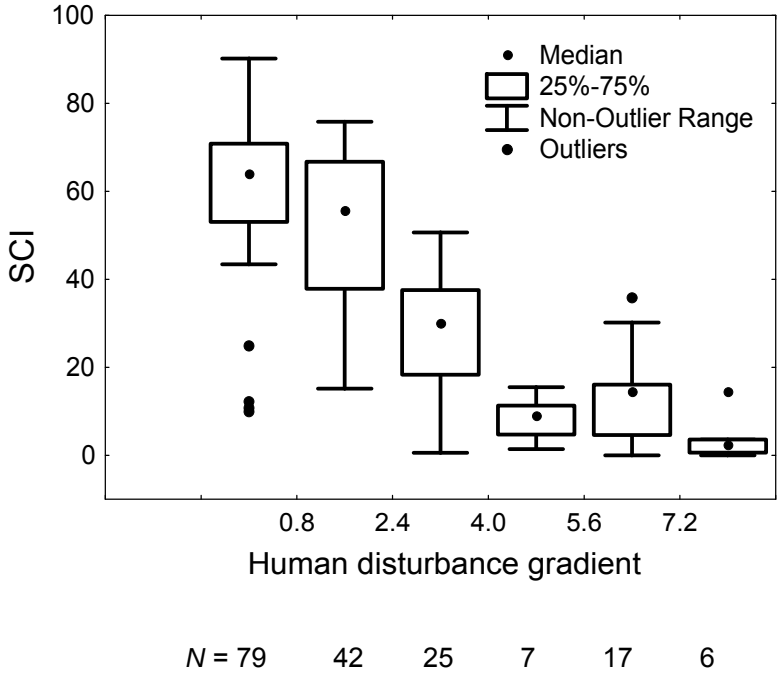

Figure 4. SCI declined as human disturbance increased. Index values were averaged for each of the 176 sites. Only samples with 75-175 individuals are shown. Number of sites in each box are shown (*N*).

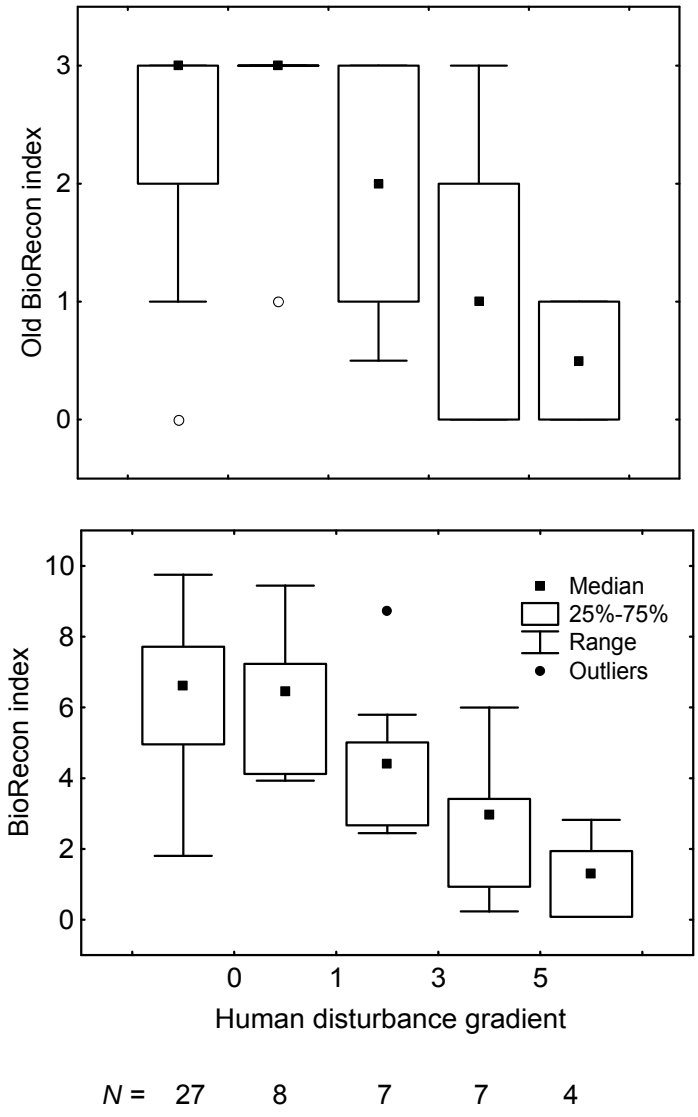

Figure 5. Original BioRecon index based on three metrics (total taxa, EPT taxa, and Florida index; upper panel) and current BioRecon index based on six metrics (lower panel). Both indexes declined as human disturbance increased, but the 6-metric index has a broader range of response to disturbance. Values in boxes are <= value on x-axis.

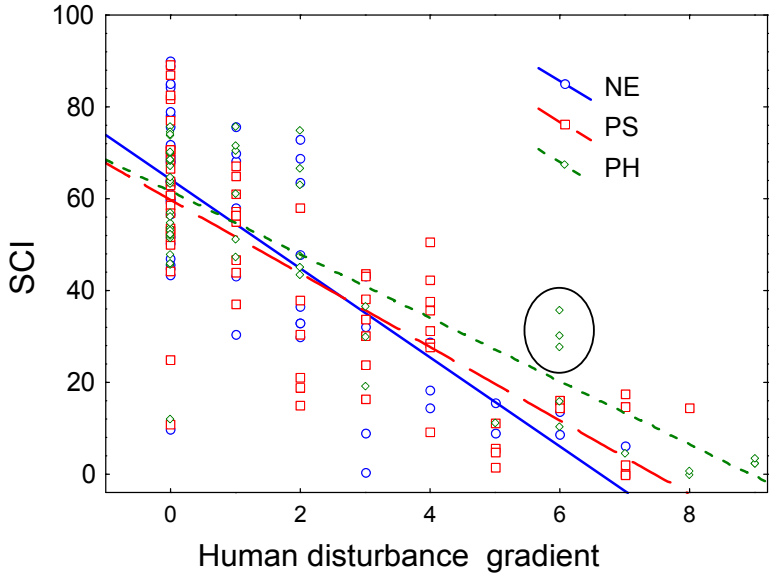

Figure 6. SCI declined as human disturbance increased. Regression lines for the peninsula and northeast had nearly identical slopes indicating a similar index response across the gradient. Three visits to two sites with higher values than predicted (circled) increased the slope for panhandle sites somewhat. Index values were averaged for each of the 176 sites. Only samples with 75-175 individuals are shown and were included in the regression. *N* = 58 (NE), 66 (PS), and 52 (PH).

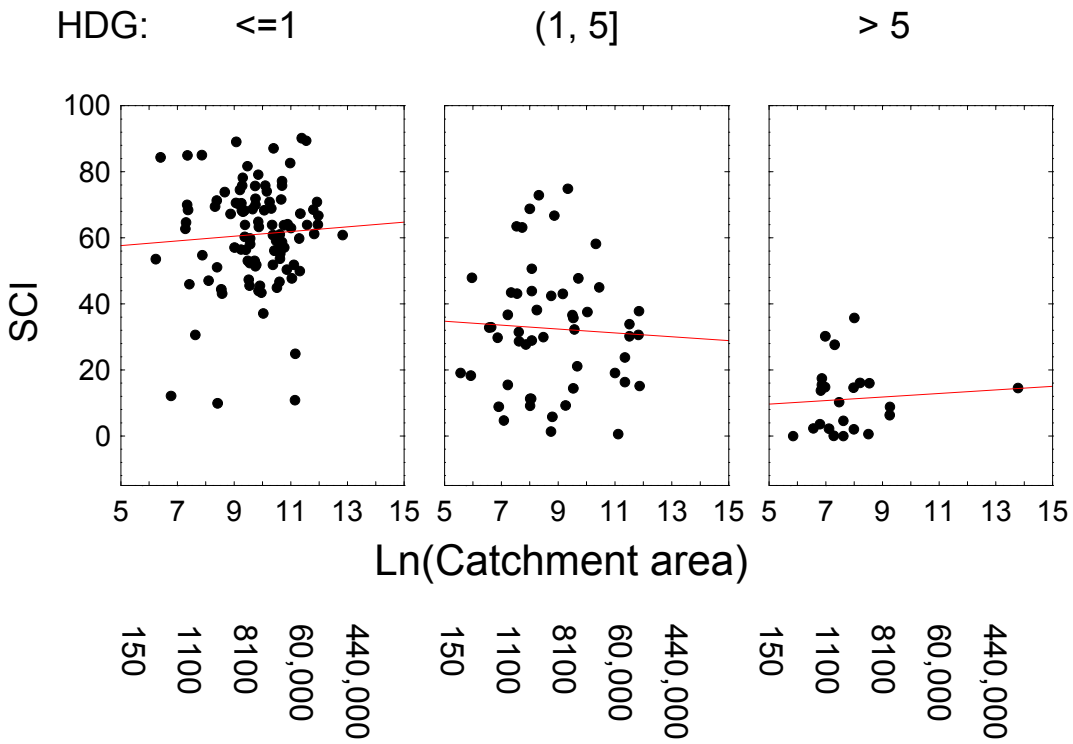

Figure 7. SCI was not significantly associated with watershed size (catchment area) when correlation was tested separately for low, moderate, and high levels of human disturbance (HDG). Highly disturbed sites tended to be small in catchment area and this relationship created a spurious correlation between SCI and catchment size. Shown are average SCI values for 176 sites. Actual catchment area shown below figures (in acres).

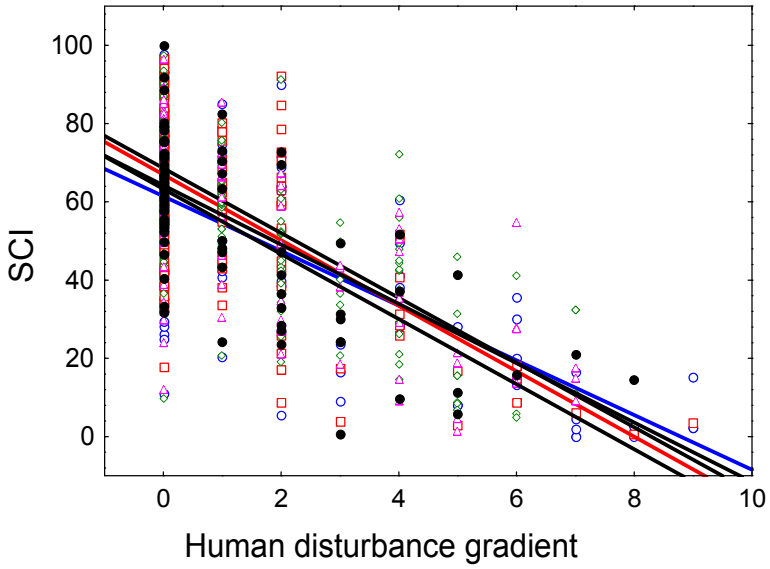

Figure 8. SCI showed a similar response to human disturbance for different years. Shown are five regression lines representing data from 1992-3, 1994-5, 1996-7, 1998-9, and 2000-1. The nearly identical slope lines indicate a consistent relationship between SCI and human disturbance for different time periods.

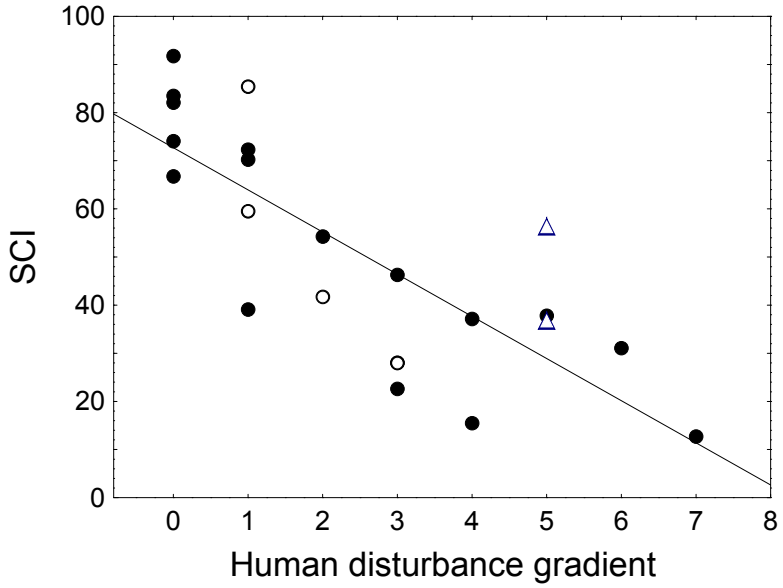

Figure 9. Correlation between SCI and the human disturbance gradient was high for an independent test of 23 sites not included in any previous analyses (Spearman's *r* = -0.81, p<<0.01). Four sites were missing data for NH3 (open circles) and two sites had >300 individuals (open triangles).

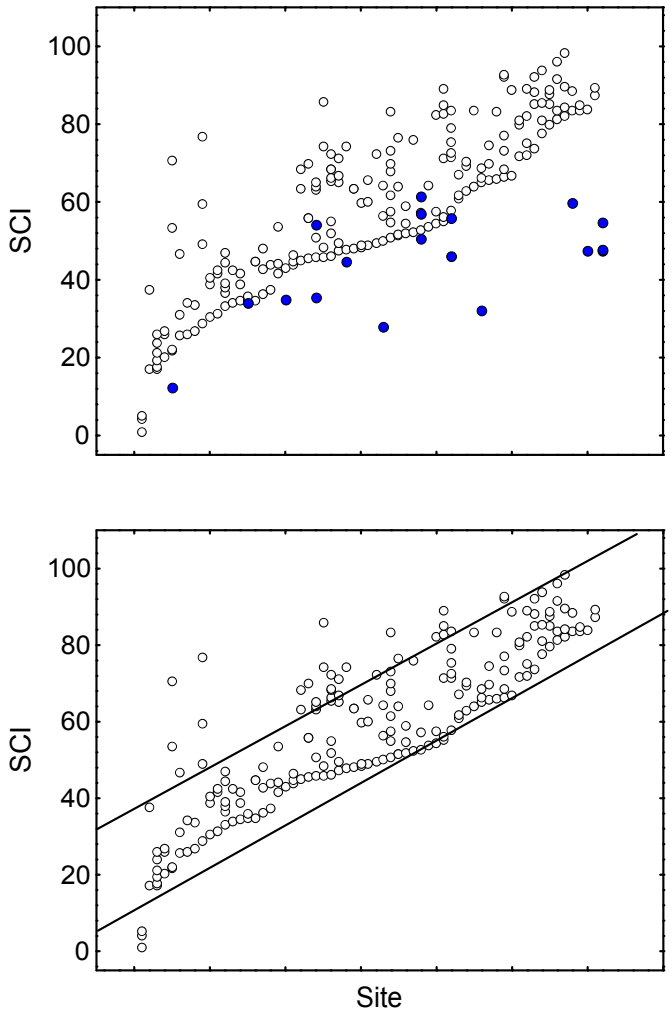

Figure 10. SCI values for repeat visits to 62 sites are shown. Sites were first sorted according to SCI value. Individual site names are not shown, but all SCI values for each site are plotted at a single point on the x-axis. Thus, vertical lines of points represent the range of SCI values at each site. Samples with < 75 individuals are indicated by solid points (upper panel). Lines (lower panel) represent the +/- 15 point confidence interval for the SCI when small samples were eliminated.

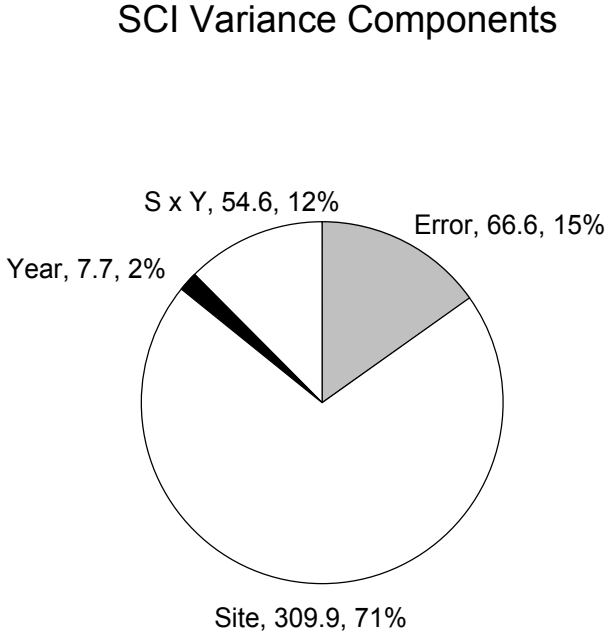

Figure 11. Estimate of variance components and their relative percentage for SCI. The largest percentage of variance was associated with site differences. Replicates collected within a single year (primarily on the same day) accounted for approximately 15% of the total observed variance. Year differences accounted for 4% of the variance. The interaction of site and year accounted for 16% of the total variance.

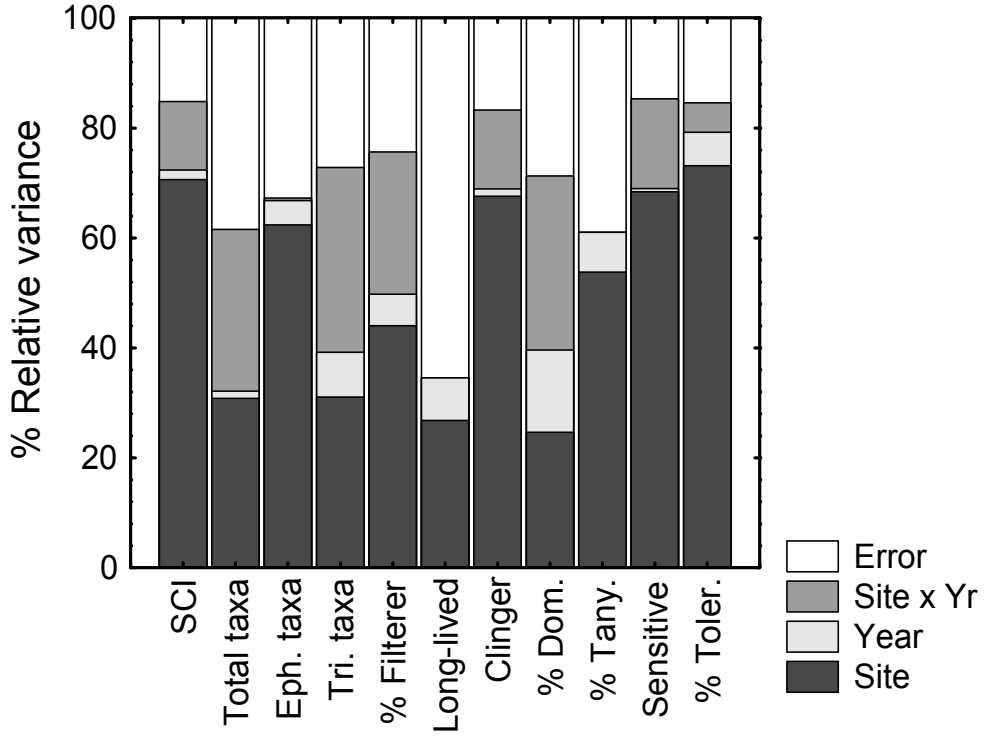

Figure 12. Variance components for SCI and its metrics. For the index and several of the metrics, variance associated with site differences represented the largest component of variance. Error variance associated with repeat visits within years (primarily on the same day) was highest for long-lived taxa richness. The least variable metrics were Ephemeroptera and sensitive taxa richness, and percent very tolerant individuals.

For the seasonal comparison, sites were chosen to represent locations with minimal human disturbance, in order to eliminate this potentially confounding source of variability from the analysis. Unfortunately, over half the SCI values for these site visits were < 70 (the "exceptional" category), indicating the seasonal dataset represented a broad range of conditions. Highly degraded sites (SCI < 20) were not included in the seasonality analysis. SCI values were on average 3.5 points higher in winter than summer (paired *t*-test, $p = 0.049$). The observed difference was greater in the northeast (6.1 points) than the panhandle (1.6 points) or peninsula (3.6 points; **Figure 13**).

Using the same data, we evaluated metrics to determine which were contributing to the seasonal differences in the SCI. Five metrics had values in the winter indicating better biological condition; these were Ephemeroptera taxa richness, % filterers, % Tanytarsini, sensitive taxa richness, and % very tolerant. Long-lived taxa richness had higher values in the summer, the opposite of the SCI. Although significant, most metric differences were rather small compared with the overall range of the metric **(Table 10).** The paired *t*-test is a very powerful test and can detect very small differences because it compares each site with itself. The exception was % Tanytarsini. With a difference of 4.6% compared with a possible range of 0 to 26%, it had the largest relative change from summer to winter. We did not modify metric scoring to correct for seasonal differences, because most metric differences were very small and the difference between SCI summer and winter values was small (3.5%) relative to the site differences through time.

To evaluate the influence of laboratory subsampling on the SCI, we used data that included multiple subsamples from the same site visit. We estimated variance using ANOVA with stream site-visit (n = 59) as a single factor and the 3 subsamples from each visit to estimate the error variance. Error variance for this model was 32.86, which represents about half the error variance calculated above when error was defined as differences associated with repeat visits to the same site (32.86/66.6 * 100 = 49%). In other words, of the variance associated with repeat visits to the same site, about half the variability is due to laboratory subsampling. However, because the variance estimates were from different datasets, the comparison should be cautiously applied. After these variance estimates for this report were completed, laboratory subsampling methods were tested and modified to address this variability. (See results and discussion in **Appendix G**.)

The BioRecon Index was calculated as the sum of the 6 scored metrics; therefore, the sum of the scores ranged from 0 to 6. We transformed the index to a range of 0 to 10 by dividing the sum by 6. The dataset for this ANOVA model was unbalanced, with many site-year combinations missing; therefore, the site x year interaction component of variance could not be estimated. The components of variance were similar to those for the SCI, in that the largest component of variance was associated with site differences and year variance was again small **(Figure 14).** The error variance for this model was 1.46, with error derived from repeat visits to the same sites. A 90% confidence interval for the BioRecon Index yielded a limit of +/- 2, or a length of 4.0 points. Dividing the range of the BioRecon index (0–10) by 4 translated into 2.5 categories of biological condition that the BioRecon could statistically distinguish**.** This result supported the decision to identify three assessment categories for the BioRecon index in a regulatory context (see Chapter 4: *Application of Biomonitoring Tools* below).

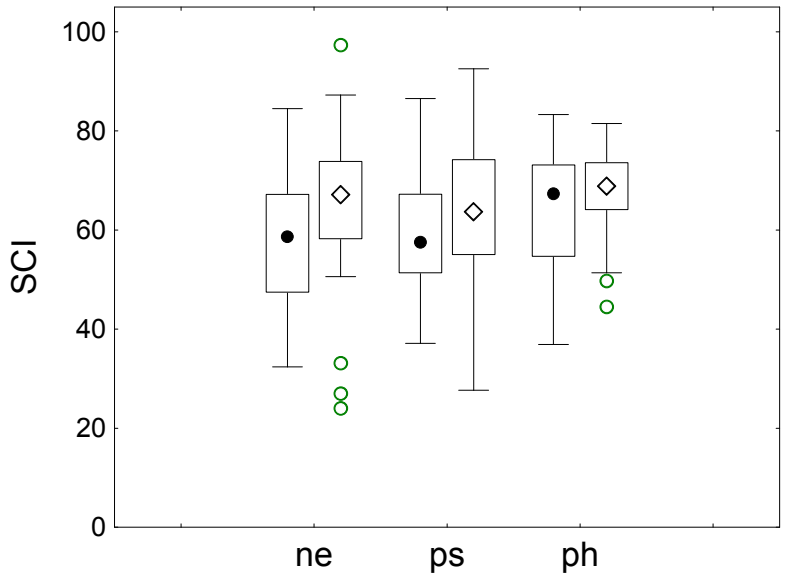

Figure 13. SCI values by region (northeast, peninsula, and panhandle) and season. Summer samples (circles in center of box) tended to be lower than winter samples (diamond centers) in the northeast and peninsula regions. *N* = 23 (NE), 34 (PS) and 31 (PH).

