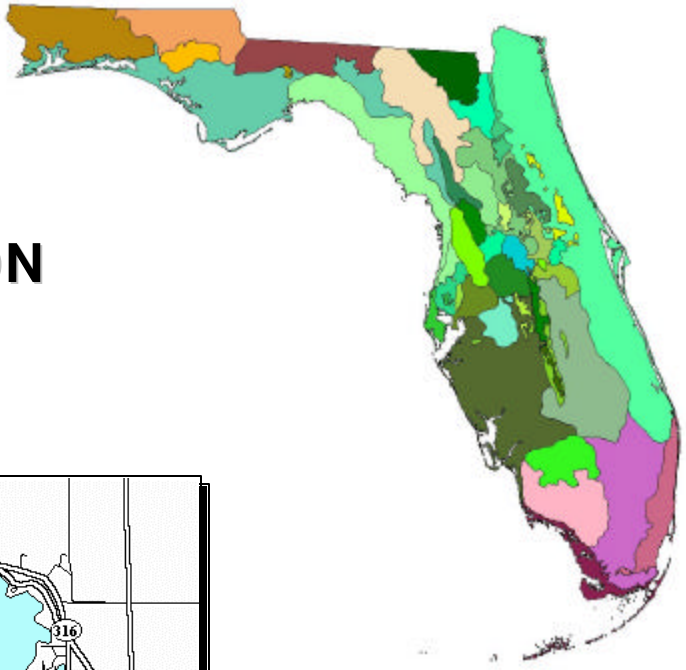
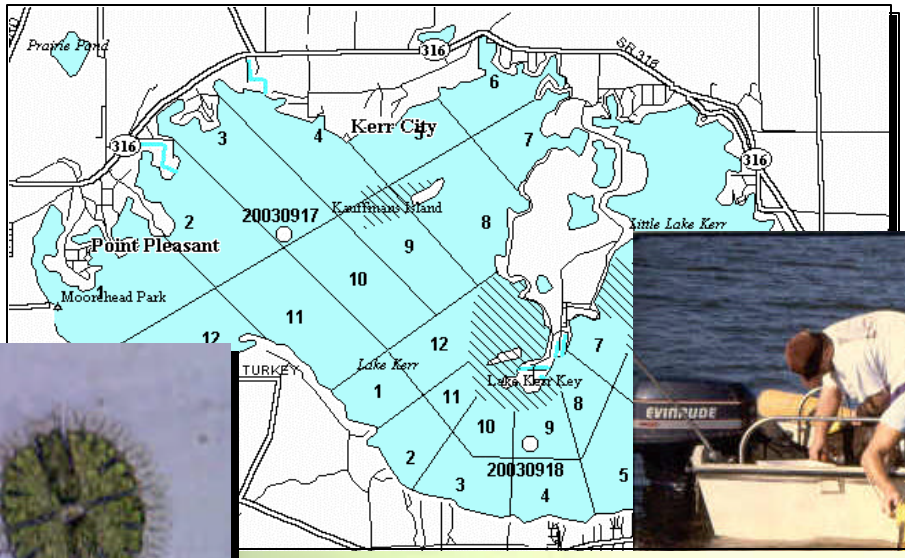
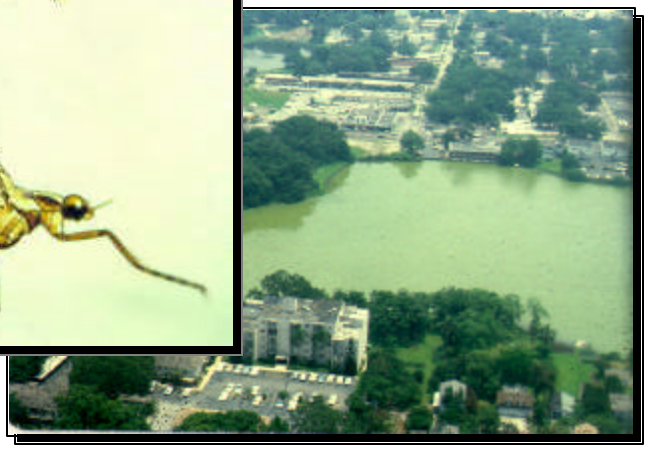
DEVELOPMENT OF LAKE CONDITION INDEXES (LCI) FOR FLORIDA