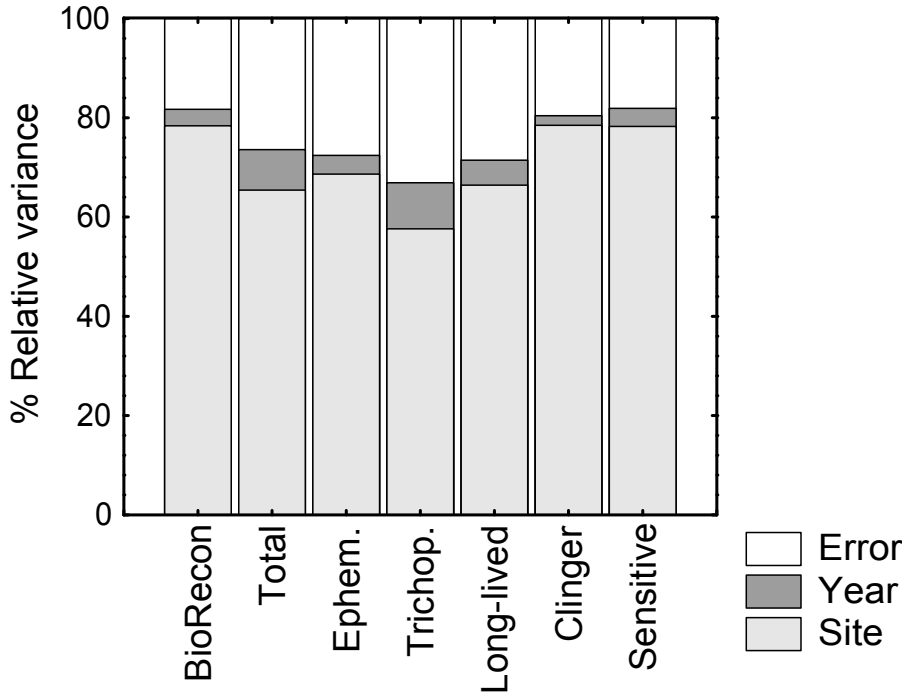

Figure 14. Variance components for BioRecon and its component taxa richness metrics. Variance associated with site differences represented the largest component for metrics and index. Error variance associated with repeat visits within years was similar across metrics ranging from 20-30% of the total. Variability associated with a specific year was relatively small for the index and metrics.

Table 10. Results for seasonal comparison of the SCI and its component metrics

Differences between summer and winter samples (negative value indicates higher winter values). Significant differences noted (p < 0.05, paired t-test).*

Index/metric	Difference
SCI	–3.5 *
Total taxa	0.1
Ephemeroptera taxa	–0.4 *
Trichoptera taxa	0.3
% Filterer	–5.2 *
Long-lived taxa	0.6 *
Clinger taxa	–0.2
% Dominance	–1.9
% Tanytarsini	–4.6 *
Sensitive taxa	–0.9 *
% Very tolerant	2.0 *

These categories for the BioRecon may be somewhat conservative, particularly for sites with good biological condition, because the variability estimated from repeat visits was much higher for moderately disturbed sites than for sites with minimal disturbance **(Figure 15).** If variance were estimated using only minimally disturbed reference sites, the error variance would be smaller. To determine whether high variability was associated with natural variability or changes in human activities, we selected the sites with the most variable BioRecon values through time and asked regional biologists to note any change in human activities at the site **(Table 11).** Four of the sites improved where roads had been paved to eliminate sediment runoff. Other changes were associated with pesticide spraying, the elimination of runoff from a wastewater treatment plant, road closure, and the cessation of fertilization upstream. The results from this anecdotal analysis suggest that much of the BioRecon variability was associated with real changes in human disturbance, rather than natural variability.

Variance results for the BioRecon components metrics were similar to those for the SCI metrics, with site differences contributing the largest component of the total variance **(see Figure 14).** In general, the metric variance due to nuisance sources of variability (year or error) was smaller for three of the BioRecon metrics than for the same SCI metrics. Lower error variance for total taxa, Trichoptera, and long-lived taxa richness suggests that the BioRecon protocol may provide more reliable estimates of these metrics. Direct comparisons of the results from different datasets should be cautiously applied, particularly for relative variance estimates, because they depend on the magnitude of the site differences.

Table 11. STORET site code, region, station nickname, observed change in BioRecon values through time, and possible reason for change

Listed sites represent locations with the highest variability observed for BioRecon values.

STORET	Region	Station	Change	Reason
20010521	Peninsula	JIMREF	Declined	Unknown
20010525	Peninsula	LILHAW@40	Declined	Unknown
20030341	Northeast	CECFLD7	Improved	Unknown
32030024	Panhandle	SFBEARREF	Improved	Intermittent pesticide spraying
33020064	Panhandle	PBRNCSTLR	Improved	Section 319 nonpoint source (NPS) restoration project to pave a road and stop sediment
33020065	Panhandle	BRSTWRKB	Improved	Section 319 NPS restoration project to pave a road and stop sediment
33020067	Panhandle	CNOECPBRNR	Improved	Section 319 NPS restoration project to pave a road and stop sediment
33020082	Panhandle	SANHOLTST	Improved	Section 319 NPS restoration project to pave a road and stop sediment
33030039	Panhandle	TRKLNHDCM	Improved	Partnership with state forest and the Nature Conservancy closed logging road and eliminated sediment
33030102	Panhandle	TURKEYCR	Improved	Summer aerial pesticide spraying
33040016	Panhandle	WILLIAMTST	Improved	Runoff from wastewater treatment plant sprayfield eliminated
33040017	Panhandle	DDFLCWC189	Improved	Ended fertilization upstream of sites in state forest
24030013	Peninsula	HILSTP4REF	Improved	Unknown
22020062	Panhandle	OKLREF	Variable	Unknown
27010050	Peninsula	MOSESUS1	Variable	Unknown
27010583	Peninsula	LITLTOMOKA	Variable	Unknown

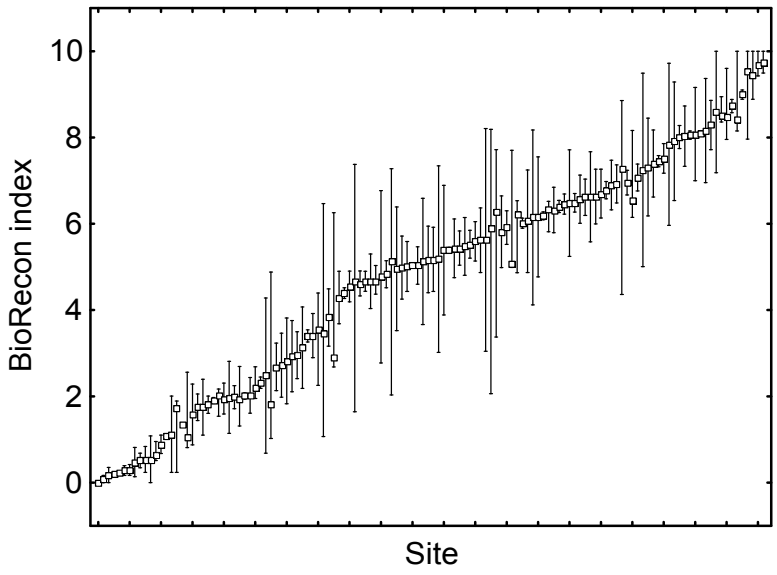

Figure 15. Range of BioRecon index values for repeat visits to 128 sites. Site names are not shown; however, each vertical line represents a single site with the minimum and maximum index values plotted as the endpoints. Note that high and low index values were less variable for repeat visits through time.

CHAPTER 3: DISCUSSION

The SCI and BioRecon Index, along with their component metrics, were developed to assess the biological condition of stream sites using samples from the stream macroinvertebrate assemblage. These assessment tools will be used primarily to define biological criteria for freshwater streams and to evaluate the effectiveness of best management practices (BMPs) (Vowell, 2001). Three results support the use of the SCI for this purpose. First, the component metrics showed a strong and consistent response to an independent measure of human disturbance (HDG). The SCI was also highly correlated with the HDG using an independent dataset for verification. Second, the SCI was independent of watershed size and geographic region. This result means that the SCI can be used across the state to compare stream sites. Third, the influence of seasonal and annual differences on the SCI was relatively small. Index values were about 3.5% higher for winter samples, and about 2% of the total variance of the SCI was associated with year of sampling. Although low, these sources of variance should continue to be evaluated with more complete sampling designs because the influence of both could potentially be eliminated by adjusting index values according to season or year.

Human disturbance gradient

The HDG simplified several aspects of metric and index testing. First, it provided a more stringent test of metric association with disturbance than a comparison of reference versus test sites. Percentage Diptera individuals serves as an example of this. This metric was included in the old SCI because it increased at disturbed sites; however, across a gradient of disturbance, it failed to consistently respond. Second, the HDG was independent of biological condition because no measures of biological condition were included. Therefore, metric expectations could be defined for equivalent levels of disturbance within each region. As a consequence, it was not necessary to correct the multimetric index for regional differences. Third, objective selection criteria based on association with the HDG were used to define individual taxa as sensitive or very tolerant. Finally, the HDG resolved a spurious correlation between watershed size and the SCI. During initial analysis, the SCI appeared to be correlated with watershed size because the most disturbed sites happened to be very small. After controlling for the HDG, the correlation between watershed size and the SCI was eliminated. The HDG can also be used to describe the intensity of disturbance associated with specific biological losses, as measured by the SCI and its component metrics.

Biological indicators

Multimetric indexes strive to integrate measures from a diverse set of biological categories for two reasons. First, monitoring different aspects of the biological assemblage improves the likelihood of detecting changes associated with different types of disturbance. Second, the potential exists to define metric signatures, that is, associations between specific metrics (or suites of metrics) that correspond to specific human activities (Norton et al., 2000; Yoder and DeShon, 2002). Metric signatures are particularly relevant to the TMDL process, which allocates responsibility for degraded stream condition among the various human activities in the watershed (Karr and Yoder, 2004; EPA, 2000).

Though not specific to any particular group, total taxa richness represents a general measure of the biological complexity found at a site. This metric is one of the most widely applied in biomonitoring programs because of its consistent decline with human disturbance for stream invertebrates (Kerans and Karr, 1994; Fore et al., 1996; Karr and Chu, 1999; Klemm et al., 2002), as well as fish (Hughes et al, 1998), terrestrial invertebrates (Kimberling et al., 2001), and birds (Bryce et al., 2002). Ephemeroptera and Trichoptera taxa richness have also been widely applied, though often combined with Plecoptera into a single (EPT) metric. Splitting these taxonomic groups provides the opportunity for metric signatures

associated with different types of disturbance. For example, Ephemeroptera taxa are known to be particularly sensitive to metals and will disappear before other taxa (Clements et al., 2000; Fore, 2002). On the other hand, an increase in Ephemeroptera taxa may indicate increased nutrients (Miltner and Rankin, 1998). Filterers are expected to decline in response to disturbance because of the increase in sediment and silt that can damage or clog nets. Long-lived taxa are expected to decline as human disturbance alters the natural flow regime, because these taxa require water in the channel year-round. Pollution events of short duration may also eliminate these taxa, while other taxa may colonize from unaffected sites.

Clinger taxa have morphological and behavioral adaptations that allow them to cling in fast water. Human development near stream sites in Florida often translates into eroding sand that can smother habitat and eliminate these taxa. Other studies have found these taxa to be sensitive to disturbance associated with mining and urbanization (Fore et al., 2001; Mebane, 2002). Percentage dominance of the most abundant taxon increases with disturbance as the natural taxonomic diversity declines and very tolerant taxa dominate samples. This metric represents a measure of the overall structure of the assemblage and has been associated with disturbance in several regions (Klemm et al., 2002). The Tanytarsini midges are used as indicators by Ohio because of their general sensitivity to human disturbance (DeShon, 1995). Three Tanytarsini midge taxa were also selected for inclusion in the list of sensitive taxa for this study.

Several of the sensitive taxa agreed with Beck's (1954) designations used to calculate the Florida Index; however, a few reversals were noted for taxa that satisfied the criteria for very tolerant. The primary difference between the sensitive taxa list and the Florida Index list was the exclusion of many rare taxa that could not be tested. The list of sensitive taxa is now shorter; nonetheless, the objective criteria used to select these taxa make these metrics easier to justify in a regulatory context.

The set of metrics selected for Florida represents a convergence with similar studies and programs in other states (Karr, 1998). Many of the Florida metrics also had a strong correlation with disturbance in Colorado, Idaho, Washington, Tennessee, and Japan, where they responded to a diverse set of human activities including timber harvest (Fore et al., 1996), recreation (Karr, 1998), urbanization (Fore et al., 2001; Karr and Morishita Rossano, 2001; Morley and Karr, 2002), agriculture (Kerans and Karr, 1994), and mining (Mebane, 2001; Fore, 2002; Mebane, 2002). The emergence of a core set of metrics across a variety of geographic contexts further supports the use of these metrics as biological indicators.

Sources of variance

We evaluated five sources of nuisance variance for the SCI and two for the BioRecon Index. For the SCI, we estimated variance associated with laboratory subsampling, repeat visits within a year (error), different seasons, different years, and site x year interaction. For the BioRecon, we estimated variance due to repeat visits within a year (error) and different years.

One of the largest contributors to the differences observed for the SCI values collected from the same site within the same year was the variability associated with laboratory subsampling. Subsampling represented approximately 49% of the variance due to repeat visits to a site within the same year. Unfortunately, the two variance estimates were derived from different datasets; thus, this percentage should be interpreted with caution. Nonetheless, subsampling in the laboratory seems to be a significant source of variability. Furthermore, the subsampling method for this small study was more carefully controlled than for routine sampling, in that the target of 100 individuals per sample that was in use at the time of this study was more frequently attained. Thus, the subsampling analysis did not include any additional variability associated with variable sample sizes; consequently, variance due to subsampling may actually be higher for routine samples. (See **Appendix G** for analysis and changes made to the subsampling protocol after the evaluation of sources of variance was completed for this document.)

Site by year interaction represents the variability associated with some unique site-year combinations. This source of variance represented index values from sites that increased (or decreased) from one year to the next in a different or more extreme manner than did other sites during those same years. The differences could be due to natural variability, e.g., the effect of a dry year on some sites but not others, or actual changes in biological condition associated with human influence. These two sources could not be distinguished using the current data, because all sources of human disturbance were not quantified. For example, agriculture is a dominant land use in Florida. Pesticide treatments represent a potentially significant impact to stream invertebrates but could not be evaluated for this study. Nonetheless, the higher variability in both the SCI and BioRecon Index values observed for sites with moderate index values compared with sites with high index values suggests that both indexes may be measuring real changes associated with human land use, rather than nuisance variance. Other studies have found a similar pattern of increased variance associated with greater human disturbance (Karr et al., 1987; Steedman, 1988). For the BioRecon Index, variability for several sites was explained by changes in human land use. Road paving to eliminate erosion, the elimination of toxic runoff, and the discontinuation of fertilization were all associated with improved BioRecon values.

For the SCI, winter index values were significantly higher than summer values collected from the same sites; however, the difference was small (3.5%). We did not adjust metric scores to eliminate the seasonal difference in the SCI because individual metric differences were relatively small and the differences in SCI values was due to a cumulative effect across several metrics. The seasonal difference was also relatively small compared with the variance due to other sources, such as repeat visits within a year or laboratory subsampling. The influence of seasonality awaits a better dataset that compares index values by season for sites with little or no change in human disturbance.

For the BioRecon Index, the variance associated with repeat visits within years was somewhat higher than the variance observed for the SCI. This is largely because the site x year interaction term could not be estimated (due to incomplete data), and this variance was included in the error term. In addition, the BioRecon data included mostly samples from different days within the year, rather than the predominantly same-day samples used for the SCI. As a consequence, the variance estimate for the BioRecon Index may be somewhat high and the precision of the index underestimated.

The higher variability noted for the BioRecon may be due to human disturbance at the site, different levels of sampling effort applied by field biologists, the smaller number of metrics in the index (6 for the BioRecon versus 10 for the SCI), or some combination. In the field, biologists examine the sample until all unique taxa have been identified. They also bring back examples of each taxon for laboratory verification. Biologists may vary both in the amount of time they spend searching for unique taxa and in the number of taxa returned to the lab. The protocol specifies that a minimum of 5 examples of each unique taxon be returned for verification. If multiple examples of each taxon are returned, the probability of identifying additional taxa increases as a function of the number of individuals returned. This is because many field identifications can only be made to the genus level, while in the lab more taxa can be identified to the species level. The precision of this index could likely be improved by applying the same level of effort to each sample in the field. In addition, the number of individuals returned to the laboratory for verification should be made consistent for each sample. (Note: these issues have been addressed in the current version of the BioRecon SOP.)

CHAPTER 4: APPLICATION OF BIOMONITORING TOOLS

This section takes the results from the previous chapters and describes how the biological monitoring tools can be integrated into a regulatory framework for managing streams under the Clean Water Act. States are required to report to Congress the water bodies that are failing to support their designated uses. FDEP intends to use biological criteria along with physical and chemical criteria to determine whether stream sites are meeting their designated uses, particularly uses related to aquatic life use support. In this section the regulatory framework of the Clean Water Act is summarized; an approach for defining more specific designated uses for aquatic life use is described; and biological criteria based on the SCI and BioRecon Index are proposed.

Two approaches were used to define categories of biological condition. The primary approach involved the U.S. EPA's Biological Condition Gradient, which we used as a framework for defining biological standards in a regulatory context (EPA, 2005; Davies and Jackson, 2006). A secondary, but parallel approach, adopted by many states, defines biological standards based on measurements, such as SCI, collected from a set of reference sites (EPA, 2006a, 2006b). We examined the reference site data and the variability associated with the SCI determination as a secondary line of evidence to support the impairment threshold decision. Additionally, the reference site data was used as the primary basis for proposing the biological criteria threshold for sites that may be considered of "exceptional" quality.

Regulatory framework

The regulatory context for the management of surface waters is derived from the mandates of the Clean Water Act (CWA; EPA, 2005; Davies and Jackson, 2006). Within this framework, states and tribes must adopt water quality standards to protect their waters. Water quality standards (WQS) include three parts: 1) designated uses, 2) numeric and narrative criteria that protect the uses, and 3) antidegradation policies to prevent deterioration of high-quality waters (EPA, 2006a). The CWA authorizes the U.S. EPA to determine appropriate minimum levels of protection and provide national oversight to state programs; however, considerable flexibility and discretion are left to states and tribes to design their own programs and establish levels of protection beyond the national minimums (EPA, 2005). WQS are part of state law and define the water quality goals for a water body by designating the use(s) and setting criteria necessary to protect the use(s).

Thus, individual state WQS provide the foundation for the management and protection of surface waters and pollution control programs. WQS provide the basis for determining attainment or nonattainment of water bodies in order to identify impaired waters and prioritize water bodies for TMDL (Total Maximum Daily Load) establishment. Historically, states and tribes have taken different approaches to defining their WQS. Different approaches are acceptable to EPA as long as a minimum standard is protected. In other words, states are encouraged to be more protective than the national minimum. Once WQS are in place, states are authorized to implement monitoring programs that allow them to report on the attainment of those standards and to identify and prioritize waters not attaining standards.

Designated uses

All of the water quality protections available under the CWA follow from the water body's designated use. All state water bodies have designated uses as specified in water quality standards, whether or not the uses are currently being attained. When classifying water bodies and determining designated uses, a state must take into consideration public water supplies, protection and propagation of fish, shellfish, and wildlife, recreation in and on the water, and agricultural, industrial, and other purposes, including navigation. The language of the CWA includes a general mandate for protecting waters for

fishing and swimming and the protection and propagation of aquatic life. States differ in their approaches to defining designated uses under this general mandate for the protection of aquatic life.

Some states designate a general "aquatic life use" or have narrative biological criteria, which is often a general statement such as "aquatic life communities shall be maintained similar to aquatic life as naturally occurs". Florida's current description for Class III waters provides an example of this approach: "recreation and propagation and maintenance of a healthy, well-balanced population of fish and wildlife". For states with a general narrative biological criterion, single thresholds are typically set based on a multimetric index of biological integrity, with expectations determined according to regional reference conditions (U.S. EPA, 2006a; Stoddard et al., 2006). Water bodies are then assessed to determine whether they support or fail to support a single general use for aquatic life.

Currently in Florida, FDEP has convened a Designated Uses Policy Advisory Committee (DUPAC) to explore an alternative system of designated use classification for Florida. The DUPAC is considering the environmental, social, and economic consequences of use designation, and includes the interested public, the regulated community, water quality managers, and scientists. The scheme currently proposed through the DUPAC involves the separation of human and aquatic life uses and recommends a tiered system of aquatic life uses.

To be consistent with the anticipated direction of the FDEP DUPAC, this chapter proposes three categories for the SCI. Subject to future rule making decisions, those three categories may be conceptually interpreted as "exceptional" (Category 1), "healthy" (Category 2), and "impaired" (Category 3). In this way, exceptional stream resources are identified to support the intent of the anti-degradation component of the water quality standards (see below). Additionally, the "impaired" category supports decisions needed by other regulatory programs, such as the TMDL program.

Water quality criteria

Water quality criteria should be closely tied to a specific designated use and should provide a meaningful assessment of whether the use is supported. The assumption is that when criteria are met, the designated use is protected. Water quality criteria may be either narrative statements that describe the conditions needed for a water body to achieve its designated use, or numeric levels of pollutants or chemicals that should not be exceeded so as to protect the designated use. While all States have adopted *narrative* criteria for the protection of aquatic life, only some have adopted *numeric* criteria such as constituent concentration levels for various pollutants or specific biological index values. Biocriteria refer specifically to criteria based on biological aspects of the water body, typically measures of fish, macroinvertebrates, plants or algae.

Anti-degradation policy

This component of the WQS has traditionally received less attention than the other two components. An anti-degradation policy is required to ensure 1) water quality is sufficient to support existing uses; 2) where water quality is better than that needed to support aquatic life uses, that level of quality must be protected unless a public process determines that quality can be lowered to allow economic or social development; and 3) the water quality of water bodies with exceptional recreational or ecological significance must be maintained and protected (EPA, 1985). Thus, states are required to provide a framework for managing water bodies when their water quality changes. It is anticipated the DUPAC will establish an anti-degradation policy to continue to maintain the water quality and biological health of exceptional water bodies.

Tiered aquatic life uses

In 2001, the National Research Council published a report on *Assessing the TMDL Approach to Water Quality Management* in which the authors recommended *tiering* designated uses for improving the decision-making related to setting water quality standards (NRC, 2001). The NRC found the CWA's goals to be too broad to provide the operational definition of designated uses needed to support aquatic life, and recommended greater specificity in defining aquatic life uses. For example, rather than stating that a water body needs to be "fishable," the designated use should specifically describe the expected fish assemblage (e.g., cold water fishery, warm water fishery, or salmon, trout, bass, etc.). *Tiered aquatic life uses* (TALUs) are bioassessment-based statements of expected biological condition in specific water bodies that allow more precise and measurable definitions of *designated aquatic life uses* (EPA, 2005).

Designated uses are written in qualitative, narrative terms; therefore, the challenge is to relate a water quality criterion to the designated use. Establishing this relationship is more straightforward when the water quality measure, or criterion, is closely and meaningfully related to the designated use. For this reason, the NRC recommended the use of biological information to define more appropriate aquatic life uses. The TALU approach provides an interpretative framework for developing a technical program that will tighten the linkage between narrative use statements and numeric biological criteria.

Designated aquatic life uses represent a state's biological goals for their water bodies. Tiered aquatic life uses rely on biological information to more precisely define these goals relative to natural conditions. Bioassessments can then be used to measure attainment of the goals (EPA, 2005). Thus, the TALU approach is designed to support the implementation of biocriteria, and the scientific knowledge of aquatic ecology that they represent, into state and tribal water quality standards. The driving motivation here is to ensure that listings of impaired waters are scientifically defensible and to identify and protect high quality water resources.

Determining the impairment threshold using the Biological Condition Gradient model

The objective of the Clean Water Act is to "restore and maintain the physical, chemical and biological integrity of the Nation's waters." To achieve this, a consistent, uniform interpretation of biological condition must be applied to data derived from any assessment methodology (Davies and Jackson, 2006). This interpretive framework must define ecological condition, allow for variability exhibited in the reference condition, and take into account the designated use of a given waterbody. The U.S. EPA has outlined a tiered system of aquatic life use designation, along a Biological Condition Gradient (BCG), that illustrates how ecological attributes change in response to increasing levels of human disturbance. The BCG is a conceptual model that assigns the relative health of aquatic communities into one of six categories. The BCG is based on fundamental ecological principles and has been extensively tested and verified by aquatic biologists throughout the U.S.

The BCG utilizes ten biological attributes of aquatic systems which respond predictably to increasing pollution and human disturbance. While these ten attributes are measurable, some are not routinely quantified in monitoring programs (e.g., rate measurements such as productivity), but may be inferred via the community composition data (e.g., abundance of taxa indicative of organic enrichment). The attributes are:

I. Historically documented, sensitive, long-lived or regionally endemic taxa
II. Sensitive and rare taxa
III. Sensitive but ubiquitous taxa
IV. Taxa of intermediate tolerance

V. Tolerant taxa
VI. Non-native taxa
VII. Organism condition
VIII. Ecosystem functions
IX. Spatial and temporal extent of detrimental effects
X. Ecosystem connectance.

The gradient represented by the BCG is divided into six levels (tiers) of condition that were defined during a series of national workshops with experienced aquatic biologists from across the U.S. (Davies and Jackson, 2006). The six tiers are described as:

1. Natural or native condition
2. Minimal changes in structure of the biotic community and minimal changes in ecosystem function
3. Evident changes in structure of the biotic community and minimal changes in ecosystem function
4. Moderate changes in structure of the biotic community with minimal changes in ecosystem function
5. Major changes in structure of the biotic community and moderate changes in ecosystem function
6. Severe changes in structure of the biotic community and major loss of ecosystem function.

Davies and Jackson (2006) recommend that regional biological experts adapt the conceptual tiers described by the BCG to local conditions using data from a regional monitoring program. The tiers can then be used in assessment and biocriteria. To develop a BCG for Florida streams, a panel of 22 experienced aquatic biologists were convened as part of a workshop. Participants were asked to apply and calibrate the general BCG model to stream data collected from Florida streams **(Table 12; Appendix H)**. Results from the workshop were used to define biological thresholds for the SCI.

The panel of experts was provided with macroinvertebrate taxonomic lists and biological metrics for 15 reference stream sites and 30 test samples, equally divided into Florida's three bioregions (Panhandle, Northeast, and Peninsula). Data collection and metric calculation followed standard FDEP protocols. The panel was NOT given the SCI value associated with any of these sites, or any physical, chemical, or habitat descriptions of the sites (other than the Bioregion location). Detailed descriptions of the BCG attributes and tiers were provided to the panel prior to the meeting. On the day of the meeting, a presentation reiterated these definitions, accompanied by extensive discussion (Frydenborg and Miller, 2007).

The panel of 22 Florida scientists assigned the 30 test samples to BCG Tiers 1 through 6, generating a total of 660 individual sample assessments. After discussion, there was general agreement that it was difficult to identify Tier 1 sites (pristine, natural condition). This was primarily due to inadequate information in the data sets to properly characterize BCG Attribute 1, "*Historically documented, sensitive, long-lived or regionally endemic taxa."* The 20 dip net sweep sampling method, along with the laboratory sub-sampling procedure, do not result in a complete biological census of any given site. This lack of data on rare, endemic taxa made it difficult for the group to judge a "pristine" condition. Even given this constraint, there were approximately twenty instances where panelists assigned a score of Tier 1 to a sample, although none of the samples was unanimously assigned to Tier 1 by all 22 experts. However, there was complete agreement among the panelists that Tier 2 represented the minimally disturbed reference condition, which was considered, for practical purposes, to be almost indistinguishable from "natural."

Table 12. BCG Workshop Participants

Name of regional expert, affiliation, and years of experience in benthic macroinvertebrate identification,

Expert name		Affiliation	Years
Jerrell	Daigle	DSA, Inc.	>20
Dana	Denson	FDEP Central District	10-15
Doug	Durbin	Biological Research Associates	>20
John	Epler	John Epler Consulting	>20
Julie	Espy	FDEP Tallahassee	5-10
David	Evans	Water and Air Research	>20
Michael	Heyn	FDEP Tallahassee	>20
Joy	Jackson	FDEP Tallahassee	10-15
Deron	Lawrence	Biological Research Associates	10-15
Laura	Line	Water and Air Research	10-15
Rob	Mattson	St. Johns River Water Management District	>20
Peggy	Morgan	FDEP Southwest District	>20
Patrick	O'Connor	FDEP Northeast District	>20
Manuel	Pescador	Florida Agricultural and Mechanical University	>20
Marianne	Pluchino	Reedy Creek Improvement District	>20
Eric	Pluchino	FDEP Orlando District	>20
Donald	Ray	FDEP Northwest District	>20
Johnny	Richardson	Leon County	10-15
Gitta	Schmitt	FDEP Southwest District	5-10
Mary	Szafraniec	Southwest Florida Water Management District	5-10
Albert	Walton	FDEP South District	>20
Gary	Warren	Florida Fish and Wildlife Commission	>20

During the workshop, the 22 panelists BCG scores were tabulated for each test sample and the panel discussed the results. In all cases, the majority of panelists' scores were assigned to the same tier, with a small number of the adjacent tiers represented. After discussion on specifics associated with the data set, the panelists were given the opportunity to modify their score.

During the national workshops sponsored by EPA, different groups of experts independently concurred that the ecological characteristics described by Tiers 1–4 corresponded to how they interpret the Clean Water Act (CWA)'s interim goal for protection and propagation of aquatic life. They further identified Tiers 1 and 2 as indicative of biological integrity (U.S. EPA, 2005). Subsequent to these national workshops, several states have adopted a line of impairment near the threshold between Tiers 4 and 5 (U.S. EPA, 2006b). The Florida panel of experts was similarly asked to offer their opinion as to where along the 6 tiered rankings they believed the interim goal of the Clean Water Act (CWA) was met. In other words, sites below a selected tier threshold would be considered "impaired." There was unanimous agreement that Tier 2 represented reference conditions, and that Tier 3 clearly met the CWA interim goal for biological integrity. There was also unanimous agreement that Tier 5 did not meet the CWA biological integrity goal. Approximately half the group thought the impairment threshold occurred between Tiers 4 and 5. The other half of the Florida experts proposed the impairment line to be drawn between Tiers 3 and 4. Those panelists expressed a concern that management action may not be taken on sites until they were considered "impaired." In other words, those panelists wanted the impairment line to include a buffer to allow more time for management action to prevent sites from becoming impaired. Taking the

recommendations of all 22 experts, we calculated the mid-point to define the line of impairment to be below Tier 4.

From this definition for the line of impairment, BCG scores were related to SCI values to determine a corresponding numeric criterion for the support of aquatic life use. Two statistical approaches were used: linear regression and change point analysis. Using linear regression, we found that an SCI value of 34 corresponded to a BCG score of 4 **(Figure 16)**. Based on this analysis, the impairment threshold could be set at an SCI value < 34. Step function regression, also known as change point analysis, identified a significant difference (change or break point) corresponding to an SCI score of 36 (**Figure 17; Appendix I;** Niu and Miller, 2007). Based on this analysis, the impairment threshold could be set at an SCI value < 36. Thus, the two different statistical methods yielded very similar results. We averaged the SCI values derived from the two approaches to define the line of impairment to occur at SCI < 35. Using results from the BCG approach and the recommendations of 22 regional experts, SCI values from 0-34 are proposed as Category 3 (provisionally interpreted as "impaired"). SCI scores >= 35 represent Categories 2 and 1 (provisionally interpreted as "healthy" and "exceptional"; see below).

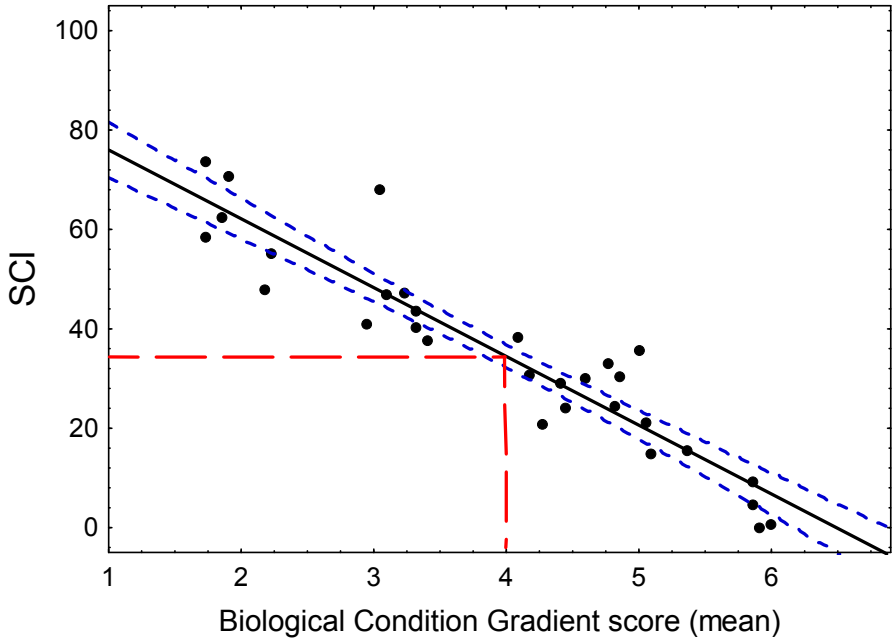

Figure 16. SCI values were highly correlated with the BCG scores assigned by the experts. Shown are the regression line (solid black) and its 90% confidence intervals (dashed blue lines). Red dashed lines indicate the SCI threshold value of 34.4 based on a BCG score of 4 as the line below which biological impairment was defined.

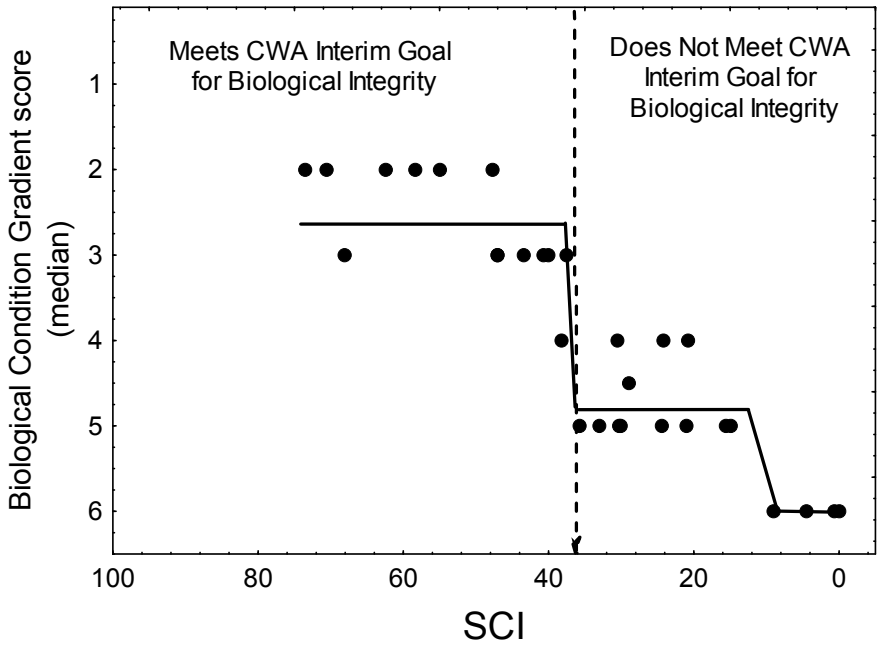

Figure 17. Change point model showing the relationship between the median BCG scores for each test sample and the corresponding SCI scores (change points = 35.8 and 9.1, alpha < 0.05).

Defining biocriteria using reference site condition

Another U.S. EPA-recommended approach for setting the standard for biological condition involves analyzing reference sites. Reference sites may be defined as those with minimal human disturbance, approximating the "natural" condition of a region (U.S. EPA, 2006a, b; Hughes, 1995 Stoddard et al. 2006). From a set of reference sites, measures of site condition, such as a multimetric index of biological condition, can be used to define thresholds for acceptable and unacceptable biological condition.

Lower percentiles, e.g., the 10th or 25th percentile, of the distribution of biological index values observed in reference sites are used by many states to define biological criteria. The selection of the specific percentile depends on the relative confidence associated with the initial selection of reference sites.

Defining the limit of reference condition using a subset of reference sites recognizes that natural stressors can affect the biology of a stream, introducing variability into the reference site data distribution. In Florida, natural drought will cause dry or stagnant flow conditions, which may be associated with lower SCI values. Substrate diversity at some reference sites may be naturally limiting, which also may reduce SCI values. For Florida streams, our confidence was high that reference sites truly represented reference condition; therefore, we selected the 10th (rather than the 25th) percentile.

Reference site selection

To develop the HDG, FDEP biologists selected sites from each of their regions that represented the broadest possible range of human influence, including sites that represented the very best or minimally disturbed sites in their regions. In the early years of FDEP's bioassessment program, every effort was made to identify the best examples of reference sites in the state. Over time, program

emphasis has shifted to other sampling needs, including status and trend monitoring, TMDL assessment, and springs studies **(Figure 18)**. Due to the extensive experience and knowledge of DEP regional biologists, most of the minimally disturbed stream sites have already been identified and sampled. In contrast, other states that started their biological monitoring programs later may have identified their reference sites from a probabilistic sampling design. The criteria for selecting reference sites may be the same for both data sets, but the results may differ. Specifically, hand-picked reference sites in Florida may represent the very best sites available while other sites meeting the criteria for reference sites may have lower SCI values due to natural variability or minimal human disturbance.

Reference sites were initially selected as sites with HDG = 0. Recall that HDG = 0 does not mean *no* human disturbance, but minimal human disturbance. The criteria for HDG = 0 were:

- NH_3 < 0.1 mg/L
- Habitat Assessment Index > 65% of total points (scored from 0–100% of total points)
- Hydrological index < 6 (scored from 0–10), and
- LDI < 2.

Sites meeting these initial conditions were further scrutinized to eliminate sites potentially influenced by human activities not captured by the HDG. Additional criteria applied to reference sites included:

- Habitat Assessment Index > 75% of total points
- Site average conductivity < 600 umhos/cm
- No receiving sites for NPDES-permitted wastewater treatment facility discharges, based on a review of GIS coverage information from the Water Facilities Regulation (WAFR) database, and
- The number of individuals identified ranged from 100-175 (inclusive).

These criteria were established to select the very best reference sites from among sites with a HDG score of 0. The habitat assessment index criterion was raised from 65% to 75% because sites scoring 75% or greater were equivalent to all or most of the habitat assessment parameters scoring as “Optimal” or “Suboptimal” (FDEP, 2004). The < 600 umhos/cm criterion for conductivity was based on results uncovered while evaluating a revision of the Class III freshwater specific conductance criterion (Chapter 62-302.530(61) F.A.C.) This study found that for a specific conductance of 20 umhos/cm approximately half of the taxa within a SCI sample would be sensitive taxa and for 600 umhos/cm the proportion of sensitive taxa decreased to almost none (FDEP, 2005). For the third criterion, sites could not be located below an active facility discharge based on review of GIS coverages and information from district biologists. A lower limit of 100 individuals was set as the minimum sample size for reference sites in order to be consistent with other state monitoring programs. This minimum of 100 individuals is consistent with a target of 100 organisms used by a majority of other state agencies (54% of 43 state programs surveyed; Carter and Resh, 2001). These criteria were established for selection of reference sites only; the application of the SCI is not limited to sites or samples conforming to these reference site criteria.

Based on these criteria, 64 reference sites with a total of 205 site-visits were selected **(Appendix J).** For the 64 vetted reference sites, statistics were calculated from the distribution of reference site SCI values based on the median SCI value observed for each site. SCI values for reference sites ranged from 43.4–92.6 representing the natural variability associated with the SCI at sites with minimal human disturbance **(Table 13)**. The lower 10^{th} percentile SCI value for the reference site distribution was 48.8.

Note that the 10^{th} percentile could have been derived in more than one way from the reference site scores. When we calculated these alternate statistics they provided very similar SCI values. Examples include the 10^{th} percentile of SCI for all site-visits (44.9, N = 205); the 10^{th} percentile for

reference site mean values (SCI = 50.5, N = 64); the 10th percentile for the most recent site-visit (SCI = 50.2, N = 64).

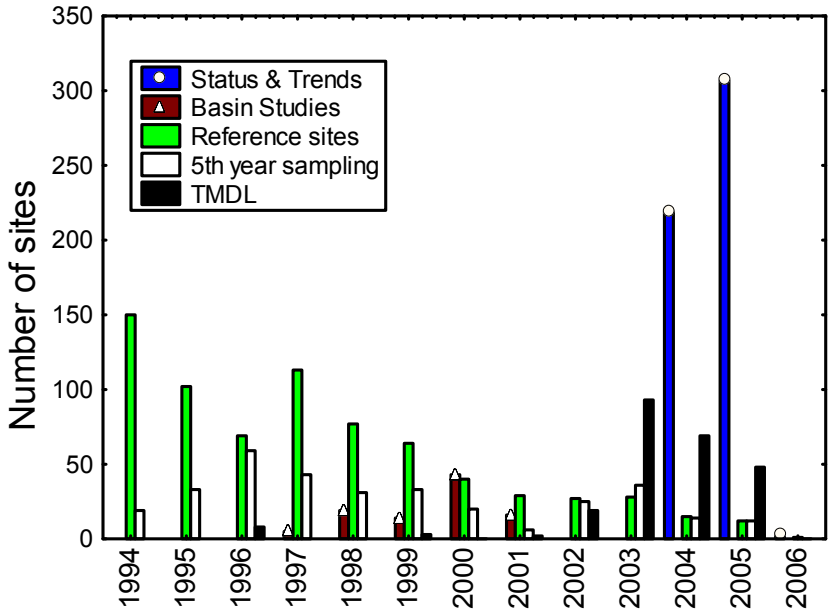

Figure 18. Number of stream sites sampled for SCI by year and program.

Table 13. Statistics and percentile values for the SCI reference sites.

Statistics and values for the distribution of SCI values at reference sites (N = 64).

Statistic	Value
N	64
Minimum	43.4
10th percentile	48.8
25th percentile	56.7
Median	67.3
Average	66.3
Standard Deviation	12.6
75th percentile	75.2
90th percentile	84.0
Maximum	92.6

Matching the approach of other states (EPA, 2006b), we defined a buffer of SCI values below the 10th percentile to represent the natural variability associated with field sampling that could be due to water level fluctuation, weather, or microhabitat differences. We used the MDD (calculated above in Chapter 2: *Statistical Precision of Indexes*) to estimate the variability in SCI associated with repeat sampling of the same stream site. Specifically, we subtracted half the MDD interval from the 10th percentile to obtain a

lower limit of reference value of SCI = 35 (48.8 – 13.4 = 35.4). Thus, an SCI value of 35 represented the lowest SCI value we expect to obtain from a reference site.

Distinguishing between Category 1 and 2

We used SCI values observed at reference sites to propose a threshold between Category 1 ("exceptional") and Category 2 ("healthy"). The upper 90th percentile of SCI values for the 64 reference sites was selected to represent exceptional sites (SCI score of 84.0). As for the impairment threshold definition above, we defined a buffer below the 90th percentile using half the MDD interval (13.4) to yield an SCI value of 71 as the limit of Category 1 (84.0 – 13.4 = 70.6). The MDD interval provides a buffer around the point estimate of the lowest SCI value representing exceptional reference condition.

As above, we note that the 90th percentile could have been derived in more than one way from the reference sites. And, again as above, when we calculated these alternate statistics they provided very similar SCI values. Examples include the 90th percentile of SCI for all site-visits (86.7, N = 205); the 90th percentile for reference site mean values (SCI = 83.8, N = 64); the 90th percentile for the most recent site-visit (SCI = 83.9, N = 64).

Biocriteria for streams

The above analyses support the establishment of three categories of aquatic life use for Florida streams, described as Category 1 ("exceptional", with SCI = 71–100), Category 2 ("healthy", with SCI = 35–70), and Category 3 ("impaired", with SCI = 0–34). **Table 14** provides a narrative description for the numeric ranges of SCI**.**

The threshold values for the SCI were also used to define biocriteria for the BioRecon Index. Data were collected using both protocols at 112 sites. A linear regression equation was used to equate threshold values of SCI to corresponding values of the BioRecon Index. This analysis was used to define Category 1 ("exceptional", with BioRecon = 6.5–10), Category 2 ("healthy", with BioRecon = 3.1–6.4), and Category 3 ("impaired", with BioRecon = 0–3.0) stream condition.

To have confidence in the final stream assessment for regulatory decisions, two separate, temporally independent site-visits resulting in a Category 3 evaluation are required to list a stream site as failing to support aquatic life use.

Table 14. Proposed aquatic life use categories, corresponding SCI values, and descriptions of biological conditions typically found for that category

Narrative metric descriptions were not used to score metrics; rather, they describe values associated with a range of SCI values.

Aquatic life use category	SCI Range	Description
Category 1	71–100	Higher diversity of taxa than for Category 2, particularly for Ephemeroptera and Trichoptera; several more clinger and sensitive taxa than found in Category 2; high proportion for Tanytarsini; few individuals in the dominant taxon; very tolerant individuals make up a very small percentage of the assemblage
Category 2	35–70	Diverse assemblage with 30 different species found on average; several different taxa each of Ephemeroptera, Trichoptera, and long-lived and, on average, 5 unique clinger and 6 sensitive taxa routinely found; small increase in dominance by a single taxon relative to Category 1; very tolerant taxa represent a small percentage of individuals, but noticeably increased from Category 1
Category 3	0–34	Notable loss of taxonomic diversity; Ephemeroptera, Trichoptera, long-lived, clinger, and sensitive taxa uncommon or rare; half the number of filterers than expected; assemblage dominated by a tolerant taxon; very tolerant individuals represent a large proportion of the individuals collected

CHAPTER 5: CONCLUSIONS

The correlation between biological metrics and the independently derived human disturbance gradient (HDG) provided strong evidence in support of the relationship between human influence and biological condition. The high correlation between the SCI and HDG was verified using an independent set of data. In addition, the pattern of biological response to human disturbance was repeated across all 3 regions in Florida (panhandle, peninsula, and northeast), indicating that the multimetric index was independent of geographic context. Finally, the index was consistent across years, with a nearly identical relationship between the SCI and HDG for 10 years of data.

To document every source of human disturbance is prohibitively expensive; consequently, the HDG was inevitably incomplete. We had no information on metals, pesticides, dredging, or other pollutants. Aerial pesticide application is a frequent practice on agricultural lands and can be very damaging to stream invertebrates, as pesticides are concentrated in rain runoff (Hutchens et al., 1998). This lack of information may likely explain why low SCI values were observed for sites with no known source of disturbance. In sharp contrast, sites with known disturbance never had high values for the SCI. This result supports the idea that much of the lack of fit between disturbance and biological condition is largely due to measurement error associated with quantifying human disturbance, rather than the variability of biological measures (Bryce et al., 1999; Karr and Morishita Rossano, 2001; Fore, 2003). In short, multimetric indexes accurately synthesize the influence of human activities over time, while measures of human disturbance are at best approximations of the complex ways that human activities influence the environment.

The SCI had good statistical precision and could detect 3.7 categories of biological condition based on a single sample and 5 categories based on 2 samples. The BioRecon was not as precise but had adequate precision to detect 2.5 categories of biological condition based on a single sample and 3.5 categories for 2 samples. Strong correlation with an independent measure of disturbance, good statistical precision, immunity to regional differences or watershed size, and minimal sensitivity to seasonal or yearly differences all contribute to the usefulness of these indexes for the biological assessment of Florida's streams.

We followed U.S. EPA guidance to translate index values into biocriteria to be included as part of the water quality standards for the state. Two approaches were used to define categories of biological condition. The primary approach involved convening a panel of regional aquatic scientists to apply a nationally endorsed conceptual model of biological condition to Florida stream data, and was used to define the threshold of impairment (line between biological condition Categories of 2 and 3). The second approach used data from minimally disturbed areas (reference sites) to define expectations for reference condition and to define the threshold of exceptional sites (line between biological condition Categories of 1 and 2). Based on these results, three categories of biological condition are proposed to represent Category 1 ("exceptional"), Category 2 ("healthy"),and Category 3 ("impaired") biological condition for Florida stream site assessments. The results reported here provide the scientific basis for managing and protecting Florida's stream resources.

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APPENDICES

Appendix A. HDG calculation

This appendix details the calculations and interim steps involved in deriving an HDG value for each site. The HDG combined 4 measures of human disturbance (NH_3, habitat index, hydrologic index, and LDI) by first converting them to unitless scores (representing low, medium and high disturbance) and then summing the scores. When multiple site visits occurred at a single sampling location, the individual component measures were averaged for each site and then converted to unitless scores. For a site with multiple visits, NH_3, the habitat index, and, in a few instances, the hydrologic index, could change. LDI could not change because it was calculated only once for each site. When component values were missing, they were assumed to be in good condition and scored as 0.

Coding for NH_3

Many of the reported NH_3 values had qualifiers associated with them. In some cases the qualifiers were omitted and the results were used as reported and in others the value was simply set to 0 to simplify coding for the HDG (Table A-1). Another more common approach is to record half of the detection limit rather than 0, but for calculating the HDG this interim recoding did not matter because either value would be scored as a 0 for the HDG.