July 2000

Nonpoint Source
Bioassessment Program

DEPARTMENT OF ENVIRONMENTAL PROTECTION
FLORIDA

DEVELOPMENT OF LAKE CONDITION INDEXES (LCI) FOR FLORIDA

Prepared for:

Florida Department of Environmental Protection
Under Contracts WM 565 and WM 655

Prepared by:

Jeroen Gerritsen, Ben Jessup, Erik W. Leppo, and
Jeffrey White

Tetra Tech, Inc.
10045 Red Run Boulevard
Suite 110
Owings Mills, MD 21117

This project was funded by Section 104(b)(3) and Section 319(h) grants from the U.S. Environmental Protection Agency through a contract with the stormwater/Nonpoint Source Management Section of the Florida Department of Environmental Protection.

July 2000

ACKNOWLEDGMENTS

This project would not have been possible without the crucial support of many persons in the Florida Department of Environmental Protection (DEP). Mike Scheinkman of DEP was work assignment manager of the project and provided critical guidance and assistance. Jim Hulbert and Ellen McCarron of DEP had the vision to support the first statewide implementation of EPA's lake bioassessment protocols, and Jim Hulbert also designed much of the program. Russel Frydenborg developed the sampling protocols, trained many of DEP's lake biologists, and provided critical assistance for the program. Glenn Griffith and Jim Omernik of USEPA developed the lake regionalization that we relied upon for this project. Bill Beers and Kim Schildt of DEP developed the land use analysis of lake buffer zones. Lou Ley of DEP coordinated data input to the statewide database. Eric Pluchino of DEP volunteered help with lake sampling and taxonomic identification. Finally, the project depended on the DEP lake biologists who sampled hundreds of lakes throughout Florida, developed the data, and prepared reports that formed the basis for these analyses: Bob Rutter, Randy Payne, Mike Hollingsworth, Stefan Schulze, and Kate Orellana.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF FIGURES (CONTINUED)

1.0 EXECUTIVE SUMMARY

The Florida Department of Environmental Protection (DEP) began developing a lake bioassessment protocol to be able to monitor and assess the biological condition of Florida lakes. The Florida DEP monitors state waters to protect and manage ecosystem health. The lake bioassessment protocol is a tool for the ambient monitoring program, in support of Florida's water quality standards. In this document, we describe data analysis of the lake assessment monitoring, and show development of indexes for lake biological assessment.

The framework for bioassessment consists of characterizing reference conditions upon which comparisons can be made, and identifying appropriate biological attributes with which to measure the condition. Reference conditions are selected to be the "best available" conditions for a particular region or area, and are intended to be representative of sustainable ecosystem health. They do not necessarily represent pristine conditions uninfluenced by human activities.

An earlier geographic regionalization based on topography, natural water chemistry, lake origin, lake hydrology, and soils identified 47 lake regions in Florida (Griffith et al. 1996). This report summarizes lake data collected from 1993 to 1997 from 122 reference and 84 non-reference lakes within 36 of the lake regions. Macroinvertebrate species composition was related to several environmental variables: Secchi depth, Kjeldahl nitrogen, total nitrogen, total phosphorus, chlorophyll *a*, water color, and pH.

Classification of Florida lakes, using both chemical water quality and biological species composition, revealed that Florida lakes can be best classified according to water pH, water color, and ecoregion of the lake basin. A convenient classification is to divide the lakes into 4 water chemistry groups: acid-clear, acid-colored, alkaline-clear, and alkaline-colored. Benthic macroinvertebrate species composition is most strongly affected by lake water color, and somewhat less by water pH and the geographic ecoregion of the lake.

Thirty potential benthic invertebrate metrics were tested that were relevant to attributes of lake ecology. Six metrics were included in 3 alternative multimetric indexes: total number of taxa, number of EOT taxa (ephemeropterans, odonates, trichopterans), Shannon-Wiener index, % EOT, % Diptera, and the invertebrate Hulbert index (HI). In addition to the benthic macroinvertebrate index, 2 alternative trophic state biotic indexes were developed using chlorophyll *a*, Secchi depth and other water quality measures.

The principal stressors present in the data set were nutrient enrichment and eutrophication. Among the 5 alternative indexes, two emerged as most effective for discriminating unimpaired reference lakes from stressed lakes. An additive multimetric index for macroinvertebrates was most effective at discriminating reference from stressed lakes for clear lakes (water color # 20 PCU), and an index on trophic condition (chlorophyll *a* and Secchi depth) was most effective for colored lakes (water color >

20 PCU). These two indexes could successfully discriminate 71% and 77% of non-reference lakes, respectively.

The report further recommends:

- Adoption of two Lake Condition Indexes (LCIs) for bioassessment of Florida lakes; a benthic macroinvertebrate LCI for clear lakes (# 20 PCU), and a trophic LCI for colored lakes ($ 20 PCU). The indexes can be the basis for lake biocriteria.

- Testing the indexes with an independent data set, with emphasis on stressed, acid-clear lakes.

- Further examination of the 20 PCU color threshold for use of the two indexes.

- Use of the indexes to assist in the development of nutrient criteria for Florida lakes.

2.0 INTRODUCTION

2.1 Background

Assessment of ecosystem health is becoming ever more important in managing water resources nationwide. In support of ecological assessment, the Florida Department of Environmental Protection (DEP) developed an index for assessing stream health, the Florida Stream Condition Index (SCI; Barbour et al. 1996), based on current practices recommended by the U.S. EPA and several states (e.g., Gibson et al. 1996; Barbour et al. 1999).

In 1998, the U.S. EPA published a draft guidance document extending the bioassessment concept to lakes (Gerritsen et al. 1998). In 1993-97, Florida DEP implemented the first full-scale field application of lake bioassessment as proposed by EPA. This document presents the data analysis from that implementation, and describes lake groups for Florida based on benthic macroinvertebrates, potential metrics to use for operational bioassessment of Florida lakes, and two proposed biological indexes for Florida lakes.

Biological assessment is a powerful tool for determining the condition of waterbodies. Resident biota in a watershed function as continual natural monitors of environmental quality, responding to the effects of both episodic and cumulative pollution and habitat alteration. The assessment of ecosystem health cannot proceed without measuring the attainment of biological integrity (Gibson et al. 1996).

The multimetric approach to bioassessment defines an array of measurements, each of which represents a measurable characteristic of the biological assemblage that changes in a predictable way with increased or decreased environmental stress (Karr et al. 1986, Gibson et al. 1996). When integrated, a multimetric index functions as an overall indicator of biological condition. Multimetric assessment typically includes several measurements of at least three out of four ecological properties:

- Health of individuals or populations
- Species structure and composition
- Trophic structure
- System function

Biological assessment of waterbodies depends on our ability to define, measure, and compare biological integrity among similar systems. Impairment of a waterbody is judged by its departure from an expected, or reference condition. Reference conditions are in turn established by identifying least impacted reference sites, characterizing the biological condition of the reference sites, and setting thresholds for scoring measurements.

Biological integrity makes the explicit assumption that natural, undisturbed systems are healthier than those degraded by human activities. Because biological integrity is defined relative to unimpaired conditions, it must also be measured relative to those conditions. The four classes of ecological properties listed above are measurable relative to natural or unimpaired conditions.

Because there is tremendous variation in the physical, chemical and biological characteristics of lakes nationwide, the first step in defining reference conditions is to classify lakes so that comparisons can be made within, and not across, lake classes. Classification should reflect the inherent, natural properties of lakes independent of human influence and therefore must be made on the basis of measurements that do not change as the result of human activities. Finally, the classification scheme should also reflect differences in biota among lake classes (Gerritsen et al. 1998).