Table A-1. Laboratory codes associated with water chemistry values, their description, and the action used to convert them to simple numbers. All the codes are listed but J and Q were not found for the NH_3 values.

CODE	Description	ACTION
A	A-Value reported is the mean of two or more determinations	omitted A qualifier and used result as reported
I	I- The reported value is between the laboratory method detection limit and the laboratory practical quantitation limit	set result to 0
J	J-Estimated Value	omitted J qualifier and used result as reported
Q	Q-Sample held beyond normal holding time	omitted Q qualifier and used result as reported
U or BDL	U-Material analyzed for but not detected; value reported is the minimum detection limit	set result to 0
T	T- below detection limit; estimate	set result to 0

Derivation of habitat index values

Data for this study were collected from 1991–2001. During that time the habitat assessment protocol was modified and additional measurements were added. During these different time periods the maximum possible total score changed. To make comparisons of the habitat index across years, we divided habitat index values by the total maximum score that was possible for that protocol. In this way the habitat index was transformed into a percentage measure that ranged from 0–100%.

Derivation of hydrologic index values

At the April, 2002, Biocriteria Meeting, a training session on the hydrologic index scoring process was conducted. The purpose for assigning the hydrologic modification scores was to develop one of several components of the HDG. FDEP biologists were instructed to use their knowledge of human activities within a watershed and upstream of selected sampling sites in order to assign a score from 1 to 10, following the specific criteria presented (see Table 2, main document). The key issues for consideration in the scoring process were:

- *The stream's hydrograph response to a rain event.*
- *The amount of ditching or impoundments within the watershed that would alter normal water deliveries to the stream.*
- *Topographic alterations in the watershed that would adversely affect water deliveries to the stream.*
- *Water withdrawals (consumptive use) in the area that may reduce water to the stream.*
- *The relative amount of impervious surfaces in the watershed that would "spike" the hydrograph.*

Since the participants had simultaneously visited certain streams as part of previous Biocriteria Meeting training and quality assurance activities, appropriate hydrologic index scores for these example streams were discussed, to promote consistency in the application of the process.

Scoring component measures of the HDG

In order to calculate the HDG, the 4 component measures were first transformed into unitless scores (see Table 5, main document, for scoring rules). The first round of data was used to derive the break points for scoring the measures. The graphs used to define the break points are shown below for each of the 4 component measures. To define the scoring rules, natural break points in the data were used. When no break points were obvious, the range of the data was divided into approximately equal parts.

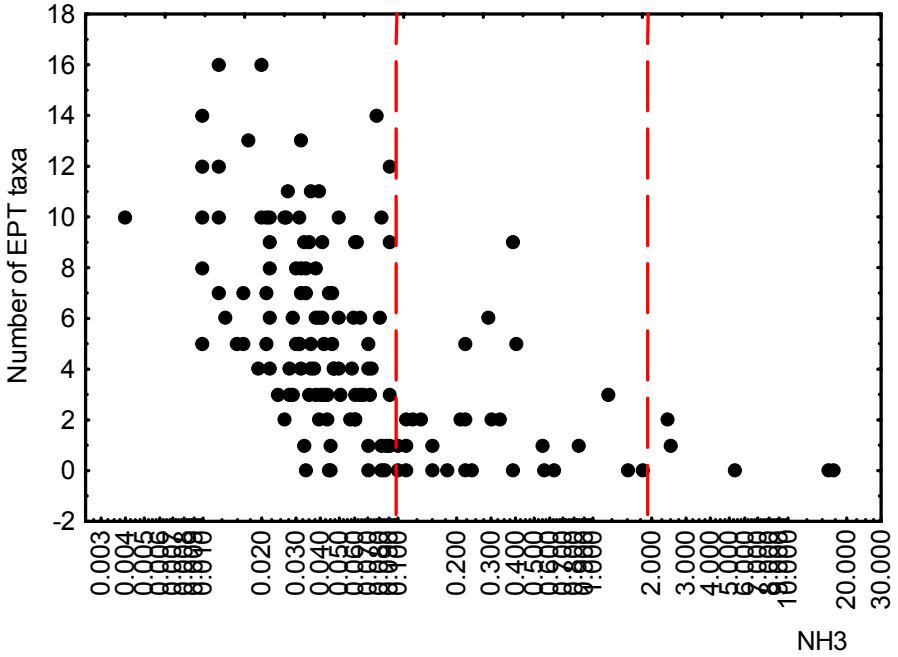


Figure A-1. Scoring rules for NH_3 for inclusion in the HDG. NH_3 plotted against a logarithmic axis.

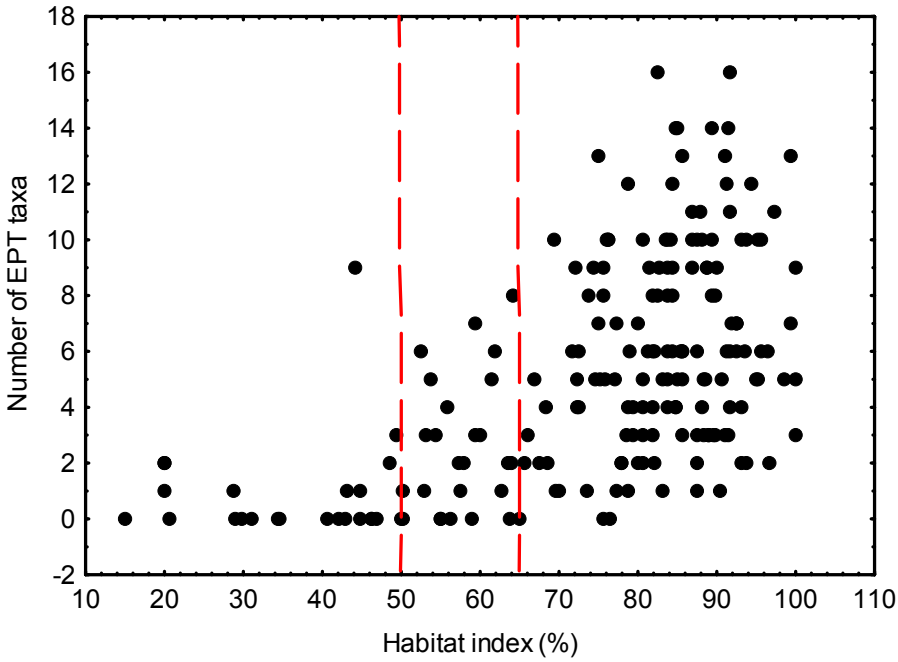


Figure A-2. Scoring rules for the habitat index for inclusion in the HDG.

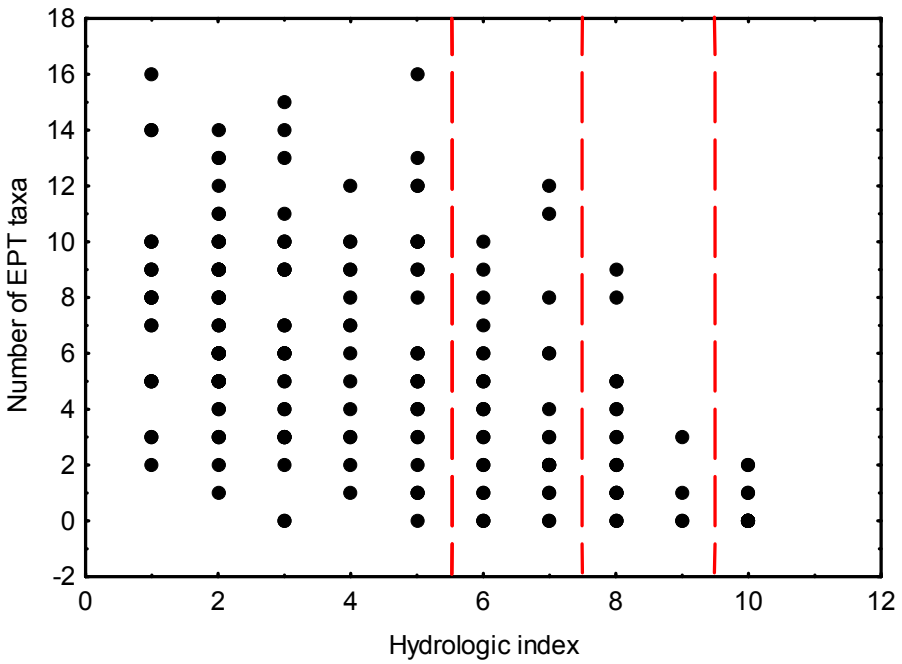


Figure A-3. Scoring rules for hydrologic index for inclusion in the HDG.

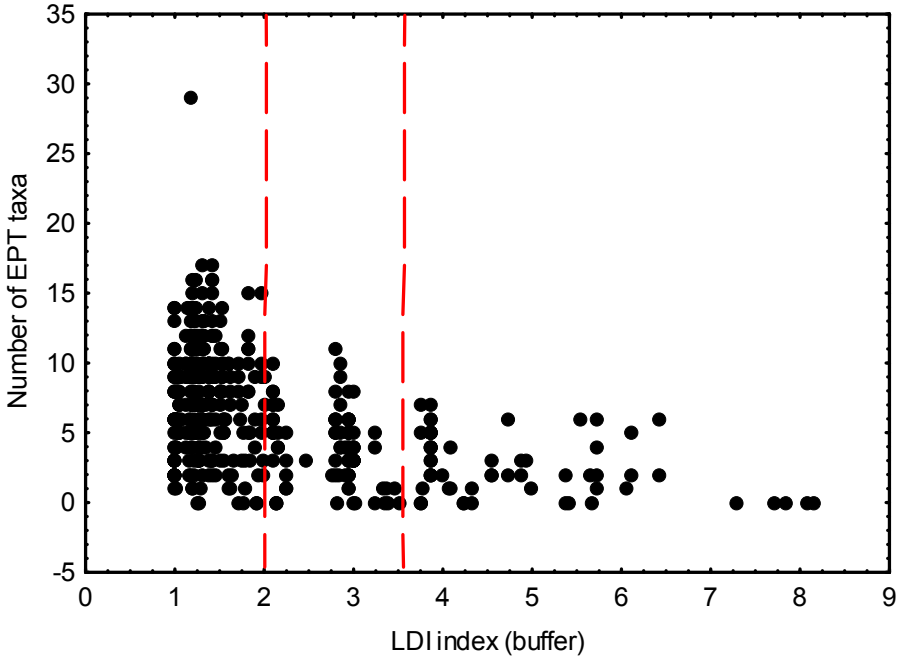


Figure A-4. Scoring rules for the LDI index for inclusion in the HDG.

Appendix B. Long-lived taxa

Taxonomic order, family, genus, or species name of taxa used to calculate long-lived taxa richness. Taxonomic synonyms present in the current Florida database are also shown. Long-lived (semivoltine) taxa require more than one year to complete their life cycles.

Order	Family	Genus	Species (Long-lived)	Synonyms
Decapoda			All families, genera, and species	
Basommatophora	Lymnaeidae	*Fossaria*	All species	
Mesogastropoda	Ampulariidae	*Pomacea*	All species	
Veneroida	Corbiculiidae	*Corbicula*	*Corbicula fluminea*	*Corbicula manilensis*
Unionoida	Unionidae		All genera and species	
Odonata	Aeshnidae	*Basiaeschna*	*Basiaeschna janata*	
		Boyeria	All species	
	Cordulegastridae		All species	
	Gomphidae	*Gomphus*	*All species*	*Gomphurus*
		Haegenius	*Hagenius brevistylus*	
		Progomphus	All species	
	Libellulidae	*Macromia*	All species	
		Somatochlora	All species	
		Tetragoneuria	All species	*Epitheca*
	Petaluridae	*Tachopteryx*	*Tachopteryx thoreyi*	

Order	Family	Genus	Species (Long-lived)	Synonyms
Plecoptera	Pteronarcidae	*Pteronarcys*	*Pteronarcys dorsata*	
	Leuctridae	*Leuctra*	All species	
	Peltoperlidae	*Tallaperla*	*Tallaperla cornelia*	
	Perlidae	*Acroneuria*	All species	
		Agnetina	*Agnetina annulipes*	
		Eccoptura	*Eccoptura xanthenes*	*Acroneuria xanthenes*
		Neoperla	All species	
		Paragnetina	All species	*Banksiana*
				Banksiella
	Perlodidae	*Clioperla*	*Clioperla clio*	*Isoperla clio*
		Perlinella	*Perlinella drymo*	*Atoperla ephyre*
			Perlinella ephyre/zwicki	
		Isoperla	*Isoperla orata*	
Megaloptera	Corydalidae		All genera and species	
Trichoptera	Brachycentridae	*Brachycentrus*	*Brachycentrus americanus*	*Oligoplectrum amercanum*
		Micrasema	All species	
	Calamoceratidae	*Heteroplectron*	*Heteroplectron americanum*	
	Hydropsychidae	*Diplectrona*	*Diplectrona modesta*	
		Macrostemum	*Macrostemum carolina*	*Macronema carolina*
				Macronemum carolina
	Lepidostomidae	*Lepidostoma*	All species	*Alepomyia, Alepomyiodes,*
				Arcadopsyche, Atomyia,
				Jenortha, Mormomyia,
				Neuropsyche, Nosopus,
				Notiopsyche, Olemira,

Order	Family	Genus	Species (Long-lived)	Synonyms
				Oligopsyche, Phanopsyche,
				Pristosilo, Athripsodes
	Leptoceridae	*Ceraclea*	All species	
	Limnephilidae	*Ironoquia*	*Ironoquia punctatissisma*	
		Pycnopsyche	All species	
	Molannidae	*Molanna*	All species	
	Phryganeidae	*Banksiola*	*Banksiola concatenata*	*Neuronia concatenata*
		Ptilostomis	*Ptilostomis postica*	*Neuronia postica*
		Agrypnia	*Agrypnia vestita*	
	Psychomyiidae	*Lype*	*Lype diversa*	
	Rhyacophilidae	*Rhyacophila*	All species	
	Uenoidae	*Neophylax*	All species	

Appendix C. Sensitive taxa

Taxonomic order, family, genus or species name; taxonomic synonyms in the current Florida database; whether the taxon was included in the Florida Index (2 = very sensitive; 1 = sensitive); and species name of Florida Index taxa are listed for taxa identified as sensitive to human disturbance.

Order	Family	Genus	Species (sensitive)	Synonyms	Florida index	
Acariformes	Lebertiidae	*Lebertia*	All species			
Isopoda	Asellidae	*Caecidotea*	*Caecidotea*	Asellus	1	*Caecidotea* spp.
Amphipoda	Crangonyctidae	*Crangonyx*	*Crangonyx*			
Ephemeroptera	Baetidae	*Acerpenna*	*Acerpenna pygmaea*	*Baetis pygmaeus*		
	Ephemerellidae		All genera and species			
	Heptageniidae		All genera and species		1	*Stenacron interpunctatum* *Stenonema mexicanum integrum*
					2	*Stenonema exiguum* *Stenonema smithae*
	Leptophlebiidae		All genera and species			
	Leptohyphidae		All genera and species			
Odonata	Libellulidae	*Macromia*	All species		2	*Macromia* spp.
Plecoptera			All families, genera and species		2	All Plecoptera
Trichoptera	Hydropsychidae	*Hydropsyche*	All species		2	*Hydropsyche* spp.
	Leptoceridae	*Triaenodes*	All species			
	Philopotamidae	*Chimarra*	*Chimarra*		2	*Chimarra* spp.

Order	Family	Genus	Species (sensitive)	Synonyms	Florida index	
	Psychomyiidae	*Lype*	*Lype diversa*			
Coleoptera	Elmidae	*Ancyronyx*	*Ancyronyx variegatus*			
		Gonielmis	*Gonielmis dietrichi*			
		Macronychus	*Macronychus glabratus*			
Diptera	Chironomidae	*Microtendipes*	All species			
		Parametriocnemus	All species			
		Polypedilum	*Polypedilum aviceps*			
		Rheocricotopus	All species		2	*Rheocricotopus robacki*
		Stempellinella	All species			
		Tanytarsus	*Tanytarsus sp. d epler*			
		Tanytarsus	*Tanytarsus sp. m epler*			
		Tribelos	*Tribelos jucundum*			
	Empididae	*Hemerodromia*	*Hemerodromia*			
	Simuliidae		All genera and species		2	*Simulium* spp.

Appendix D. Very tolerant taxa

Taxonomic order, family, genus or species name; taxonomic synonyms in the current Florida database; whether the taxon was included in the Florida Index (2 = very sensitive; 1 = sensitive); and species name of Florida Index taxa are listed for taxa identified as very tolerant to human disturbance.

Order	Family	Genus	Species (very tolerant)	Synonyms	Florida Index	
Hoplonemertea	Tetrastemmatidae	*Prostoma*	*Prostoma rubrum*			
Haplotaxida	Naididae	*Bratislavia*	*Bratislavia unidentata*			
		Dero	All species			
		Nais	*Nais communis* complex	Nais communis		
				Nais elinguis		
				Nais pardalis		
				Nais variabilis		
		Pristina	*Pristina synclites*			
	Tubificidae	*Haber*	*Haber speciosus*			
		Limnodrilus	All species	*Camptodrilus californicus*		
				Limnodrilus aurantiacus		
Lumbriculida	Lumbriculidae	*Lumbriculus*	All species			
Rhynchobdellida	Glossiphoniidae		All genera and species			
Basommatophora	Ancylidae	*Hebetancylus*	*Hebetancylus excentricus*			
		Laevapex	All species			
	Physidae	*Physella*	All species			
	Planorbidae		All genera and species			

Order	Family	Genus	Species (very tolerant)	Synonyms	Florida Index	
Mesogastropoda	Hydrobiidae	*Pyrgophorus*	*Pyrgophorus platyrachis*	*Pyrogophorus platyrachis*		
	Thiaridae	*Melanoides*	All species			
Odonata	Libellulidae	*Pachydiplax*	*Pachydiplax longipennis*			
	Coenagrionidae	*Argia*	*Argia sedula*		2	*Argia sedula*
		Enallagma	*Enallagma cardenium*	*Enallagma coecum*		
		Ischnura	All species	Anomalagrion		
Heteroptera	Mesoveliidae		All genera and species			
Lepidoptera	Pyralidae		All genera and species			
Coleoptera	Haliplidae	*Peltodytes*	All species			
Diptera	Chironomidae	*Larsia*	All species		1	*Larsia lurida*
					1	*Larsia decolorata*
		Chironomus	*All species*	*Tendipes Chaetolabis*		
		Cladopelma	*Cladopelma*			
		Cricotopus	All species		2	*Cricotopus bicinctus*
		Cryptochironomus	All species			
		Dicrotendipes	*Dicrotendipes modestus*			
			Dicrotendipes neomodestus			
		Glyptotendipes	All species			
		Goeldichironomus	All species			
		Kiefferulus	*Kiefferulus*			

Order	Family	Genus	Species (very tolerant)	Synonyms	Florida Index	
		Parachironomus	All species			
		Polypedilum	*Polypedilum beckae*	*Asheum beckae* *Pedionomus beckae*		
			Polypedilum illinoiense grp.	*Polypedilum illnoense*	1	*Polypedilum illinoiense grp.*
			Polypedilum tritum	*Chironomus tritum*		
		Tanypus	All species	Pelopia		
		Tanytarsus	*Tanytarsus sp. f epler*			
	Stratiomyidae	*Odontomyia*	*Odontomyia*	Eulalia		
	Tipulidae	*Tipula*	*Tipula*	*Nippotipula* *Playttipula* *Yamatotipula*		

Appendix E. Regional calibration of SCI metric scores

After metrics were selected based on their correlation with the human disturbance gradient (HDG), they were transformed to unitless scores to compensate for differences in their ranges of values. For example, percentage filterer might range from 0 to 60% while long-lived taxa range only from 0 to 7. The 5th and 95th percentiles for each metric were used to determine the metric values that would be defined as 0 and 10 **(Table E-1)**. To control for differences due to natural variability associated with different bioregions, the percentiles were calculated within each of the 3 bioregions.

The next step tested for regional differences in metrics scores by regressing metric scores for each bioregion against the HDG and comparing the slopes of their lines. If the regression lines for each bioregion failed to match closely, the endpoints for the metrics that defined values of 0 and 10 were adjusted away from the 5th and 95th percentiles and tested again until the regression lines for the different bioregions yielded a similar relationship with HDG **(Figure E-1; Table E-2)**. In some cases, the 5th percentile values were very close and they were simply averaged across bioregions for simplicity.

After determining which metric values define the scores of 0 and 10, the remaining metric values between those endpoints must also be scored (**Table E-3;** FDEP, 2007). As an example, for taxa richness in the northeast bioregion the interval between the 2 endpoints is 42 – 16 which equals 26. If the lowest endpoint is 16, we would first subtract 16 from the total taxa richness observed at a site, divide by the interval length of 26, and multiply the result by 10 to obtain scores that range from 0 to 10. Taxa richness values less than 16 would be scored as 0 and values greater than 42 would be scored as a 10.

Table E-1. SCI metric name, 5th and 95th percentile values by bioregion for site-visits with 75–175 individuals. Underlined values indicate that the metric values used to define a score of 0 or 10 were slightly different from the percentile value.

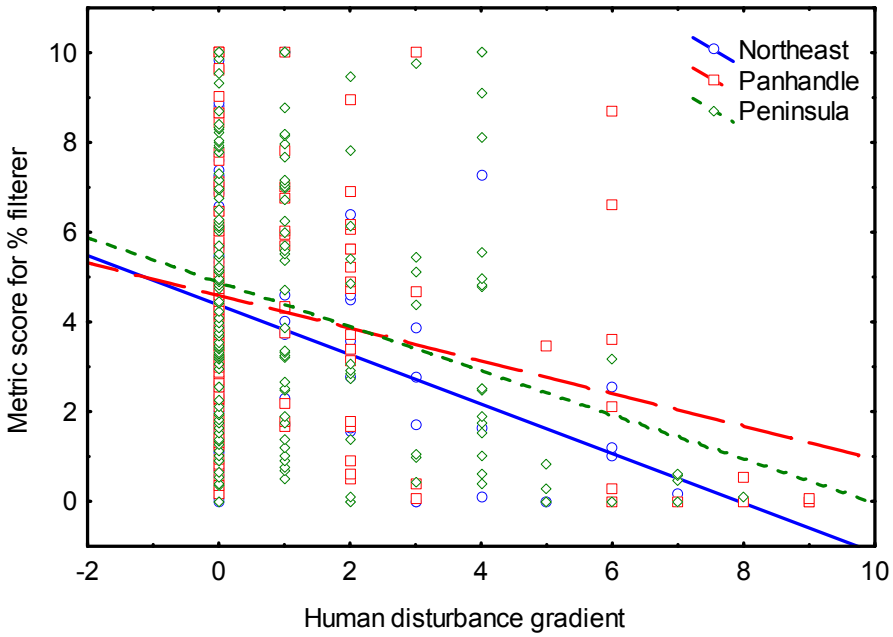
SCI Metric	NE 5th %tile	PH 5th %tile	PS 5th %tile	NE 95th %tile	PH 95th %tile	PS 95th %tile
Total taxa	17.0	16.0	16.0	42.0	46.0	41.0
Ephemeroptera taxa	0.0	0.0	0.0	4.0	6.0	5.0
Trichoptera taxa	0.0	0.0	0.0	7.0	7.0	7.0
% Filterer	1.4	1.3	0.9	52.0	45.1	40.0
Long-lived taxa	0.0	0.0	0.0	3.0	5.0	4.0
Clinger taxa	0.0	0.0	0.0	10.0	14.0	8.0
% Dominance	10.9	9.6	11.6	47.2	42.6	54.5
% Tanytarsini	0.0	0.0	0.0	27.9	22.8	26.8
Sensitive taxa	0.0	0.0	0.0	11.0	16.0	9.0
% Very tolerant	0.0	0.0	0.8	78.2	36.3	58.5

Table E-2 (Table 8 from main SCI document). SCI metric name, adjusted metric values used to scores metrics (endpoints of the range represent scores of 0 and 10), and comment describing how the values differed from the 5th and 95th percentiles. Underlined values correspond to the underlined values in Table E-1.