Following classification, metrics are selected from the set of potential biological measurements. Metrics that are responsive to stressors are selected by comparing their values between the set of reference sites and a second set of stressed lakes (e.g., by urban runoff, agricultural drainage, contamination). Responsive metrics will show a clear difference between reference and stressed (test) lakes. Scoring criteria for each metric are determined from the distribution of metric values in the reference lakes, and the index is the sum of the scores of the selected metrics.

2.2 Objectives

The DEP sampled benthic macroinvertebrates and phytoplankton in lakes throughout Florida from 1993 to 1997. Parallel to the sampling program, an ecoregionalization project defined and delineated Florida lake regions (Griffith et al. 1997) based on soils, geology, hydrology, and climate. Reference lakes were identified in the lake regions, and reference lakes were sampled from 1994 through 1997.

Objectives of the analysis phase were:

- Determine an optimal classification of Florida lakes based on benthic macroinvertebrates, water column biotic measures, and the Florida lake regions;
- Characterize reference conditions for each biological lake class
- Select candidate metrics for a lake invertebrate index
- Optimize lake sampling design for cost-effective monitoring
- Identify a gradient of human stress for lakes based on land use in the lake watershed, and determine the response of lake biological indicators to the stress gradient.

3.0 METHODS

3.1 Florida DEP Field Methods

Reference and test lakes have been sampled by Florida DEP since summer 1993 (Frydenborg 1994, Payne 1995, Rutter 1995, 1996, Schulze 1996). Following the 1993 sampling, lake regions were delineated for the state (Griffith et al. 1997), but the lake regions did not coincide with the subecoregions that had been developed earlier for streams. For the 1994 and 1995 sampling efforts, reference lakes were selected by DEP personnel to represent the least impaired lakes within a lake region. Lake regions were further refined and revised in 1996 (Griffith et al. 1997). All lakes in the data set were reassigned to the correct respective lake region as of August 1996 for subsequent analyses.

Two index periods were defined for sampling benthic macroinvertebrates, summer (July-October), and winter (December - March). Several lakes were sampled in both summer and winter index periods in 1994 and 1995, and a few lakes were sampled in more than a single year.

Two sampling protocols were used for benthic macroinvertebrates. All macroinvertebrate samples were taken with either Petite PONAR or Ekman grab samplers in 2-4m water depth, if possible below the littoral macrophyte zone. In 1993 and 1994, one to three sites were selected in each lake, and six sediment grabs were taken at each site. Three of the grabs were mixed, processed and subsampled randomly until at least 100 organisms had been sorted. If the three grabs yielded less than 100 organisms, then subsequent grabs (fourth to sixth) were added and sorted until at least 100 organisms had been sorted. Each site (one to three in each lake) was intended to be kept separate for subsequent analysis.

In 1995, DEP adopted a new sampling protocol to obtain more representative samples of each lake, in part based on results from the 1993-94 samples (Florida DEP 1996). Lakes greater than 1000 acres were divided into two or more basins (R. Frydenborg, 1995 memo), usually by separating at constriction points or between bathymetrically identifiable basins (Fig. 3-1). The 2-4m sublittoral zone of each lake basin was divided into 12 equal segments, and a grab was taken in each segment with a Petite PONAR or Ekman sampler (0.02 m^2) (Fig. 3-1). Positions of segments and sampling sites were estimated by eye in the field. The 12 grabs were combined into a single composite sample, and each sample was randomly subsampled to a count of at least 100 organisms, which were identified to the lowest practical taxonomic level. Basins (in lakes greater than 1000 acres) were retained as separate sample units. Lakes smaller than 1000 acres were represented by a single 100-organism sample.

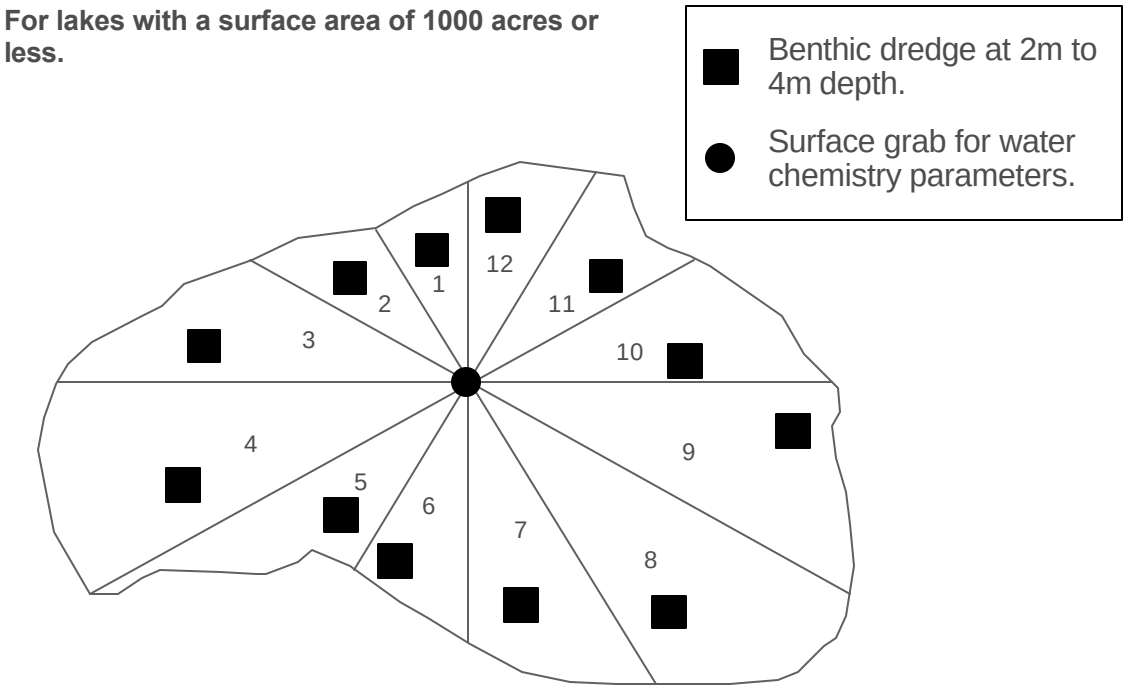


Figure 3-1. Lake sampling scheme (after Florida DEP 1996)

In fixed organism subsampling, a targeted number of organisms (typically 100 to 500) is identified. If fixed organism subsampling for benthos is conducted in an unbiased manner using a random selection method , the resulting information on richness and relative abundance is comparable among samples. For benthic samples, the targeted number is reached by randomly choosing several fractions or "grids" from a pan; all organisms enclosed within the grids are sorted to avoid bias toward large and easily seen individuals. Ideally, several (4 or more) grids are sorted to ensure proper representation.

Water chemistry samples and phytoplankton samples were taken near the center of each lake. Observations included field measurements and laboratory analyses (Table 3-1), and identification of phytoplankton to genus.

Table 3-1. Lake measurements

Measurement	Sampling	Analysis
benthic macroinvertebrates	sublittoral, 2-4 m depth	species and counts of 100 organisms
phytoplankton	mid-lake	genera and counts of natural units (cells or colonies), to 100 of the dominant taxon
chlorophyll a	mid-lake	
algal growth potential	mid-lake	
secchi depth	mid-lake	
water chemistry	mid-lake	alkalinity conductivity DO (surface and bottom) pH turbidity total NH_3 NO_3 and NO_2 total Kjeldahl N total Orthophosphorus total P
sediment fractions (1993 - 94 only)	sublittoral, with macroinvertebrate grabs	fine gravel coarse sand medium sand fine sand very fine sand fine particles % organic matter

3.2 Data Analysis

Development of biological indicators as part of a bioassessment program is an iterative process where site classification and metric selection are revisited at various stages of analysis. Index development requires a classification framework to partition natural variability and to evaluate metrics. Metrics representing various attributes of the targeted aquatic assemblages can either be aggregated into an index, or retained as individual measures (Gerritsen et al. 1998, Barbour et al. 1999). Data analysis consisted of (1) data reduction and storage; (2) development of a classification of relatively unstressed Florida lakes to account for natural variations in the aquatic biota; (3) identification and evaluation of potential metrics; (4) aggregation of selected metrics in an index; (5) examining associations between metric values and potential sources of stress; and (6) selecting thresholds for assessment of condition (Barbour et al. 1999).