SCI Metric	Northeast	Panhandle	Peninsula	Comment
Total taxa	16–42	16–49	16–41	Averaged the 5th %tile and used same value in all bioregions
Ephemeroptera taxa	0–3.5	0–6	0–5	Lowered expectations for 95th %tile for NE
Trichoptera taxa	0–6.5	0–7	0–7	Lowered expectations for 95th %tile for NE
% Filterer	1–42	1–45	1–40	Lowered expectations for 95th %tile for NE
Long-lived taxa	0–3	0–5	0–4	
Clinger taxa	0–9	0–15.5	0–8	Lowered expectations for 95th %tile for NE; raised for PH
% Dominance	54–10	43–10	54–10	Averaged lower 5th %tile;
% Tanytarsini	0–26	0–26	0–26	Raised expectations for 95th %tile for NE
Sensitive taxa	0–11	0–19	0–9	Raised expectation for 95th %tile for PH
% Very tolerant	78–0	36–0	59–0	

Table E-3. SCI metric scoring formulae for converting metric values to a metric score ranging from 0 to 10. In each equation, 'X' stands for the raw metric value; "ln" stands for the natural log.

SCI metric	Northeast	Panhandle	Peninsula
Total taxa	10 * (X–16)/26	10 * (X–16)/33	10 * (X–16)/25
Ephem. taxa	10 * X /3.5	10 * X /6	10 * X /5
Trichoptera taxa	10 * X /6.5	10 * X /7	10 * X /7
% Filterer	10 * (X–1)/41	10 * (X–1)/44	10 * (X–1)/39
Long-lived taxa	10 * X /3	10 * X /5	10 * X /4
Clinger taxa	10 * X /9	10 * X /15.5	10 * X /8
% Dominance	10 – (10 * [(X–10)/44])	10 – (10 * [(X–10)/33])	10 – (10 * [(X–10)/44])
% Tanytarsini	10 * [ln(X + 1) /3.3]	10 * [ln(X + 1) /3.3]	10 * [ln(X + 1) /3.3]
Sensitive taxa	10 * X /11	10 * X /19	10 * X /9
% Very tolerant	10 – (10 * [ln(X + 1)/4.4])	10 – (10 * [ln(X + 1)/3.6])	10 – (10 * [ln(X + 1)/4.1])



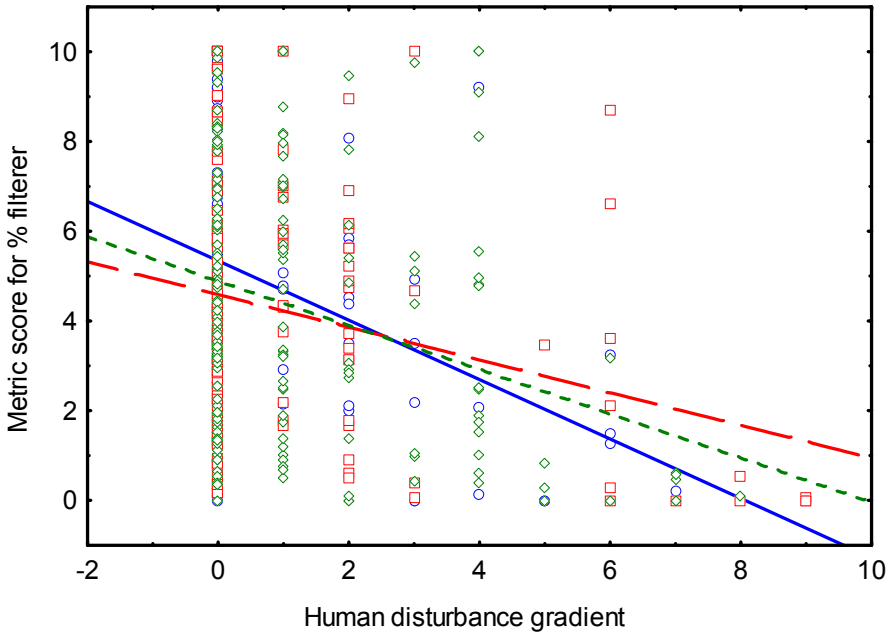


Figure E-1. Metric scores for percent filterer by bioregion. The upper panel shows metrics scored according the 5th and 95th percentiles and the lower panel shows scores after adjustment. The regression line for the NE bioregion was lower than the other 2 regions initially and plotted higher and closer to the other 2 lines after adjustment.

References

Florida Department of Environmental Protection (FDEP) 2007. Department of Environmental Protection Standard Operating Procedures for Laboratory Activities. Table LT7200-1 of LT 7200 Stream Condition Index (SCI) Determination in DEP-SOP-002/01. LT 7000 Determination of Biological Indices. Available: http://www.dep.state.fl.us/labs/sop/index.htm.

Appendix F. ANOVA output and regression results for the SCI and BioRecon

Table F-1. General linear model output for the SCI versus HDG by region (northeast, panhandle, or peninsula)

Neither the region nor the interaction of the HDG and region was statistically significant. Test failed to detect a difference in SCI values due to region.

Univariate Tests of Significance for AvgOfSCI6_100 (1NGM_4)					
	SS	**df**	**MS**	**F**	**p**
Intercept	403623.3	1	403623.3	1832.253	0.000000
Region	361.6	2	180.8	0.821	**0.441846**
HDG2	62750.7	1	62750.7	284.858	0.000000
Region*HDG2	1142.9	2	571.5	2.594	**0.077660**
Error	37449.0	170	220.3		

Table F-2. General linear model output for the SCI versus HDG by year class (1992–93, 1994–95, 1996–97, 1998–99, and 2000–01)

The interaction term was not significantly different for year class and the HDG, suggesting the lines were parallel (equal slopes). The year class alone, however, was significant, with values for 1994–95 being slightly higher for the SCI.

Univariate Tests of Significance for SCI6_100 (1NGM_4)					
	SS	**df**	**MS**	**F**	**p**
Intercept	1627060	1	1627060	5835.657	0.000000
Year class	2948	4	737	2.644	**0.032766**
HDG2	134497	1	134497	482.390	0.000000
Year class*HDG2	791	4	198	0.709	**0.586005**
Error	171749	616	279		

Table F-3. ANOVA estimates of SCI variability for repeat visits to the same site within a year

Error estimate used to calculate confidence interval is underlined.

ANOVA for SCI (SCI6_100) (Same_day_SCI)							
	Effect	**df**	**MS**	**df**	**MS**	**F**	**p**
{1}STORET	Random	60	1211.636	39.41542	188.7279	6.420014	0.000000
{2}Year	Random	6	269.430	35.76223	170.2402	1.582648	0.180727
1*2	Random	53	134.481	82.00000	**66.5952**	2.019385	0.002054

Table F-4. ANOVA estimates of SCI variability for laboratory subsampling

Error estimate used is underlined.

ANOVA Results: SCI subsampling (Metrics)							
	Effect	**df**	**MS**	**df**	**MS**	**F**	**p**
{1}Sitename	Random	58	1393.986	118	**32.86312**	42.41795	0.00

Table F-5. ANOVA estimates of BioRecon variability for repeat visits to the same site within a year

Error estimate used to calculate confidence interval is underlined.

ANOVA for BioRecon (BR3_10) (New metrics multiple_2)							
	Effect	**df**	**MS**	**df**	**MS**	**F**	**p**
{1}STORET	Random	126	15.91691	117.3771	1.696009	9.384924	0.000000
{2}year	Random	8	6.02260	156.0000	**1.458840**	4.128352	0.000171

Appendix G. Optimal sample size for calculating SCI

D. Whiting, J. Espy, and L. Wolfe (FDEP)

Taxa richness increases with the number of individuals identified from a sample. This relationship is well documented for many studies (Larsen and Herlihy, 1998), and is further supported by recent analyses conducted for FDEP which are described below. Because the SCI includes several metrics that quantify taxa richness, the concern here is that SCI values may be artificially increased or decreased depending on the number of individuals identified from a sample. Therefore, laboratory methods were modified in recent years (2004–2006) to ensure that the number of individuals identified is more consistent across samples.

The SCI data used to develop and test the SCI and BioRecon were collected between 1991–2001. The laboratory protocols used during that time mandated a target number of 100 individuals. In practice, however, this target was often exceeded due to the nature of the laboratory subsampling methods (many samples had several hundred individuals). During this early time period, FDEP did subsample material collected from the field; however, laboratory protocols did not allow multiple divisions of the material to achieve the 100-individual target. For the data set used to test and develop SCI, only site-visits with 75–175 individuals were used in order to more closely approximate the target number of individuals. Thus, metric expectations and scoring rules for SCI were based on samples with between 75 and 175 individuals (mean 122, median 119). Restricting the index development data set to samples with only 100 to 110 individuals would have reduced the number of samples available for the recalibration effort dramatically, thereby reducing confidence in the resulting biomonitoring tool.

The target number of organisms for the 2004 laboratory protocols for the SCI was 100 organisms (acceptable range 100-110). Such a narrow acceptable range was made possible by modifying the 20-dip net sweep composite sample preparation Standard Operating Procedure. This modification resulted in the samples consistently being on target.

Random site sampling in 2004 from the ambient monitoring program yielded an adequate number of samples (n = 201) to compare the effect of the more accurate laboratory subsampling methods on metric values. We compared the percentiles of the metric values used to define the scoring rules for the SCI and the percentiles of metric values from this more recent data set. For the 1992-2001 sampling, 420 samples with between 75 and 175 individuals were used. For the 2004 sampling, 196 samples were used. Approximately even numbers of samples were available for the 2004 data in the peninsula and panhandle regions; not enough sites were sampled in the northeast to calculate percentiles.

This comparison was not ideal because the sampling designs were very different. The 2004 sites were selected randomly while the earlier sites used in this study were selected for a variety of specific reasons (e.g., references sites, 5th years inspections, special studies, etc.). Thus, the distribution of percentile values may be shifted due to the laboratory methods or the types of sites sampled. If both data sets had been created from random sampling, the comparison would more clearly isolate the effect of the change in laboratory methods.

As expected, total taxa richness showed the largest differences with lower values observed in 2004 **(Table G-1)**. Other taxa richness metrics were also lower at the 95th percentile. Ephemeroptera and long-lived taxa were lower in peninsula region, Trichoptera had a similar distribution, and clinger taxa were lower in the panhandle region. The 95th percentiles for sensitive taxa were lower in both regions.

Developing scientific protocols for use in a regulatory framework is inevitably an iterative process. Our understanding of the sources of variability and the influence of precision on assessment methods has evolved rapidly in recent years. If we were to start over with no data on

hand and develop a biological monitoring protocol, the number of individuals to identify from each sample might be one of the first elements that we would define. The iterative nature of this process derives from the fact that knowledge gained regarding one aspect of the analysis methods often necessitates that methods related to another aspect of the analysis be reconsidered. A large portion of the variability in SCI values was tied to laboratory methods, and subsequently, improvements have been made in that area. This change should be understood as a *calibration* of the monitoring protocol. The fundamental relationships between biological metrics and human influence are unaffected by this issue.

A recent analysis by the FDEP Central Laboratory biologists has found that averaging the SCI scores from two 150-organism subsamples provides a more reliable assessment than a single 100-organism subsample. The objective of this analysis was to evaluate how laboratory processing and/or the number of individuals identified affects SCI score variability. For this analysis, all the organisms found in one-third (33%) of the material brought to the laboratory for each of three samples were identified. Using those data, 250 random draws were conducted for each sample for each of six different target numbers of individuals: 100, 125, 150, 175, 200, and 300 **(Figures G-1 to G-3)**

Table G-1. Percentiles of metric values for the development data set and random sampling in 2004

Shown are the 5th and 95th percentile values for SCI metrics from sites sampled during 1992–2001 (SCI development data set) and 2004 (ambient sampling).

	Panhandle				Peninsula			
	1992-2001	2004	1992-2001	2004	1992-2001	2004	1992-2001	2004
Percentile	**5th**	**5th**	**95th**	**95th**	**5th**	**5th**	**95th**	**95th**
Total taxa	16.0	13.0	46.0	37.0	16.0	13.0	41.0	35.0
Ephemeroptera taxa	0.0	0.0	6.0	5.0	0.0	0.0	5.0	3.0
Trichoptera taxa	0.0	0.0	7.0	6.0	0.0	0.0	7.0	7.0
% Filterer	1.3	0.9	45.1	45.7	0.9	0.0	40.0	32.0
Long-lived taxa	0.0	0.0	5.0	5.0	0.0	0.0	4.0	2.0
Clinger taxa	0.0	0.0	14.0	9.0	0.0	0.0	8.0	7.0
% Dominance	9.6	11.9	42.6	55.3	11.6	13.2	54.5	68.0
%Tanytarsini	0.0	0.0	22.8	31.8	0.0	0.0	26.8	20.0
Sensitive taxa	0.0	0.0	16.0	11.0	0.0	0.0	9.0	7.0
% Very tolerant	0.0	0.0	36.3	48.1	0.8	1.0	58.5	79.8

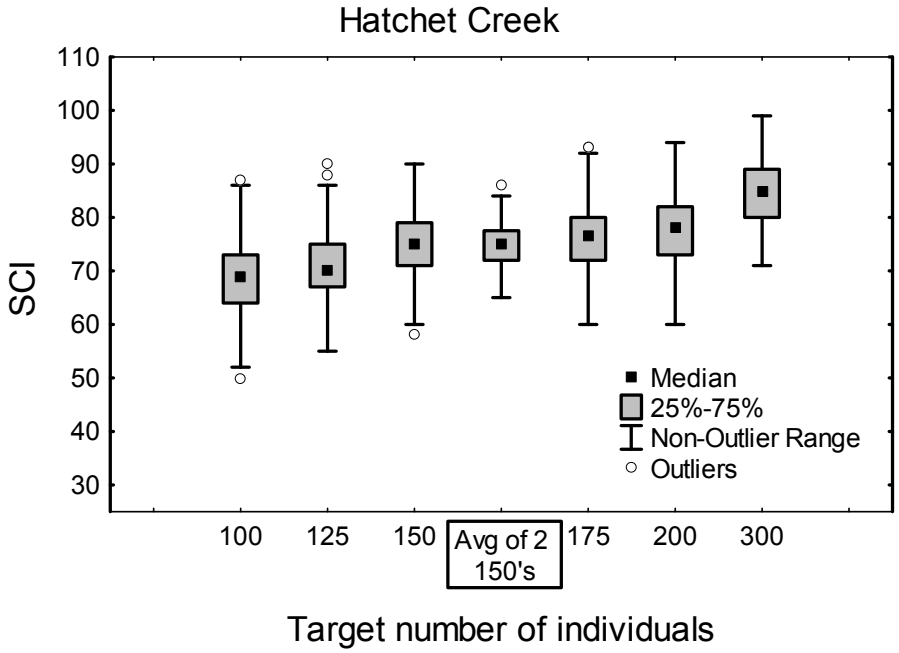


Figure G-1. Box plots showing the change in SCI score with target number of individuals counted for 250 random draws of one-third of a sample from Hatchet Creek. Shown also is the box plot for the average SCI score from 2 150 subsamples.

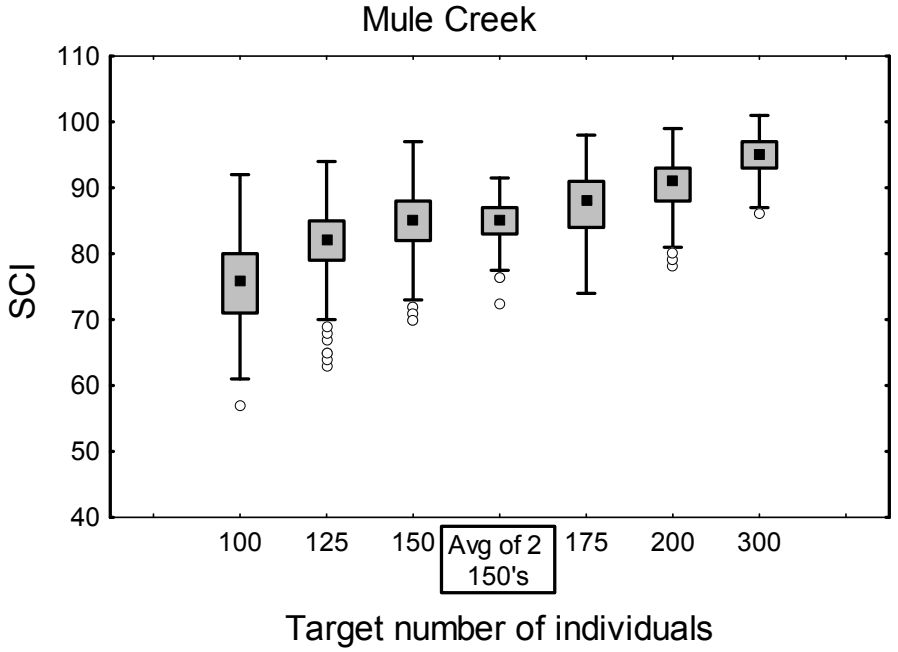


Figure G-2. Box plots showing the change in SCI score with target number of individuals counted for 250 random draws of one-third of a sample from Mule Creek. Shown also is the box plot for the average SCI score from 2 150 subsamples.

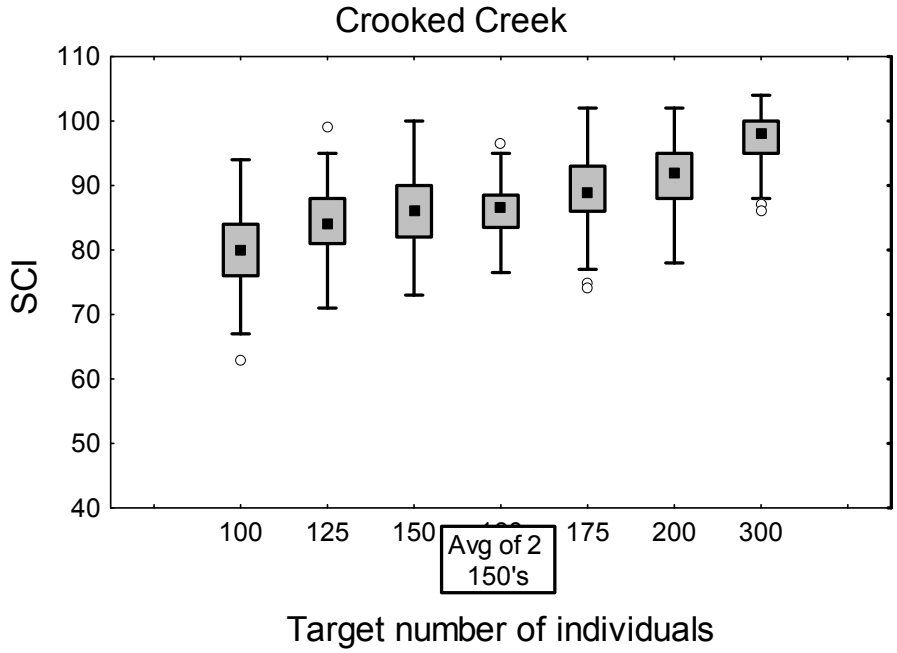


Figure G-3. Box plots showing the change in SCI score with target number of individuals counted for 250 random draws of one-third of a sample from Crooked Creek. Shown also is the box plot for the average SCI score from 2 150 subsamples.

FDEP Central Lab biologists believe the increase in precision associated with larger and multiple subsamples is worth the cost, as it makes the calculated result less variable and provides greater confidence in the tool. Prior to the sample preparation SOP being modified to more accurately achieve the target number of organisms, the sample processing time at the FDEP Central Laboratory averaged 18 hours per sample. The revision to the SOP resulted in the processing time being reduced by 56% (approximately 8 hours). While the increase from 100 organisms to 300 organisms (150x2) results in a significant increase in laboratory workload (average processing time based upon preliminary data is about 14 hours), the increase is balanced by the earlier changes to the sample preparation SOP that result in the analyst more accurately achieving the target number of organisms. The result is the identification of three times as many organisms for approximately a 75% increase in laboratory effort.

A survey of 43 state agencies' programs found that approximately 54% of those programs targeted 100 organisms (Carter and Resh, 2001). The next most commonly used target number of organisms was 300 (26%). All other target number of organisms were used by $\leq$ 7% of the programs. Additionally, U.S. EPA has used a target number of organisms of 300 for the National Wadeable Streams survey (EPA, 2006).

The FDEP decision to use 150X2 subsamples was made based upon four key points:

- First, the 150x2 subsampling will result in 300 organisms being identified, which was the second most common subsampling target number in the survey, and which is consistent with U.S. EPA minimum subsampling target used for the National Wadeable Streams survey
- Second, the use of 150x2 subsamples rather than one 300 target subsample maintains scoring within the range used to calibrate the SCI (75-175)

- Third, targeting 150 organisms in each subsample, rather than 125 organisms (approximately the mean number of organisms in the calibration dataset samples used to score the SCI metrics), results in a slightly higher score at good quality sites, while poor quality sites scores do not appear to increase similarly. This effect results in slightly better discrimination between good, moderate, and poor quality sites
- Fourth, the use of 150x2 subsamples reduces the variability in the resulting SCI score by using the average of two 150-organism-based. This reduction in variability improves consistency with regard to the assessment of category thresholds.

References

Carter, J. L. and V. H. Resh. 2001. After site selection and before data analysis: sampling, sorting, and laboratory procedures used in stream benthic macroinvertebrate monitoring programs by USA state agencies. Journal of the North American Benthological Society 20:658-682.

Larsen, D. P. and A. T. Herlihy. 1998. The dilemma of sampling streams for macroinvertebrate richness. Journal of the North American Benthological Society 17:359-366.

U. S. Environmental Protection Agency (EPA). 2006. *Wadeable Streams Assessment: A* Collaborative Survey of the Nation's Streams. EPA 841-B-06-002. Washington, D. C.: Office of Water. Available: http://www.epa.gov/owow/streamsurvey/.

Appendix H. FDEP Biological condition gradient workshop summary

Russel Frydenborg and Denise Miller (FDEP)

Background

Florida Department of Environmental Protection (FDEP) staff evaluated stream macroinvertebrate data from the stream condition index (SCI) recalibration dataset with the goal of refining delineations of biological/ecological condition based on the SCI 2004 scores. Several approaches were taken for this evaluation, among them a Biological Condition Gradient (BCG) Workshop which was conducted by FDEP staff on October 24, 2006. This document provides a summary of FDEP's BCG workshop. A more detailed description of the workshop and materials is provided in Frydenborg and Miller, 2007.

In preparing for this workshop, FDEP staff used the framework of prior such workshops conducted by Susan Davies of the Maine Department of Environmental Protection, Susan Jackson of the U.S. Environmental Protection Agency (Davies and Jackson, 2006), and Jeroen Gerritsen of Tetra Tech, Inc. Each of those individuals also contributed thoughtful commentary during the planning of this workshop.

For the FDEP BCG workshop, a group of experts in aquatic biology and stream macroinvertebrate identification were requested to review taxonomic data from a subset of samples from the SCI 2004 recalibration data set and to provide scores for those samples using the BCG framework (scoring based on descriptions of BCG attributes per experts' review of the taxonomic data). Each expert would thus provide their assessment of the biological condition of each of 30 samples selected by FDEP to span the range of SCI scores from the SCI 2004 recalibration data. Experts did not know the SCI scores for the samples. FDEP evaluated the experts' BCG scores together with SCI scores for the samples as one line of evidence in determining what delineations or thresholds of SCI scores should be used in regulatory decision-making (e.g., what distribution of scores signifies that a site is meeting designated use or not meeting designated use).

FDEP invited participants for this workshop who have expertise in Florida stream macroinvertebrate identification, and who hail from local and state regulatory agencies, universities and the private sector.

Methods

Prior to the workshop, experts were provided with background literature on the biocondition gradient concept (Davies and Jackson, 2006; Stoddard et al., 2006) and descriptions from FDEP of the intended process (Frydenborg and Miller, 2007: Attachment A). Experts were informed that they would be asked to review SCI sample data that would be provided to them at the workshop and to assign each sample a score of 1 to 6, signifying their assessment of the biological condition represented by the sample (1 best to 6 worst based on the 6 tiers of the conceptual BCG summarized from Davies and Jackson, 2006: Figure 2):

- Score of 1: Native or natural condition
- Score of 2: Minimal loss of species; some density changes may occur
- Score of 3: Some replacement of sensitive-rare species; functions fully maintained
- Score of 4: Some sensitive species maintained but notable replacement by more-tolerant taxa; altered distributions; functions largely maintained

- Score of 5: Tolerant species show increasing dominance; sensitive species are rare; functions altered
- Score of 6: Severe alteration of structure and function.

At the workshop, Russel Frydenborg, FDEP's workshop moderator, gave a presentation describing the BCG process and attributes, and distributed materials from the presentation for reference by the experts (Frydenborg and Miller, 2007: Attachment B).

During the workshop, experts were given the opportunity to discuss their rationale for scoring of a site, and, if they desired, to change their score for a site based on their assessment from the discussions (Delphi method). Scoring sheets were provided to each expert for recording both initial and final sample scores. Experts were requested to indicate what relevance they ascribed to their BCG scores. More specifically, they were asked to record the minimum BCG score(s) (1 being best, 6 worst) that they believed met the interim goals of the Clean Water Act (meets designated use), and what maximum score(s) they believed did not meet the interim goals of the Clean Water Act (impaired site; does not meet designated use). In other words, sites below a selected tier score would be considered "impaired."

Sample Selection

Sample data were drawn from the SCI recalibration data and were limited to those where the total number of individual organisms counted in the sample was between 100-175, inclusive.

Five (5) reference site (minimally disturbed condition site) samples from each of three (3) Florida Bioregions (Panhandle, Peninsula, Northeast) were selected to allow the experts an assessment of reference expectations separately for each region because prior SCI analysis indicated differences in expectations for some metrics between the bioregions **(Table H-1)**. The 15 reference site samples were selected from sites already identified as reference based on criteria defined independently of the biological condition (see *Chapter 4: Reference site selection*). The 5 reference samples from each bioregion were selected to span the range of SCI scores for all of the reference site samples from that bioregion, including minimum and maximum SCI 2004 scores, and samples with scores closest to the 25th percentile, median, and 75th percentile values from each bioregion reference range. SCI scores were used for sample selection, but were not provided to the experts. Additionally, experts were not provided with information about the location or description of the selected sample sites, other than the bioregion from which the samples were collected.

Additionally, 10 samples were selected for each bioregion, and identified to bioregion, for analysis of macroinvertebrate data and scoring by the experts. Sites were selected to span a range of conditions using existing information on SCI scores and Human Disturbance Gradient (HDG), and, as needed, LDI from the SCI recalibration data set. The specific 10-sample selection methodology for each bioregion was as follows **(Table H-2)**:

- 4 samples selected based on those having SCI scores closest to the 10th, 25th, median and 75th percentile SCI scores for the non-reference sites for the bioregion;
- 2 samples randomly selected from non-reference sites for the bioregion having SCI scores in the range of 35-50 (sorted on SCI score), to ensure scrutiny of some samples in this score range;
- 3 samples randomly drawn from HDG ranges of 1-2, 3-5, and/or 6-9, based on the assessment of the HDG scores already represented from the prior 6 sample selection (sorted on HDG scores);
- For the Northeast bioregion samples only, there were insufficient sites with the target HDG ranges (i.e., no HDG scores of 5 through 9, and only one HDG 4 site). Therefore, 2 of the intended HDG-based samples were randomly drawn based on the

range of LDI scores for the samples, after an assessment of the LDI scores already represented from the prior 7-sample selection. Specifically, there were 6 samples in the 1 to < 2 LDI range, and 1 sample in the LDI 6 to < 7 range. Therefore, 2 samples were randomly selected from the LDI 2 through 9 range (sorted on LDI scores), excepting sites with LDIs >= 6 and < 7; and,

- 1 sample randomly drawn from the entire non-reference dataset for the bioregion (sorted on HDG score).

Table H-1. BCG workshop reference sites

Site identification information, bioregion location, date of sampling, SCI value, and LDI value.

BCGSampleID	STORET	Station Nickname	Bioregion	Sample Date	SCI	LDI
NERef01	20030341	CECFLD7	Northeast	3/5/1996	70.4	1.90
NERef02	21010018	FALLNGREF	Northeast	7/27/1999	39	1.37
NERef03	19010099	PIGEONREF	Northeast	2/1/2000	58	1.27
NERef04	21010056	ROBCKCR131	Northeast	3/13/2000	79.2	1.35
NERef05	20030456	THOMASREFB	Northeast	3/14/1996	92.4	1.25
PanhRef01	33040014	BIGHORSREF	Panhandle	8/23/1994	67	1.23
PanhRef02	32010024	LIMEREF	Panhandle	7/14/1998	74.5	1.33
PanhRef03	33010065	RSTAREARUN	Panhandle	2/14/1996	54.7	1.19
PanhRef04	22030010	STMARKSREF	Panhandle	9/18/2001	40.5	1.29
PanhRef05	31010051	SWTREF	Panhandle	7/8/1992	97.8	1.31
PenRef01	28020237	CYPCKUNK	Peninsula	7/21/1999	55.7	1.18
PenRef02	20030414	GLDHEADREF	Peninsula	1/12/2000	100	1.00
PenRef03	24010002	MANAREF	Peninsula	11/21/2000	80.2	1.31
PenRef04	20020012	OKLRIVREF	Peninsula	2/15/2000	68.1	1.01
PenRef05	20030253	PUTNA44	Peninsula	3/16/1998	24	1.15

Table H-2. BCG workshop samples

BCG SampleID	STORET	Station Nickname	Bioregion	Sample Date	SCI	HDG	LDI	Selection Rationale
NE01	19020027	ALIGTRREF	Northeast	4/11/2001	47.1	1	1.4	SCI [35-50] random
NE02	19010049	BLDWIN REF	Northeast	2/14/2000	32.9	2	3.22	Random
NE03	19020053	CALAHANTST	Northeast	9/24/2001	24.4	3	1.41	SCI 10th Percentile (22.96)
NE04	19020053	CALAHANTST	Northeast	7/15/1996	40.1	3	1.41	SCI [35-50] random
NE05	20030549	CECFLD6	Northeast	3/6/1996	73.6	1	2	LDI [2-9] random
NE06	20030332	JAXHEITTST	Northeast	5/5/1992	28.9	4	6.11	HDG [3-5] random
NE07	21030059	LKBUTTST	Northeast	9/15/1992	9.1	3	5.26	LDI [2-9] random
NE08	19010073	MCCLNYTST	Northeast	3/22/1999	68	0	1.55	SCI 75th Percentile (68.27)
NE09	20030573	MILOG209	Northeast	1/26/1998	30.6	1	1.61	SCI 25th Percentile (30.39)
NE10	19020059	YULEEREF	Northeast	10/28/1996	47	0	1.28	SCI 50th Percentile (45.09)
Panh01	22020078	ABHOPTST	Panhandle	6/4/1996	20.7	3	1	HDG [3-5] random
Panh02	33040073	CANEYCBELL	Panhandle	8/22/2000	30	3	1.71	HDG [3-5] random
Panh03	31010125	CHATTATST	Panhandle	10/13/1998	62.5	0	1.62	SCI 50th Percentile (62.40)
Panh04	31020071	CHTMDLCRKD	Panhandle	7/23/1996	70.5	1	1.72	SCI 75th Percentile (70.50)
Panh05	31020072	CHTMDLOTTR	Panhandle	7/23/1996	43.5	2	1.42	Random
Panh06	33010112	COFECSTLR	Panhandle	2/16/1999	47.7	2	1.05	SCI [35-50] random
Panh07	31020042	DOZREF	Panhandle	9/1/1992	30.2	6	2.83	SCI 25th Percentile (30.10)
Panh08	31020043	DOZTST	Panhandle	9/1/1992	35.8	6	2.75	SCI [35-50] random
Panh09	22030064	FLAMINREF	Panhandle	3/8/1993	4.6	7	8.08	SCI 10th Percentile (4.92)
Panh10	22050003	TAYLO4	Panhandle	4/10/2000	0.6	3	2.28	HDG [3-5] random
Pen01	20030585	BUNCNTRL	Peninsula	9/8/1998	15.5	6	5.29	HDG [6-9] random
Pen02	25020550	DESOT34	Peninsula	9/3/1997	37.5	4	2.91	SCI [35-50] random

BCG SampleID	STORET	Station Nickname	Bioregion	Sample Date	SCI	HDG	LDI	Selection Rationale
Pen03	27010580	GROOVRARPT	Peninsula	8/22/2000	24.2	1	1.79	SCI 25th Percentile (24.55)
Pen04	24040117	GULFTST	Peninsula	10/8/1998	14.8	7	7.85	SCI 10th Percentile (14.45)
Pen05	24030044	HILSTP5REF	Peninsula	8/18/1993	55	1	2.12	HDG [1-2] random
Pen06	20010384	LONGBRANCH	Peninsula	7/13/1999	38.2	3	2.53	SCI [35-50] random
Pen07	28010224	NWFRLOXREF	Peninsula	9/20/1995	40.8	4	3.87	SCI 50th Percentile (40.81)
Pen08	25020014	OAKREF	Peninsula	8/22/1995	58.3	1	2.1	SCI 75th Percentile (58.70)
Pen09	20020145	SWEETCK	Peninsula	8/10/1998	21.1	2	1.3	Random
Pen10	20020420	WGCITREF	Peninsula	12/1/1992	0	7	8.32	HDG [6-9] random

Data Content

Data provided for each sample, whether for reference samples or samples for BCG scoring, consisted only of the biological data for each sample and an identification of the sample to the Bioregion of its collection. Other data regarding the sample and site (e.g., more specific location, collection date, information on physical/chemical characteristics, human disturbance metrics such as LDI, hydrologic alteration) were not provided to the experts, but were available for analysis with the scoring data from the workshop.

Biological data that were provided for each sample were retrieved from the FDEP Statewide Biological database (SBIO). Those data included the collapsed taxa list with the number of individuals of each taxon counted in that sample and a suite of metrics. Details regarding the SBIO taxa collapsing procedure are provided in the SBIO database manual (Wolfe and Lurding, 1999: p. 84, and Appendix 2). Frydenborg and Miller, 2007 (Attachment C) provides a copy of each sample sheet of the taxa list and metrics that was provided to experts, a copy of a list of synonyms that was provided to assist in their review of the taxa list, and a copy of the sample scoring sheet.

Results

Each expert provided a BCG score for each site sample **(Table H-3)** and an assessment of the interpretation that should be applied to each BCG score (e.g., meets designated use, does not meet designated use; **Table H-4**). In several cases, experts reported a “gray” area in the relevance they ascribed to their BCG scores. Several experts identified BCG scores they judged as clearly meeting designated use, those they judged as clearly not meeting designated use, and left a gap between those scores signifying their uncertainty about where such a line could be

drawn. Responses were unanimous that a BCG score of 3 or lower (1-3) meets the CWA interim goal for biological integrity and that BCG scores of 5 and 6 do not meet the CWA biological integrity goal. Approximately half the panel thought the impairment threshold occurred between BCG scores of 4 and 5. The other half of the panel wished the impairment line to be drawn between BCG scores of 3 and 4, with many of those panelists expressing the concern that management action may not be taken on sites until they were considered "impaired."

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Table H-3. BCG workshop scores summary

Expert Code	E01	E02	E03	E04	E05	E06	E07	E08	E09	E10	E11	E12	E13	E14	E15	E16	E17	E18	E19	E20	E21	E22	Median BCG Score	SCI Score
BCGSampleID	**Final BCG Score**																							
NE01	3	4	3	4	3	3	3	3	3	4	3	3	4	3	4	3	3	3	3	2	3	4	3	47.1
NE02	5	5	5	5	5	5	5	5	5	5	4	4	5	5	5	5	5	4	4	4	5	5	5	32.9
NE03	4	5	5	5	5	5	5	5	5	5	5	5	5	5	5	4	5	5	5	5	3	5	5	24.4
NE04	3	4	4	3	4	4	3	4	3	3	3	3	3	3	3	3	3	3	3	3	4	4	3	40.1
NE05	2	1	2	2	2	1	2	2	2	2	2	2	2	1	2	2	2	1	2	1	1	2	2	73.6
NE06	4	5	4	5	3	5	5	5	3	5	5	5	4	5	5	4	4	4	4	5	4	4	5	28.9
NE07	6	6	5	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	5	6	5	6	6	9.1
NE08	3	3	3	2	3	4	5	3	3	3	4	3	2	3	2	3	3	3	3	3	3	3	3	68
NE09	4	4	5	4	5	3	4	4	5	5	4	4	4	4	5	4	4	4	4	4	4	4	4	30.6
NE10	3	3	4	2	3	4	4	3	3	3	2	3	3	3	4	3	3	3	3	3	3	3	3	47
Panh01	5	4	4	5	5	5	5	4	4	4	3	5	4	4	5	4	4	5	4	3	4	4	4	20.7
Panh02	5	4	4	4	5	5	5	5	4	5	4	4	5	5	5	4	5	5	5	4	4	5	5	30
Panh03	2	2	2	2	2	1	2	2	2	2	2	2	2	1	2	2	1	2	2	2	3	1	2	62.5
Panh04	2	2	2	2	2	2	2	2	2	2	2	3	2	1	2	1	1	2	2	2	2	2	2	70.5
Panh05	4	4	3	2	4	4	3	4	3	3	4	3	4	3	3	3	3	3	4	3	3	3	3	43.5
Panh06	3	2	2	2	2	2	2	3	2	2	2	2	3	2	2	2	2	2	2	2	3	2	2	47.7

Expert Code	E01	E02	E03	E04	E05	E06	E07	E08	E09	E10	E11	E12	E13	E14	E15	E16	E17	E18	E19	E20	E21	E22	Median BCG Score	SCI Score
Panh07	5	5	5	5	5	5	5	5	5	5	4	5	5	5	5	5	5	5	5	4	4	5	5	30.2
Panh08	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	6	5	4	5	5	5	35.8
Panh09	6	6	6	6	5	6	5	6	6	6	6	6	6	6	6	6	6	6	5	6	6	6	6	4.6
Panh10	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	0.6
Pen01	5	5	6	5	5	6	5	6	5	5	5	5	5	6	5	6	6	5	5	5	6	6	5	15.5
Pen02	3	3	3	4	3	3	3	4	4	4	3	3	4	4	3	4	4	3	4	3	3	3	3	37.5
Pen03	5	4	4	4	5	4	4	4	5	5	4	4	5	5	4	5	4	5	5	4	5	4	4	24.2
Pen04	5	5	5	5	5	5	5	5	5	5	4	5	5	6	5	6	5	5	5	5	5	6	5	14.8
Pen05	3	2	2	2	2	2	2	3	2	2	2	2	2	2	2	2	3	3	3	2	2	2	2	55
Pen06	4	4	4	3	5	4	4	4	5	4	4	4	4	5	4	4	4	4	4	4	4	4	4	38.2
Pen07	3	3	2	2	3	3	3	3	3	3	3	3	3	3	4	3	3	3	3	3	3	3	3	40.8
Pen08	2	2	1	1	2	2	2	1	1	2	1	2	1	2	2	2	2	2	2	2	2	2	2	58.3
Pen09	5	5	5	5	5	4	5	6	5	5	5	5	5	5	5	5	5	5	5	5	6	5	5	21.1
Pen10	6	6	6	6	6	5	6	6	6	6	6	5	6	6	6	6	6	6	6	6	6	6	6	0

Table H-4: Experts' categorization of BCG workshop scores

Expert Code:	Not Impaired/ Meets Goal	Gray Area	Does Not Meet Goal/Impaired	Comments
E01	3		4	
E02	3	4	5	
E03	3	Not impaired 3-4, closer to 4, but not below 4	5	
E04	4		5	
E05	4		5	
E06	3		4	
E07	3		4	
E08	3		4	
E09	3		4	
E10	3		4	
E11	3		4	
E12	4		5	
E13	3		4	
E14	4		5	Impairment line 4-5, but concerned that the category 5 sites will not be improved and category 4 sites will slip into category 5 and then may be clumped with the other 5's as "irretrievable"
E15	3		4	
E16	3	line between 4 and 5	5	
E17	3	Impairment Line 4-5, 4 probably meets	5	
E18	4		5	
E19	4		5	
E20	4		5	
E21	4		5	
E22	4		5	

Appendix I. Change Point Analysis of Stream Condition Index and Biological Condition Gradient Data

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Background

For the purpose of scrutinizing Stream Condition Index (SCI) macroinvertebrate data with the goal of refining delineations of biological/ecological condition, Florida Department of Environmental Protection (FDEP) staff conducted a biological condition gradient (BCG) workshop in October 2006 (Frydenborg and Miller, 2007). Participants in the BCG workshop (the panel) consisted of twenty-two (22) experienced aquatic biologists in Florida who agreed to assist FDEP as part of this delineation effort.

During the BCG workshop, the panel was provided with species lists and metrics for 15 reference streams and 30 test samples, equally divided into Florida's three bioregions (Panhandle, Northeast, and Peninsula). The panel was not given the SCI scores associated with any of the sites, or any physical, chemical, or habitat descriptions of the sites (other than the Bioregion location). Decisions were based on biological community composition data collected via the 20-dip net sweep method associated with SCI sampling. The scores (1 through 6) were based on the six tiers of the conceptual biological condition gradient (BCG) from Davies and Jackson, 2006, listed below:

- Score of 1: Native or natural condition
- Score of 2: Minimal loss of species; some density changes may occur
- Score of 3: Some replacement of sensitive-rare species; functions fully maintained
- Score of 4: Some sensitive species maintained but notable replacement by more-tolerant taxa; altered distributions; functions largely maintained
- Score of 5: Tolerant species show increasing dominance; sensitive species are rare; functions altered
- Score of 6: Severe alteration of structure and function

The analyses conducted in this report were based on the results from the BCG workshop (BCG scores for 30 samples by each of 22 panelists) and the SCI scores for those same samples to address FDEP's goal of refining delineations of biological/ecological condition for SCI scores.

The Detection Procedure

Niu et al. (2000) introduced an iterative procedure for detecting and modeling level-shift change points. The procedure is similar to that suggested by Chang (1982) and further developed by Chang et al. (1988) for detecting outliers and level shifts in time series analysis. Statistical details of this procedure can also be found in Pankratz (1991, Chapter 8).

For simplicity, let us consider a response variable *Y*, after an appropriate transformation. Suppose that observations $\{(X_i, Y_i),\ i = 1, 2, \ldots, n\}$ are available where *n* is the sample size and *X* is an independent variable. Moreover, we assume that the observations are arranged in the following manner:

- The values $\{X_i, \quad i = 1, 2, \ldots, n\}$ are distinct. If several $Y_i's$ are corresponding to a single *X* value, the median of the $Y_i's$ is taken to be the response value for the *X* value.
- $\{(X_i, Y_i),\ i = 1, 2, \ldots, n\}$ are sorted according to the values of *X* from least to greatest.

For each integer $l > 1$, define the step variable $S_i(l) = 0$ for $i < l$ and $S_i(l) = 1$ for $i \geq l$.

Step 1. Fit the linear regression model:

$$Y_i = \beta_0(l) + \beta_1(l) S_i(l) + \varepsilon_i(l), \qquad i = 1, 2, \ldots, n, \tag{1}$$

where for a fixed *l*, the $\varepsilon_i(l)'s$ are assumed to be independent and identically distributed normal random variables with mean zero and variance $\sigma^2(l)$.

Step 2. Calculate the values $\{L(l) = \hat{\beta}_1(l) / se(\hat{\beta}_1(l)),\ l = 2, 3, \ldots, (n-1)\}$ where $se(\hat{\beta}_1(l))$ is the estimated standard error of $\hat{\beta}_1(l)$.

Step 3. Let $L(l_1) = \max\{L(2), L(3), \ldots, L(n-1)\}$ and compare $L(l_1)$ with the critical value *C*=3.0 (or *C*=3.5). The critical value *C*=3.0 (or *C*=3.5) corresponds roughly to $\alpha = 0.10$ (or $\alpha = 0.05$), or the 10% (or the 5%) significance level, based on the simulation results of Chang et al. (1988). If $L(l_1)$ is significant, we conclude that the response *Y* has a change point at X_{l_1} with a level-shift $\hat{\beta}_1(l)$.

Step 4. Let $Y_i^* = Y_i - \beta_1(l_1) S_i(l_1)$. Repeat Steps 1-3 on the new response variable Y_i^* for detecting a possible second change point. Continue the process until no further change point can be identified.

Step 5. Suppose that *k* change points are detected in the response variable *Y* and the corresponding *X* values are $\{X_{l_1}, X_{l_2}, \ldots, X_{l_k}\}$. Fit the model

$$Y_i = \beta_0 + \beta_1 S_i(l_1) + \beta_2 S_i(l_2) + \ldots + \beta_k S_i(l_k) + \varepsilon_i, \qquad i = 1, 2, \ldots, n. \tag{2}$$

Then the estimated coefficients $\{\hat{\beta}_1, \hat{\beta}_2, \ldots, \hat{\beta}_k\}$ will be the *k* estimated level-shift values.

Analysis of SCI and BCG Data

Change Point Analysis

Table I-1 contains a list of the SCI values for the 30 samples from the BCG workshop along with their median and mean BCG scores based on the individual scores given by the experts. Change-point analysis of the SCI values was conducted in this report using the median BCG scores as the dependent variable. Median BCG scores rather than mean BCG scores were used to avoid potential outlier problems.

Two change points were detected in the SCI values. The first change point was at SCI=35.8, which has the statistic $L(l_1)$ = 9.79 and is significant at the 5% level (95% confidence). The second change point occurred at SCI=9.1 which has the statistic $L(l_1)$ = 3.87 and is also significant at the 5% level (95% confidence). The R-square of the regression is 0.86, indicating that the step-function regression model fits the median BCG scores very well.

Table I-1. SCI and BCG scores for the 30 Samples

STORET Station	Sample Date	SCI Score	BCG Median Score	BCG Mean Score
20030549	3/6/1996	73.6	2	1.7
31020071	7/23/1996	70.5	2	1.9
19010073	3/22/1999	68	3	3
31010125	10/13/1998	62.5	2	1.9
25020014	8/22/1995	58.3	2	1.7
24030044	8/18/1993	55	2	2.2
33010112	2/16/1999	47.7	2	2.2
19020027	4/11/2001	47.1	3	3.2
19020059	10/28/1996	47	3	3.1
31020072	7/23/1996	43.5	3	3.3
28010224	9/20/1995	40.8	3	3
19020053	9/24/2001	40.1	3	3.3
20010384	7/13/1999	38.2	4	4.1
25020550	9/3/1997	37.5	3	3.4
31020043	9/1/1992	35.8	5	5
19010049	2/14/2000	32.9	5	4.8
20030573	1/26/1998	30.6	4	4.2
31020042	9/1/1992	30.2	5	4.9
33040073	8/22/2000	30	5	4.6
20030332	5/5/1992	28.9	4.5	4.4
19020053	9/24/2001	24.4	5	4.8
27010580	8/22/2000	24.2	4	4.5
20020145	8/10/1998	21.1	5	5
22020078	6/4/1996	20.7	4	4.3

STORET Station	Sample Date	SCI Score	BCG Median Score	BCG Mean Score
20030585	9/8/1998	15.5	5	5.4
24040117	10/8/1998	14.8	5	5.1
21030059	9/15/1992	9.1	6	5.9
22030064	3/8/1993	4.6	6	5.9
22050003	4/10/2000	0.6	6	6
20020420	12/1/1992	0	6	5.9

Change Points: 1). SCI=35.8 with the test statistic of 9.79 and confidence level > 95%;
2). SCI=9.1 with the test statistic of 3.87 and confidence level > 95%.
Highlighted numbers are the SCI-BCG Scores at the change points.

Confidence Intervals for the Change Points

In this section, we constructed confidence intervals for the detected change points based on a *modified* bootstrapping method. "Regular" bootstrapping is a statistical method for estimating the sampling distribution of an estimator by resampling with replacement from the original sample (Efron, 1979; Efron and Tibshirani, 1993). This method is widely used in parameter estimation, regression, time series, and other statistical problems.

In regression analysis, there are two basic approaches to perform bootstrapping. The first approach is bootstrapping cases. Assume that we want to fit a regression model with response variable *Y* and predictors $\{x_1, \ldots, \ldots x_p\}$, and we have a sample of *n* cases $\{Z_i = (Y_i, x_{1i}, \ldots, \ldots x_{pi}), i = 1, \ldots, n\}$. In bootstrapping, we select *n* cases randomly from the original observations with replacement. For each bootstrapping sample, we fit the regression model and save the coefficients. Confidence intervals for the regression coefficients can be constructed based on *M* (a given large number) bootstrapping samples. The second approach in bootstrapping regression analysis is resampling the residuals. In this way a regression model is fitted to the original data first. The fitted values and residuals are calculated based on the fitted model. The residuals are randomly resampled and added back to the fitted values. After generating a new set of bootstrapping data, the regression model is refitted.

Efron and Tibshirani (1993, Chapter 9, p. 113) pointed out that bootstrapping cases is less sensitive to model assumptions such as normality and constant variance than bootstrapping residuals. In this study, the bootstrapping cases method was used to construct confidence intervals for change points.