3.2.1 Data Reduction and Precision

Data files were received from Florida DEP as spreadsheets and as extracts from the DEP database. Data were maintained in Microsoft Access® for QC and data reduction. Statistica (Statsoft Inc. 1995) and PC-ORD (McCune and Mefford 1997) were used for statistical analyses. Data were checked for anomalous and nonsense values.

One of the tasks of data reduction was to reconcile the 1993-94 field sampling methods with the improved methodology introduced in 1995. The earlier (1993-94) sampling consisted of 2 or 3 sampling locations in each lake, and 3-6 grabs were taken at each site. The grabs within sites were composited, but sites within lakes were kept separate. As a result of analysis of these field methods (see Chapter 4), the methods were modified in 1995 (Figure 3-1) such that all 12 grabs from a lake were composited. In order to make the two protocols more compatible, 1993-94 samples were composited from lakes < 1000 acres, but sites within lakes > 1000 acres were kept separate. Metrics were recalculated from the composited data.

Compositing of 1993-94 site-within-lake data resulted in some very large samples (> 1000 organisms). Since taxa richness increases with the number of organisms captured, it was necessary to standardize the number of organisms so that the taxa richness metrics are comparable (Hurlbert 1971, Vinson and Hawkins 1996, Barbour and Gerritsen 1996).

If a subsample was within 20% of its target size, no adjustment was made. The number of taxa in subsamples larger than 120 organisms was recalculated according to Hurlbert's rarefaction formula (Hurlbert 1971). The adjusted value is an expected value as if the sample had been randomly re-subsampled at the correct subsample size. Rather than a single random subsample, the adjusted values use all of the information that has been collected so that the adjusted value may have a fractional value (e.g., 7.3 taxa). The adjustments apply only to taxa richness metrics, including total taxa, EOT, chironomid taxa, Florida Index, and Hulbert Index (HI). Percentage metrics are not biased by sample size, and the sample Shannon-Wiener index is only slightly biased, because its value is determined primarily by the most abundant taxa. Following adjustment of the raw benthic data, benthic metrics were calculated (Table 3-2).

Table 3-2. Macroinvertebrate metrics calculated from Florida lake data

Metric	Expected response to anthropogenic impacts
Total taxa	decrease
Shannon diversity	decrease
Hulbert Index (HI) macroinvertebrate part (Hulbert 1989)	decrease
Florida Index	decrease
Chironomidae taxa	decrease
Odonata, Ephemeroptera, Trichoptera taxa (EOT)	decrease
Orthoclad taxa	increase
% Orthoclads/total chironomidae	increase

Table 3-2 (continued). Macroinvertebrate metrics calculated from Florida lake data

Metric	Expected response to anthropogenic impacts
% Tanypodidae/total Chironomidae	decrease
% Dominance	increase
% Subsurface gatherers	no information
% Shredders	decrease
% Scrapers	decrease
% Predators	decrease
% Parasites	increase
% Surface gatherers	decrease
% Filter feeders	decrease
% Diptera	increase
% Oligochaeta	increase
% Ephemeroptera	decrease
% Trichoptera	decrease
% Odonata	decrease
% EOT	decrease
% Amphipoda	increase
% Isopoda	increase
% Gastropoda	increase
% Pelecypoda	decrease
% Mollusca	increase
% Decapoda	increase
% Trombidiformes	increase
% Crustacea	no information
% Crustacea + Mollusca	no information
% Gatherers	uncertain

The Florida Index and Hulbert Index (HI) are weighted taxa counts of intolerant taxa, with taxa weighted by their tolerance. The HI is the macroinvertebrate index of the Hulbert's Lake Condition Index (Hulbert 1989) and was developed for macroinvertebrates found in lakes. The Florida Index was developed for stream macroinvertebrates and may not be appropriate for lakes.