There are several approaches to constructing bootstrap confidence intervals for change points. The bootstrap percentile interval approach was used in this study. This approach uses the empirical quantiles of $\hat{\theta}$ to form a confidence interval $[\hat{\theta}^*_{Lower}, \hat{\theta}^*_{Upper}]$ for the change point θ, where $\{\hat{\theta}_{(1)}{}^*, \hat{\theta}_{(2)}{}^*, \ldots, \hat{\theta}_{(M)}{}^*\}$ are the ordered bootstrap estimates of the change point; $\hat{\theta}^*_{Lower}$ and $\hat{\theta}^*_{Upper}$ are the α/2 percentile and the (1 − α/2) percentile of the bootstrap estimates of the change point, respectively.

For this analysis, we had 30 pairs of SCI and Median BCG Scores. We used a modified bootstrapping method to construct confidence intervals for the change points. Specifically, we

first randomly drew 90% of the 30 pairs (or dropped 10%) from the original data and conducted change-point analysis. This procedure is also called the *drop-d Jackknife method* in statistics (Efron and Tibshirani, 1993, Page 149). Three thousand (3,000) samples were drawn from the original data, and the results are presented in Table I-2. For the first change point at SCI=35.8, the 90% and 95% confidence intervals based on the percentiles of the 3,000 estimated change points were identical.

Table I-2. Confidence Intervals (CI) for the change points of SCI scores based on *M*=3,000 drop-10% samples.

	First Change Points	Second Change Points
SCI Based on Original Data	35.8	9.1
SCI Based on Average of drop-10% Estimates	35.8	8.8
SCI Based on Median of drop-10% Estimates	35.8	9.1
Standard Error of the Change Point	1.6	2.7
SCI Percentile Interval, 95% CI	[32.9, 38.2]	[4.6, 13.4]
SCI Percentile Interval, 90% CI	[32.9, 38.2]	[4.6, 12.0]

Summary

This change-point analysis was conducted for the purpose of addressing FDEP's goal of refining delineations of biological/ecological condition for Stream Condition Index (SCI) scores. The BCG (biological condition gradient) scores that were used to evaluate changes in SCI scores were provided by a panel of 22 experienced aquatic biologists in Florida. The results of this change-point analysis indicated a first change point at SCI=35.8, with the 95% confidence interval [32.9, 38.2]. In other words, SCI scores higher (better) than 35.8 were determined to be different from lower scores based on the biological condition assigned to taxonomic data for the samples by experienced aquatic biologists. Additional details of the BCG workshop and the change-point analysis are provided in Frydenborg and Miller, 2007 and Niu and Miller, 2007, respectively.

References

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Appendix J. Reference sites

Table J-1. Reference site locations.

STORET	Site Description	Bioregion	Waterbody Name	Facility Information from GIS Coverages	Latitude			Longitude		
					Deg	Min	Sec	Deg	Min	Sec
20030245	Ates Creek @ CR 315	Northeast	BLACK CREEK	No point sources or stormwater inputs upstream	29	54	38	81	53	5
20030437	BLACK CR., NORTH PRONG, 1/4 ABOVE BIG BRANCH	Northeast	BLACK CREEK	One large stormwater constructin permit upstream, possibly connected through wetlands issued in 6/2005.	30	6	48.13	81	54	23.34
20030419	Black Creek, South Prong	Northeast	BLACK CREEK	Camp blanding is upstream of this site	29	56	3.15	81	55	9.63
20030723	N. FORK BLACK CRK ABOVE CARTER SPENCER RD.	Northeast	BLACK CREEK	Dupont Highland Mine facility discharges, but this site is above this.	30	4	9.2	81	58	43.9
20030247	South Fork Black Creek @ SR 16	Northeast	BLACK CREEK	No point sources or stormwater inputs upstream	29	58	45	81	51	10
20030570	Bradley Crk @ 739B-Peter's crk basin	Northeast	BRADLEY CREEK	Subdivision (Silver Creek) stormwater permits (2003 &2005), discharge may flow towards this site.	30	2	48.8	81	47	5.5
20030487	Bull Creek @ NE Crossing of CR 215	Northeast	BULL CREEK	No point sources or stormwater inputs upstream	30	2	3.2	81	54	6.8
19010042	Calkins Ck, off Hamp Register Rd.	Northeast	CALKINS CREEK	No point sources or stormwater inputs upstream	30	19	50.95	82	14	30.47
21010043	Deep Creek upstream of bridge @Old River Rd	Northeast	DEEP CREEK	No point sources or stormwater inputs upstream	30	22	35.7	82	38	16.1
21010018	Falling Creek @ CR 131, above falls Suwannee R.	Northeast	FALLING CREEK	KOA Domestic WWTF ~3000 m upstream of site, not NPDES permitted, design capacity = 0.0150. Not NPDES permitted, and small design capacity likely means that this is a percolation pond. District Biologist is not aware of a KOA discharge in this area.	30	15	39.47	82	40	5.19
19010064	John Rowe Br, test site for NE FL State Hosp FYI	Northeast	JOHN ROWE BRANCH	No point sources or stormwater inputs upstream; The hospital doesn't show up as permitted site on the GIS coverage	30	13	47.4	82	8	16.4

STORET	Site Description	Bioregion	Waterbody Name	Facility Information from GIS Coverages	Latitude			Longitude		
					Deg	Min	Sec	Deg	Min	Sec
20030571	Peter's Crk above Rosemary hill Rd- Basin	Northeast	PETERS CREEK	Clay Co. Dept. of Env. Services has 2 stormwater permits right at the site and 2 stormwater outfall sites	30	0	25.9	81	44	55.4
19010099	Pigeon Creek at US 1 St. Marys-Nassau River	Northeast	PIGEON CREEK	No point sources or stormwater inputs upstream	30	45	40.73	81	58	3.07
21010056	PCS Phosphate FYI5, control site	Northeast	ROBINSON CREEK	No point sources or stormwater inputs upstream	30	19	14	82	39	44.1
21010032	Rocky Ck @ Woodpecker Rd	Northeast	ROCKY CREEK	No point sources or stormwater inputs upstream	30	32	40.27	82	44	1.91
20030265	Sal Taylor Creek below Rowell Creek	Northeast	SAL TAYLOR CREEK	One large stormwater constructin permit above these sites issued in 12/2004.	30	11	59	81	54	50
21030115	Santa Fe Rv- Control A BMP	Northeast	SANTA FE RIVER	A few stormwater permits pretty far upstream	29	50	46.9	82	12	13.8
20030456	Thomas Ck, Forestry BMP Study Ref Site B	Northeast	THOMAS CREEK	No point sources or stormwater inputs upstream	29	54	57.6	81	51	44.9
19010072	McClenny WWTP upstream	Northeast	TURKEY CREEK	A few stormwater permits around, look to be small and may not actually discharge to this stream	30	16	6.4	82	7	34.9
20030341	Yellow Water Creek above Sal Taylor Creek	Northeast	YELLOW WATER CREEK	One large stormwater constructin permit above these sites issued in 12/2004.	30	12	3.2	81	55	10.35
20030342	Yellow Water Creek below Sal Taylor Creek	Northeast	YELLOW WATER CREEK	One large stormwater constructin permit above these sites issued in 12/2004.	30	11	44.45	81	55	5.8
22020070	BIG CYPRESS BRANCH-TIMBER ENERGY FYI CNTRL SITE	Panhandle	BIG CYPRESS BRANCH	No point sources or stormwater inputs upstream	30	19	0	84	48	0
33040014	BIG HORSE CREEK S.R.2 OKA.CO.	Panhandle	BIG HORSE CREEK	No point sources or stormwater inputs upstream	30	55	19.3	86	35	42.6
21030064	Cedar Head Run, above Ichetucknee River	Panhandle	CEDAR HEAD RUN	Ichetucknee Family Campground WWTF upstream of site, not NPDES permitted (?), design capacity=0.007. Subsequent additional information indicates no discharge. Stream begins in State Park.	29	58	50.2	82	45	30.92
31010050	CROOKED CREEK HWY.270 GADSDEN CO.	Panhandle	CROOKED CREEK	No point sources or stormwater inputs upstream	30	34	59.79	84	52	51.49

STORET	Site Description	Bioregion	Waterbody Name	Facility Information from GIS Coverages	Latitude			Longitude		
					Deg	Min	Sec	Deg	Min	Sec
32030023	ECONFINA CREEK AT SCOTT RD	Panhandle	ECONFINA CREEK	No point sources or stormwater inputs upstream	30	33	19.3	85	26	6
22050077	Econfina River Forestry BMP Study Ref Site B	Panhandle	ECONFINA RIVER	No point sources or stormwater inputs upstream	30	9	52.8	83	50	14.6
33040064	Jacks Branch, Forestry BMP Study Ref Site B	Panhandle	JACK'S BRANCH	No point sources or stormwater inputs upstream	30	52	10.3	86	29	21.7
32010024	Limestone Ck @ Beckridge Rd	Panhandle	LIMESTONE CREEK	No point sources or stormwater inputs upstream	30	59	21.58	86	5	9.81
32020063	LITTLE CROOKED CREEK S.R.79 BAY CO.	Panhandle	LTL CROOKED CREEK	No point sources or stormwater inputs upstream	30	24	48.4	85	52	6.5
22030062	MCBRIDE SLOUGH HWY.267 WAKULLA CO.	Panhandle	MCBRIDE SLOUGH	No point sources or stormwater inputs upstream	30	14	10	84	16	20
33010054	MCDAVID CREEK AT SR 99	Panhandle	MCDAVID CREEK	No point sources or stormwater inputs upstream	30	44	21.6	87	26	54.8
22020049	MULE CREEK S.R.12 LIBERTY CO.	Panhandle	MULE CREEK	No point sources or stormwater inputs upstream	30	30	43.55	84	49	44.1
22020062	Oklawaha Ck, SR 267, S of Quincy	Panhandle	OKLAWAHA CREEK	Sand mine operations (permitted IWF's). Subsequent additional information indicates no discharge to sampled site.	30	27	2.18	84	38	36.89
31020073	Pitts Mill Creek above conf with Cypress Ck	Panhandle	PITTS MILL CREEK	No point sources or stormwater inputs upstream	30	16	43.9	85	14	23.6
22020010	QUINCY CR above SR12, ref for Quincy STP FYI	Panhandle	QUINCY CREEK	Engelhard IWF upstream and numerous other small IWF's just S that may/may not flow towards this site. Subsequent additional information indicates no discharge to site except maybe during hurricanes.	30	35	34.6	84	33	50.3
22030010	St. Marks R approx 4 mi N of Hwy 98 (@ "rapids")	Panhandle	ST. MARKS RIVER	No point sources or stormwater inputs upstream	30	14	24.8	84	8	43.4
31010051	SWEETWATER CREEK HWY.270 LIBERTY CO.	Panhandle	SWEETWATER CREEK	No point sources or stormwater inputs upstream	30	31	26.68	84	58	25.15

STORET	Site Description	Bioregion	Waterbody Name	Facility Information from GIS Coverages	Latitude			Longitude		
					Deg	Min	Sec	Deg	Min	Sec
31020040	TEN MILE CREEK S.R.73 CALHOUN CO.	Panhandle	TEN MILE CREEK	One small WWTP and one Industrial Wastewater permitted facility upstream; Stormwater construction at sampling point. WW facilities at least 1.5 to 2 km upstream. Additional subsequent information from District Biologist is that site is appropriate for reference.	30	30	1.27	85	12	1.91
33010065	Rest Area Run Creek below I 10	Panhandle	UNNAMED CREEK	Surrounded by stormwater construction, potential inputs from Perdido landfill upstream. Subsequent additional information from District Biologist is that this site was reference at the time relevant to the SCI sampling for the calibration dataset.	30	34	5.4	87	24	10.6
22040038	Unnamed Creek above Monticello WWTP	Panhandle	UNNAMED CREEK	A few stormwater permits around, look to be small and may not actually discharge to this stream	30	30	59.5	83	51	25.3
32020066	Wright Ck, ref for Noma STP FYI	Panhandle	WRIGHTS CREEK	Site is upstream of Noma WWTP	30	58	47.7	85	35	54.4
20010455	BLACKWATER CREEK AT HWY 44A	Peninsula	BLACKWATER CREEK	No point sources or stormwater inputs upstream	28	52	38.96	81	29	19.91
28020239	Cypress brch above SR 78	Peninsula	CYPRESS BRANCH	No point sources or stormwater inputs upstream	26	48	49	81	20	7
28020237	Cypress Creek 2 mi above SR78	Peninsula	CYPRESS CREEK	Small stormwater construction permit for Wayne Homes; permit started in 9/2004	26	45	5	81	37	14
26010972	DAVENPORT CR AT SR 545 BRIDGE	Peninsula	DAVENPORT CREEK	Gas pipeline relocation stormwater permit started in 2/2005	28	16	15.2	81	35	28.06
26010593	FISHEATING CREEK ABOVE US 27	Peninsula	FISHEATING CREEK	Lykes Campground WWTF nearby, not NPDES permitted (?); Depending on flow direction could impact site. Subsequent additional information indicates that site is upstream from campground.	26	56	12	81	19	34
20030414	GOLD HEAD BR. IN STATE PARK	Peninsula	GOLD HEAD BRANCH	No point sources or stormwater inputs upstream	29	49	56.03	81	56	46.64
24030142	Hillsborough R upstrm of Southside Br, Zephyrhil	Peninsula	HILLSBOROUGH RIVER	Zephyrhills WWTF upstream of site, but can't determine where outfall is. Lots of monitoring wells around. Subsequent additional information indicates probably a percolation pond. No discharge to the site - the site was just visited.	28	11	8.4	82	10	30.9

STORET	Site Description	Bioregion	Waterbody Name	Facility Information from GIS Coverages	Latitude			Longitude		
					Deg	Min	Sec	Deg	Min	Sec
24030048	Hillsborough River at SR 39	Peninsula	HILLSBOROUGH RIVER	Zephyrhills WWTF upstream of site, but can't determine where outfall is. Lots of monitoring wells around. Subsequent additional information indicates no discharge to the site - the site was just visited.	28	11	38.7	82	10	2.8
25020111	Horse Creek @ SR 72 Bridge	Peninsula	HORSE CREEK	No point sources or stormwater inputs upstream	27	11	59.91	81	59	15.28
20010454	JUNIPER CREEK AT HIGHWAY 19	Peninsula	JUNIPER CREEK	No point sources or stormwater inputs upstream	29	12	49.41	81	39	17.5
20020004	Little Orange Creek @ Hwy 21	Peninsula	LITTLE ORANGE CREEK	Ga Pacific-Hawthorne Plywood STW discharges upstream, but may flow into Little Orange Lake. Subsequent additional information indicates no discharge to the stream.	29	32	28.42	81	57	16.67
26011019	LIVINGSTON CREEK AT RUCKS DAIRY RD.	Peninsula	LIVINGSTON CREEK	No point sources or stormwater inputs upstream	27	42	29.61	81	26	49.02
28010223	N FK LOXAHATCHEE RIVER REF BIO STATION	Peninsula	LOXAHATCHEE RIVER	No point sources or stormwater inputs upstream	27	0	4.76	80	6	58.48
24010002	Manatee R 20 m below SR64 bridge (TP1)	Peninsula	MANATEE RIVER	Wingate Creek Mine flows SE into Johnson Crk	27	28	24.14	82	12	39.48
20020012	OKLAWAHA RIVER AT SR 316	Peninsula	OKLAWAHA RIVER	No point sources or stormwater inputs upstream	29	22	24.34	81	54	6.35
20020404	ORANGE CREEK 50 YDS. UP FROM HWY. 21	Peninsula	ORANGE CREEK	Water bottling facility (IWF) right at the sampling lat/longs. Subsequent additional information from DEP Bureau of Labs staff is that sampling trips in this area indicate that the IWF facility is located downstream of this site.	29	30	33.44	81	56	47.8
20030253	RICECR #3@BR SR #3/250yds above Bardine	Peninsula	RICE CREEK	No point sources or stormwater inputs upstream	29	41	34	81	43	27
20020317	SILVER RUN AT CONFLUENCE WITH BOAT RAMP CANAL	Peninsula	SILVER RUN	One small WWTP (Sharpes Ferry Mobile Home Park) permitted facility upstream, design capacity = 0.0190. Subsequent additional information indicates the site is upstream of any discharge.	29	12	35.7	81	59	36
28020221	TELEGRAPH CREEK AT BABCOCK RANCH	Peninsula	TELEGRAPH CREEK	One large stormwater construction nearby (permitted 3/2005), but flows into Big Island Canal which is also the receiving water for Telegraph Creek	26	44	54.4	81	40	13.2

STORET	Site Description	Bioregion	Waterbody Name	Facility Information from GIS Coverages	Latitude			Longitude		
					Deg	Min	Sec	Deg	Min	Sec
26011020	TIGER CREEK AT WALKING WATERS RD.	Peninsula	TIGER CREEK	No point sources or stormwater inputs upstream	27	48	43.53	81	26	38.63
23010464	Withlacoochee R @ county park off Auton Rd (TP3)	Peninsula	WITHLACOOCHEE RIVER	Small stormwater construction permit for bike trail; permit started in 4/2005	28	21	0	82	7	28.2
23010446	WITHLACOOCHEE RIVER	Peninsula	WITHLACOOCHEE RIVER	No point sources or stormwater inputs upstream; site below WITHREF	28	18	13.9	82	4	0.1

Table J-2. Reference sites sample data

STORET	Site Description	Bioregion	Waterbody Name	SCI	HDG	LDI	Total # individuals counted	Sample Date
20030245	Ates Creek @ CR 315	Northeast	BLACK CREEK	75.8	0	1.2	153	4/17/1997
20030437	BLACK CR., NORTH PRONG, 1/4 ABOVE BIG BRANCH	Northeast	BLACK CREEK	56.1	0	1.2	103	8/1/1995
20030437	BLACK CR., NORTH PRONG, 1/4 ABOVE BIG BRANCH	Northeast	BLACK CREEK	57.7	0	1.2	108	8/8/2000
20030437	BLACK CR., NORTH PRONG, 1/4 ABOVE BIG BRANCH	Northeast	BLACK CREEK	66.9	0	1.2	117	8/8/2000
20030437	BLACK CR., NORTH PRONG, 1/4 ABOVE BIG BRANCH	Northeast	BLACK CREEK	71.5	0	1.2	122	1/30/1996
20030437	BLACK CR., NORTH PRONG, 1/4 ABOVE BIG BRANCH	Northeast	BLACK CREEK	67.3	0	1.2	123	7/20/1994
20030419	Black Creek, South Prong	Northeast	BLACK CREEK	68.4	0	1.4	103	1/24/1995
20030419	Black Creek, South Prong	Northeast	BLACK CREEK	64.3	0	1.4	106	7/20/1994
20030419	Black Creek, South Prong	Northeast	BLACK CREEK	79.3	0	1.4	106	3/20/1997
20030419	Black Creek, South Prong	Northeast	BLACK CREEK	66.1	0	1.4	119	7/11/2000
20030419	Black Creek, South Prong	Northeast	BLACK CREEK	81.3	0	1.4	150	7/7/1998
20030723	N. FORK BLACK CRK ABOVE CARTER SPENCER RD.	Northeast	BLACK CREEK	43.4	0	1.2	108	7/12/1999
20030247	South Fork Black Creek @ SR 16	Northeast	BLACK CREEK	90.2	0	1.1	123	4/17/1997
20030570	Bradley Crk @ 739B-Peter's crk basin	Northeast	BRADLEY CREEK	85.1	0	1.3	144	1/28/1998
20030487	Bull Creek @ NE Crossing of CR 215	Northeast	BULL CREEK	63.9	0	1.2	164	4/17/1997
19010042	Calkins Ck, off Hamp Register Rd.	Northeast	CALKINS CREEK	75.7	0	1.2	102	8/3/1994
19010042	Calkins Ck, off Hamp Register Rd.	Northeast	CALKINS CREEK	80.4	0	1.2	108	11/1/1994
19010042	Calkins Ck, off Hamp Register Rd.	Northeast	CALKINS CREEK	50.0	0	1.2	114	7/10/2001
21010043	Deep Creek upstream of bridge @Old River Rd	Northeast	DEEP CREEK	66.9	0	1.4	101	11/10/1997
21010018	Falling Creek @ CR 131, above falls Suwannee R.	Northeast	FALLING CREEK	51.5	0	1.4	112	7/12/1994
21010018	Falling Creek @ CR 131, above falls Suwannee R.	Northeast	FALLING CREEK	77.9	0	1.4	126	7/17/1997
21010018	Falling Creek @ CR 131, above falls Suwannee R.	Northeast	FALLING CREEK	39.0	0	1.4	132	7/27/1999
21010018	Falling Creek @ CR 131, above falls Suwannee R.	Northeast	FALLING CREEK	42.7	0	1.4	160	2/8/1994
21010018	Falling Creek @ CR 131, above falls Suwannee R.	Northeast	FALLING CREEK	87.8	0	1.4	161	1/4/1995
19010064	John Rowe Br, test site for NE FL State Hosp FYI	Northeast	JOHN ROWE BRANCH	62.7	0	1.8	142	11/1/1994
20030571	Peter's Crk above Rosemary hill Rd- Basin	Northeast	PETERS CREEK	89.0	0	1.2	110	1/26/1998

STORET	Site Description	Bioregion	Waterbody Name	SCI	HDG	LDI	Total # individuals counted	Sample Date
19010099	Pigeon Creek at US 1 St. Marys-Nassau River	Northeast	PIGEON CREEK	78.8	0	1.3	105	3/7/1994
19010099	Pigeon Creek at US 1 St. Marys-Nassau River	Northeast	PIGEON CREEK	76.8	0	1.3	108	7/25/1994
19010099	Pigeon Creek at US 1 St. Marys-Nassau River	Northeast	PIGEON CREEK	78.9	0	1.3	109	3/6/1995
19010099	Pigeon Creek at US 1 St. Marys-Nassau River	Northeast	PIGEON CREEK	58.0	0	1.3	111	2/1/2000
19010099	Pigeon Creek at US 1 St. Marys-Nassau River	Northeast	PIGEON CREEK	83.5	0	1.3	156	3/3/1993
19010099	Pigeon Creek at US 1 St. Marys-Nassau River	Northeast	PIGEON CREEK	54.8	0	1.3	157	7/9/1996
21010056	PCS Phosphate FYI5, control site	Northeast	ROBINSON CREEK	79.2	0	1.4	113	3/13/2000
21010032	Rocky Ck @ Woodpecker Rd	Northeast	ROCKY CREEK	56.7	0	1.2	152	8/24/1994
20030265	Sal Taylor Creek below Rowell Creek	Northeast	SAL TAYLOR CREEK	68.6	0	1.8	138	3/5/1996
20030265	Sal Taylor Creek below Rowell Creek	Northeast	SAL TAYLOR CREEK	35.1	0	1.8	146	10/2/1995
21030115	Santa Fe Rv- Control A BMP	Northeast	SANTA FE RIVER	75.7	0	1.5	148	11/6/1998
20030456	Thomas Ck, Forestry BMP Study Ref Site B	Northeast	THOMAS CREEK	75.7	0	1.2	118	7/2/1997
20030456	Thomas Ck, Forestry BMP Study Ref Site B	Northeast	THOMAS CREEK	82.6	0	1.2	128	2/12/1998
20030456	Thomas Ck, Forestry BMP Study Ref Site B	Northeast	THOMAS CREEK	92.4	0	1.2	129	3/14/1996
20030456	Thomas Ck, Forestry BMP Study Ref Site B	Northeast	THOMAS CREEK	86.7	0	1.2	159	2/21/1997
19010072	McClenny WWTP upstream	Northeast	TURKEY CREEK	78.2	0	1.6	160	3/22/1999
20030341	Yellow Water Creek above Sal Taylor Creek	Northeast	YELLOW WATER CREEK	70.4	0	1.9	107	3/5/1996
20030341	Yellow Water Creek above Sal Taylor Creek	Northeast	YELLOW WATER CREEK	66.4	0	1.9	147	10/2/1995
20030342	Yellow Water Creek below Sal Taylor Creek	Northeast	YELLOW WATER CREEK	43.5	0	1.8	103	10/2/1995
20030342	Yellow Water Creek below Sal Taylor Creek	Northeast	YELLOW WATER CREEK	74.8	0	1.8	166	3/5/1996
22020070	BIG CYPRESS BRANCH-TIMBER ENERGY FYI CNTRL SITE	Panhandle	BIG CYPRESS BRANCH	53.6	0	1.3	125	8/9/1999
33040014	BIG HORSE CREEK S.R.2 OKA.CO.	Panhandle	BIG HORSE CREEK	67.4	0	1.2	114	7/15/1996
33040014	BIG HORSE CREEK S.R.2 OKA.CO.	Panhandle	BIG HORSE CREEK	41.7	0	1.2	119	2/17/1993
33040014	BIG HORSE CREEK S.R.2 OKA.CO.	Panhandle	BIG HORSE CREEK	78.4	0	1.2	126	1/30/1995
33040014	BIG HORSE CREEK S.R.2 OKA.CO.	Panhandle	BIG HORSE CREEK	75.3	0	1.2	148	2/8/2001
33040014	BIG HORSE CREEK S.R.2 OKA.CO.	Panhandle	BIG HORSE CREEK	67.0	0	1.2	154	8/23/1994
33040014	BIG HORSE CREEK S.R.2 OKA.CO.	Panhandle	BIG HORSE CREEK	96.3	0	1.2	173	7/15/1998
21030064	Cedar Head Run, above Ichetucknee River	Panhandle	CEDAR HEAD RUN	42.2	0	1.0	100	8/1/1994
21030064	Cedar Head Run, above Ichetucknee River	Panhandle	CEDAR HEAD RUN	40.6	0	1.0	105	2/1/1995

STORET	Site Description	Bioregion	Waterbody Name	SCI	HDG	LDI	Total # individuals counted	Sample Date
21030064	Cedar Head Run, above Ichetucknee River	Panhandle	CEDAR HEAD RUN	46.2	0	1.0	108	2/6/1996
21030064	Cedar Head Run, above Ichetucknee River	Panhandle	CEDAR HEAD RUN	69.4	0	1.0	111	3/15/2000
31010050	CROOKED CREEK HWY.270 GADSDEN CO.	Panhandle	CROOKED CREEK	71.1	0	1.1	108	2/13/1995
31010050	CROOKED CREEK HWY.270 GADSDEN CO.	Panhandle	CROOKED CREEK	58.5	0	1.1	132	3/25/1993
31010050	CROOKED CREEK HWY.270 GADSDEN CO.	Panhandle	CROOKED CREEK	84.8	0	1.1	151	7/9/1996
32030023	ECONFINA CREEK AT SCOTT RD	Panhandle	ECONFINA CREEK	68.2	0	1.2	102	2/7/1995
32030023	ECONFINA CREEK AT SCOTT RD	Panhandle	ECONFINA CREEK	70.4	0	1.2	106	8/25/1992
32030023	ECONFINA CREEK AT SCOTT RD	Panhandle	ECONFINA CREEK	62.3	0	1.2	124	2/6/2001
32030023	ECONFINA CREEK AT SCOTT RD	Panhandle	ECONFINA CREEK	81.3	0	1.2	151	6/28/1993
32030023	ECONFINA CREEK AT SCOTT RD	Panhandle	ECONFINA CREEK	87.9	0	1.2	162	2/5/1997
22050077	Econfina River Forestry BMP Study Ref Site B	Panhandle	ECONFINA RIVER	59.8	0	1.1	135	5/13/1998
33040064	Jacks Branch, Forestry BMP Study Ref Site B	Panhandle	JACK'S BRANCH	63.4	0	1.7	111	2/22/1996
33040064	Jacks Branch, Forestry BMP Study Ref Site B	Panhandle	JACK'S BRANCH	63.6	0	1.7	140	2/25/1997
33040064	Jacks Branch, Forestry BMP Study Ref Site B	Panhandle	JACK'S BRANCH	66.9	0	1.7	173	7/1/1998
32010024	Limestone Ck @ Beckridge Rd	Panhandle	LIMESTONE CREEK	74.5	0	1.3	171	7/14/1998
32020063	LITTLE CROOKED CREEK S.R.79 BAY CO.	Panhandle	LTL CROOKED CREEK	78.6	0	1.2	100	1/21/1999
32020063	LITTLE CROOKED CREEK S.R.79 BAY CO.	Panhandle	LTL CROOKED CREEK	67.4	0	1.2	113	2/9/1995
32020063	LITTLE CROOKED CREEK S.R.79 BAY CO.	Panhandle	LTL CROOKED CREEK	62.3	0	1.2	116	8/30/1994
32020063	LITTLE CROOKED CREEK S.R.79 BAY CO.	Panhandle	LTL CROOKED CREEK	71.5	0	1.2	126	6/29/1993
32020063	LITTLE CROOKED CREEK S.R.79 BAY CO.	Panhandle	LTL CROOKED CREEK	72.6	0	1.2	129	2/23/1994
32020063	LITTLE CROOKED CREEK S.R.79 BAY CO.	Panhandle	LTL CROOKED CREEK	80.1	0	1.2	153	7/16/1996
32020063	LITTLE CROOKED CREEK S.R.79 BAY CO.	Panhandle	LTL CROOKED CREEK	52.7	0	1.2	156	3/1/1993
22030062	MCBRIDE SLOUGH HWY.267 WAKULLA CO.	Panhandle	MCBRIDE SLOUGH	41.6	0	1.0	112	7/8/1992
22030062	MCBRIDE SLOUGH HWY.267 WAKULLA CO.	Panhandle	MCBRIDE SLOUGH	60.2	0	1.0	138	3/21/1994
22030062	MCBRIDE SLOUGH HWY.267 WAKULLA CO.	Panhandle	MCBRIDE SLOUGH	57.3	0	1.0	147	2/11/1997
33010054	MCDAVID CREEK AT SR 99	Panhandle	MCDAVID CREEK	73.2	0	1.3	101	1/24/1995
33010054	MCDAVID CREEK AT SR 99	Panhandle	MCDAVID CREEK	75.6	0	1.3	115	2/22/2001
33010054	MCDAVID CREEK AT SR 99	Panhandle	MCDAVID CREEK	68.7	0	1.3	118	2/18/1993
33010054	MCDAVID CREEK AT SR 99	Panhandle	MCDAVID CREEK	48.5	0	1.3	126	8/10/1994

STORET	Site Description	Bioregion	Waterbody Name	SCI	HDG	LDI	Total # individuals counted	Sample Date
33010054	MCDAVID CREEK AT SR 99	Panhandle	MCDAVID CREEK	70.2	0	1.3	127	8/12/1997
33010054	MCDAVID CREEK AT SR 99	Panhandle	MCDAVID CREEK	76.9	0	1.3	141	8/17/1992
33010054	MCDAVID CREEK AT SR 99	Panhandle	MCDAVID CREEK	88.7	0	1.3	173	2/14/1994
22020049	MULE CREEK S.R.12 LIBERTY CO.	Panhandle	MULE CREEK	49.9	0	1.2	104	3/25/1993
22020049	MULE CREEK S.R.12 LIBERTY CO.	Panhandle	MULE CREEK	65.7	0	1.2	106	2/13/1995
22020049	MULE CREEK S.R.12 LIBERTY CO.	Panhandle	MULE CREEK	57.7	0	1.2	112	2/23/1999
22020049	MULE CREEK S.R.12 LIBERTY CO.	Panhandle	MULE CREEK	58.6	0	1.2	114	7/8/1992
22020049	MULE CREEK S.R.12 LIBERTY CO.	Panhandle	MULE CREEK	67.8	0	1.2	125	7/9/1996
22020062	Oklawaha Ck, SR 267, S of Quincy	Panhandle	OKLAWAHA CREEK	52.0	0	1.1	100	9/9/1998
22020062	Oklawaha Ck, SR 267, S of Quincy	Panhandle	OKLAWAHA CREEK	41.8	0	1.1	101	8/1/1994
22020062	Oklawaha Ck, SR 267, S of Quincy	Panhandle	OKLAWAHA CREEK	45.2	0	1.1	105	8/12/1997
22020062	Oklawaha Ck, SR 267, S of Quincy	Panhandle	OKLAWAHA CREEK	48.3	0	1.1	108	2/13/1995
22020062	Oklawaha Ck, SR 267, S of Quincy	Panhandle	OKLAWAHA CREEK	82.9	0	1.1	147	2/13/1996
31020073	Pitts Mill Creek above conf with Cypress Ck	Panhandle	PITTS MILL CREEK	68.2	0	1.5	115	7/23/1996
22020010	QUINCY CR above SR12, ref for Quincy STP FYI	Panhandle	QUINCY CREEK	45.5	0	1.5	105	11/6/1995
22030010	St. Marks R approx 4 mi N of Hwy 98 (@ "rapids")	Panhandle	ST. MARKS RIVER	40.5	0	1.3	100	9/18/2001
22030010	St. Marks R approx 4 mi N of Hwy 98 (@ "rapids")	Panhandle	ST. MARKS RIVER	56.0	0	1.3	105	3/18/1997
22030010	St. Marks R approx 4 mi N of Hwy 98 (@ "rapids")	Panhandle	ST. MARKS RIVER	46.8	0	1.3	108	8/22/2000
31010051	SWEETWATER CREEK HWY.270 LIBERTY CO.	Panhandle	SWEETWATER CREEK	69.9	0	1.3	104	2/13/1996
31010051	SWEETWATER CREEK HWY.270 LIBERTY CO.	Panhandle	SWEETWATER CREEK	75.2	0	1.3	110	3/21/1994
31010051	SWEETWATER CREEK HWY.270 LIBERTY CO.	Panhandle	SWEETWATER CREEK	97.8	0	1.3	110	7/8/1992
31010051	SWEETWATER CREEK HWY.270 LIBERTY CO.	Panhandle	SWEETWATER CREEK	59.7	0	1.3	129	8/1/1994
31010051	SWEETWATER CREEK HWY.270 LIBERTY CO.	Panhandle	SWEETWATER CREEK	66.7	0	1.3	132	3/25/1993
31010051	SWEETWATER CREEK HWY.270 LIBERTY CO.	Panhandle	SWEETWATER CREEK	70.0	0	1.3	151	2/13/1995
31010051	SWEETWATER CREEK HWY.270 LIBERTY CO.	Panhandle	SWEETWATER CREEK	84.1	0	1.3	162	7/20/1993
31020040	TEN MILE CREEK S.R.73 CALHOUN CO.	Panhandle	TEN MILE CREEK	50.5	0	1.2	107	8/26/1994
31020040	TEN MILE CREEK S.R.73 CALHOUN CO.	Panhandle	TEN MILE CREEK	72.9	0	1.2	113	8/20/1992
31020040	TEN MILE CREEK S.R.73 CALHOUN CO.	Panhandle	TEN MILE CREEK	82.0	0	1.2	124	2/7/1995
31020040	TEN MILE CREEK S.R.73 CALHOUN CO.	Panhandle	TEN MILE CREEK	55.3	0	1.2	125	2/23/1993

STORET	Site Description	Bioregion	Waterbody Name	SCI	HDG	LDI	Total # individuals counted	Sample Date
31020040	TEN MILE CREEK S.R.73 CALHOUN CO.	Panhandle	TEN MILE CREEK	76.1	0	1.2	126	2/21/1994
31020040	TEN MILE CREEK S.R.73 CALHOUN CO.	Panhandle	TEN MILE CREEK	62.0	0	1.2	129	6/24/1993
31020040	TEN MILE CREEK S.R.73 CALHOUN CO.	Panhandle	TEN MILE CREEK	67.7	0	1.2	130	2/4/1997
31020040	TEN MILE CREEK S.R.73 CALHOUN CO.	Panhandle	TEN MILE CREEK	49.4	0	1.2	141	2/10/1998
33010065	Rest Area Run Creek below I 10	Panhandle	UNNAMED CREEK	82.2	0	1.2	113	7/12/1995
33010065	Rest Area Run Creek below I 10	Panhandle	UNNAMED CREEK	54.7	0	1.2	147	2/14/1996
22040038	Unnamed Creek above Monticello WWTP	Panhandle	UNNAMED CREEK	54.7	0	1.7	153	12/12/1995
32020066	Wright Ck, ref for Noma STP FYI	Panhandle	WRIGHTS CREEK	73.9	0	1.7	123	4/29/1993
20010455	BLACKWATER CREEK AT HWY 44A	Peninsula	BLACKWATER CREEK	59.0	0	1.0	109	3/29/1994
20010455	BLACKWATER CREEK AT HWY 44A	Peninsula	BLACKWATER CREEK	56.5	0	1.0	111	7/19/1994
20010455	BLACKWATER CREEK AT HWY 44A	Peninsula	BLACKWATER CREEK	80.9	0	1.0	126	8/26/1996
20010455	BLACKWATER CREEK AT HWY 44A	Peninsula	BLACKWATER CREEK	54.0	0	1.0	127	9/28/1993
20010455	BLACKWATER CREEK AT HWY 44A	Peninsula	BLACKWATER CREEK	51.9	0	1.0	128	8/11/1992
20010455	BLACKWATER CREEK AT HWY 44A	Peninsula	BLACKWATER CREEK	59.7	0	1.0	133	2/8/1993
20010455	BLACKWATER CREEK AT HWY 44A	Peninsula	BLACKWATER CREEK	33.5	0	1.0	143	2/7/1995
28020239	Cypress brch above SR 78	Peninsula	CYPRESS BRANCH	57.6	0	1.3	135	8/20/1997
28020239	Cypress brch above SR 78	Peninsula	CYPRESS BRANCH	70.3	0	1.3	142	8/20/1997
28020237	Cypress Creek 2 mi above SR78	Peninsula	CYPRESS CREEK	55.7	0	1.2	132	7/21/1999
28020237	Cypress Creek 2 mi above SR78	Peninsula	CYPRESS CREEK	70.4	0	1.2	135	8/5/1997
26010972	DAVENPORT CR AT SR 545 BRIDGE	Peninsula	DAVENPORT CREEK	63.0	0	2.0	104	8/16/1994
26010972	DAVENPORT CR AT SR 545 BRIDGE	Peninsula	DAVENPORT CREEK	66.3	0	2.0	110	9/30/1997
26010972	DAVENPORT CR AT SR 545 BRIDGE	Peninsula	DAVENPORT CREEK	73.6	0	2.0	113	2/14/1995
26010972	DAVENPORT CR AT SR 545 BRIDGE	Peninsula	DAVENPORT CREEK	79.4	0	2.0	167	1/10/2000
26010593	FISHEATING CREEK ABOVE US 27	Peninsula	FISHEATING CREEK	60.8	0	1.1	113	3/9/1993
20030414	GOLD HEAD BR. IN STATE PARK	Peninsula	GOLD HEAD BRANCH	90.0	0	1.0	100	8/21/1996
20030414	GOLD HEAD BR. IN STATE PARK	Peninsula	GOLD HEAD BRANCH	72.2	0	1.0	102	1/24/1995
20030414	GOLD HEAD BR. IN STATE PARK	Peninsula	GOLD HEAD BRANCH	80.8	0	1.0	109	7/20/1994
20030414	GOLD HEAD BR. IN STATE PARK	Peninsula	GOLD HEAD BRANCH	100.0	0	1.0	111	1/12/2000
20030414	GOLD HEAD BR. IN STATE PARK	Peninsula	GOLD HEAD BRANCH	89.2	0	1.0	121	3/10/1998

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20030414	GOLD HEAD BR. IN STATE PARK	Peninsula	GOLD HEAD BRANCH	77.4	0	1.0	123	2/15/1994
24030142	Hillsborough R upstrm of Southside Br, Zephyrhil	Peninsula	HILLSBOROUGH RIVER	87.1	0	1.6	145	8/21/1996
24030048	Hillsborough River at SR 39	Peninsula	HILLSBOROUGH RIVER	60.8	0	1.5	109	8/21/1996
25020111	Horse Creek @ SR 72 Bridge	Peninsula	HORSE CREEK	70.4	0	1.8	110	2/17/1999
25020111	Horse Creek @ SR 72 Bridge	Peninsula	HORSE CREEK	66.6	0	1.8	147	10/24/1994
20010454	JUNIPER CREEK AT HIGHWAY 19	Peninsula	JUNIPER CREEK	55.8	0	1.1	110	1/31/1995
20010454	JUNIPER CREEK AT HIGHWAY 19	Peninsula	JUNIPER CREEK	29.6	0	1.1	115	8/3/1993
20010454	JUNIPER CREEK AT HIGHWAY 19	Peninsula	JUNIPER CREEK	75.5	0	1.1	146	3/4/1996
20020004	Little Orange Creek @ Hwy 21	Peninsula	LITTLE ORANGE CREEK	73.2	0	1.2	133	2/9/1998
20020004	Little Orange Creek @ Hwy 21	Peninsula	LITTLE ORANGE CREEK	82.5	0	1.2	134	2/12/1996
20020004	Little Orange Creek @ Hwy 21	Peninsula	LITTLE ORANGE CREEK	86.8	0	1.2	138	8/30/1994
20020004	Little Orange Creek @ Hwy 21	Peninsula	LITTLE ORANGE CREEK	82.2	0	1.2	139	2/15/1994
20020004	Little Orange Creek @ Hwy 21	Peninsula	LITTLE ORANGE CREEK	90.3	0	1.2	150	9/26/1995
20020004	Little Orange Creek @ Hwy 21	Peninsula	LITTLE ORANGE CREEK	75.3	0	1.2	161	8/15/2000
26011019	LIVINGSTON CREEK AT RUCKS DAIRY RD.	Peninsula	LIVINGSTON CREEK	71.2	0	1.5	118	9/5/2001
26011019	LIVINGSTON CREEK AT RUCKS DAIRY RD.	Peninsula	LIVINGSTON CREEK	61.5	0	1.5	156	2/18/1997
28010223	N FK LOXAHATCHEE RIVER REF BIO STATION	Peninsula	LOXAHATCHEE RIVER	34.3	0	1.0	106	9/21/1998
28010223	N FK LOXAHATCHEE RIVER REF BIO STATION	Peninsula	LOXAHATCHEE RIVER	49.2	0	1.0	115	9/22/1995
28010223	N FK LOXAHATCHEE RIVER REF BIO STATION	Peninsula	LOXAHATCHEE RIVER	59.9	0	1.0	124	10/7/1999
28010223	N FK LOXAHATCHEE RIVER REF BIO STATION	Peninsula	LOXAHATCHEE RIVER	33.9	0	1.0	134	8/20/1992
28010223	N FK LOXAHATCHEE RIVER REF BIO STATION	Peninsula	LOXAHATCHEE RIVER	54.8	0	1.0	146	8/25/1994
28010223	N FK LOXAHATCHEE RIVER REF BIO STATION	Peninsula	LOXAHATCHEE RIVER	33.8	0	1.0	158	3/22/1999
28010223	N FK LOXAHATCHEE RIVER REF BIO STATION	Peninsula	LOXAHATCHEE RIVER	44.7	0	1.0	161	2/20/1996
24010002	Manatee R 20 m below SR64 bridge (TP1)	Peninsula	MANATEE RIVER	60.3	0	1.3	109	3/17/1993
24010002	Manatee R 20 m below SR64 bridge (TP1)	Peninsula	MANATEE RIVER	76.3	0	1.3	126	2/2/1994
24010002	Manatee R 20 m below SR64 bridge (TP1)	Peninsula	MANATEE RIVER	77.2	0	1.3	129	7/17/1996
24010002	Manatee R 20 m below SR64 bridge (TP1)	Peninsula	MANATEE RIVER	96.7	0	1.3	145	2/2/1994
24010002	Manatee R 20 m below SR64 bridge (TP1)	Peninsula	MANATEE RIVER	80.2	0	1.3	172	11/21/2000
20020012	OKLAWAHA RIVER AT SR 316	Peninsula	OKLAWAHA RIVER	77.9	0	1.0	101	8/29/1995

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20020012	OKLAWAHA RIVER AT SR 316	Peninsula	OKLAWAHA RIVER	65.2	0	1.0	117	3/20/1995
20020012	OKLAWAHA RIVER AT SR 316	Peninsula	OKLAWAHA RIVER	67.2	0	1.0	117	8/10/1993
20020012	OKLAWAHA RIVER AT SR 316	Peninsula	OKLAWAHA RIVER	68.1	0	1.0	137	2/15/2000
20020012	OKLAWAHA RIVER AT SR 316	Peninsula	OKLAWAHA RIVER	80.6	0	1.0	151	8/11/1998
20020012	OKLAWAHA RIVER AT SR 316	Peninsula	OKLAWAHA RIVER	49.6	0	1.0	153	2/8/1993
20020404	ORANGE CREEK 50 YDS. UP FROM HWY. 21	Peninsula	ORANGE CREEK	93.1	0	1.4	105	8/17/1993
20020404	ORANGE CREEK 50 YDS. UP FROM HWY. 21	Peninsula	ORANGE CREEK	94.5	0	1.4	110	9/22/1992
20020404	ORANGE CREEK 50 YDS. UP FROM HWY. 21	Peninsula	ORANGE CREEK	92.1	0	1.4	111	8/30/1994
20020404	ORANGE CREEK 50 YDS. UP FROM HWY. 21	Peninsula	ORANGE CREEK	83.1	0	1.4	118	3/16/1993
20020404	ORANGE CREEK 50 YDS. UP FROM HWY. 21	Peninsula	ORANGE CREEK	91.8	0	1.4	135	8/15/2000
20020404	ORANGE CREEK 50 YDS. UP FROM HWY. 21	Peninsula	ORANGE CREEK	94.3	0	1.4	139	2/15/1994
20020404	ORANGE CREEK 50 YDS. UP FROM HWY. 21	Peninsula	ORANGE CREEK	72.0	0	1.4	144	8/15/2000
20020404	ORANGE CREEK 50 YDS. UP FROM HWY. 21	Peninsula	ORANGE CREEK	96.3	0	1.4	168	2/28/1995
20030253	RICECR #3@BR SR #3/250yds above Bardine	Peninsula	RICE CREEK	67.1	0	1.1	125	5/11/1999
20030253	RICECR #3@BR SR #3/250yds above Bardine	Peninsula	RICE CREEK	24.0	0	1.1	136	3/16/1998
20020317	SILVER RUN AT CONFLUENCE WITH BOAT RAMP CANAL	Peninsula	SILVER RUN	34.8	0	1.3	133	9/1/1992
20020317	SILVER RUN AT CONFLUENCE WITH BOAT RAMP CANAL	Peninsula	SILVER RUN	42.3	0	1.3	136	8/10/1993
20020317	SILVER RUN AT CONFLUENCE WITH BOAT RAMP CANAL	Peninsula	SILVER RUN	53.5	0	1.3	142	9/4/2001
20020317	SILVER RUN AT CONFLUENCE WITH BOAT RAMP CANAL	Peninsula	SILVER RUN	57.6	0	1.3	142	2/8/1994
20020317	SILVER RUN AT CONFLUENCE WITH BOAT RAMP CANAL	Peninsula	SILVER RUN	54.2	0	1.3	150	3/20/1995
20020317	SILVER RUN AT CONFLUENCE WITH BOAT RAMP CANAL	Peninsula	SILVER RUN	76.0	0	1.3	166	9/16/1996
28020221	TELEGRAPH CREEK AT BABCOCK RANCH	Peninsula	TELEGRAPH CREEK	84.4	0	1.0	107	9/16/1996
28020221	TELEGRAPH CREEK AT BABCOCK RANCH	Peninsula	TELEGRAPH CREEK	80.8	0	1.0	146	7/27/1994
26011020	TIGER CREEK AT WALKING WATERS RD.	Peninsula	TIGER CREEK	74.4	0	1.0	109	7/14/1992
26011020	TIGER CREEK AT WALKING WATERS RD.	Peninsula	TIGER CREEK	76.5	0	1.0	111	2/18/1997
26011020	TIGER CREEK AT WALKING WATERS RD.	Peninsula	TIGER CREEK	75.7	0	1.0	142	2/21/1995
26011020	TIGER CREEK AT WALKING WATERS RD.	Peninsula	TIGER CREEK	73.4	0	1.0	161	7/20/1999
23010464	Withlacoochee R @ county park off Auton Rd (TP3)	Peninsula	WITHLACOOCHEE RIVER	43.4	0	1.2	100	9/28/1994
23010464	Withlacoochee R @ county park off Auton Rd (TP3)	Peninsula	WITHLACOOCHEE RIVER	46.9	0	1.2	100	9/2/1993

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23010464	Withlacoochee R @ county park off Auton Rd (TP3)	Peninsula	WITHLACOOCHEE RIVER	67.8	0	1.2	120	3/9/1999
23010464	Withlacoochee R @ county park off Auton Rd (TP3)	Peninsula	WITHLACOOCHEE RIVER	50.6	0	1.2	122	7/26/1999
23010464	Withlacoochee R @ county park off Auton Rd (TP3)	Peninsula	WITHLACOOCHEE RIVER	49.6	0	1.2	125	3/11/1993
23010464	Withlacoochee R @ county park off Auton Rd (TP3)	Peninsula	WITHLACOOCHEE RIVER	66.1	0	1.2	136	2/13/1996
23010446	WITHLACOOCHEE RIVER	Peninsula	WITHLACOOCHEE RIVER	50.0	0	1.6	147	7/28/1999