3.2.2 Classification Analysis

Lake classification consisted of multivariate ordination, and testing of various classification schemes with the ordinations obtained. Classification is a subjective activity even when it is done with seemingly objective quantitative methods. There are many different quantitative methods to classify objects (e.g., divisive and agglomerative methods), each of which may have different results. Each classification method requires decisions on the similarity measure to be used, and on the number of classes to identify. The final test of a classification is whether it makes sense scientifically, and whether it accounts for variation in the data.

Ordination consisted of detrended correspondence analysis (DCA) and nonmetric multidimensional scaling (NMDS) using the species relative abundances at each site. DCA is an eigenvalue analysis of chi-square distances among sites, and is suited for modal distributions of species abundances along a gradient (Jongman et al. 1987). NMDS works on a matrix of ranked distances among sites, and thus is distribution-free and unaffected by non-normality and nonlinearity in the data (Ludwig and Reynolds 1988). Using the ranked distances, NMDS attempts to create a "map" of the data points in two or three dimensions, similar to creating a map from a set of distances among cities. Both ordination techniques tend to have similar results when robust relationships or gradients are present, and can be used as confirmation of each other. Different results of the two techniques imply weak or apparent but nonexistent relationships.

The result of both types of ordination is a final configuration, consisting of coordinates for each site in the dimensions chosen. Points close to each other in ordination space represent sites with similar species composition. Correlation of environmental variables with each axis of the ordination can provide insight on environmental gradients that may be associated with species composition of the sites. Results of the classification analysis are given in Chapter 5.

Ordination of Water Chemistry

Limnologists have long recognized that natural water quality of Florida lakes ranges from acidic to alkaline, and from crystal clear to deeply stained water (e.g., Shannon and Brezonik 1972, Canfield 1987, Canfield et al. 1983). Florida lakes also cover a wide range of trophic states, from ultra-oligotrophic to hypereutrophic, with a majority view among limnologists that most extreme cases of eutrophy are the result of anthropogenic nutrient enrichment (but see Canfield et al. 2000).

We first addressed classification of Florida lakes with principal components analysis (PCA) of lake water chemistry, using variables assembled and reported by Griffith et al. (1997): pH, color, Secchi depth, specific conductance, alkalinity, chlorophyll *a*, total nitrogen, and total phosphorus. The data consisted of mean summer estimates of the water quality variables from approximately 1100 lakes throughout Florida, from 1980 to 1996. The data had been assembled from several sources and screened for consistent and reliable methods (Griffith et al. 1997). Owing to missing data, the actual sample for the PCA was 570 lakes.

Ordination of Macroinvertebrate Assemblages

Classifications of lakes by water chemistry, and by macroinvertebrate assemblage, were reexamined with respect to each other and with respect to metric values and distributions to develop an integrated classification that accounts for biological variation among Florida lakes, where the biological variation includes both species composition and abundances (community composition), and system attributes (metrics). Integration consisted of examination of the data and ordinations for common patterns by plotting each alternative classification with the primary responses: scatterplots of sites in ordination space, and box-and-whisker plots of calculated metric values. Ordinations were examined by identifying sites with symbols for each classification alternative, and box-and-whisker plots of metric values were organized by alternative classifications.

Correlations of environmental variables with the ordination were determined, and individual lake regions were examined for consistent values of environmental variables that were correlated with the ordination. Classification was not a simple linear process, but was iterative. Successful classifying variables were those that either were associated with the positions of sites in ordination space, were associated with the distribution of species among sites, or were associated with the values of metrics calculated from the species data.

Finally, the classification obtained from ordination, as well as alternative classifications, were examined with respect to metric values. This was to ensure that the resultant biological classification reflected both species composition of the assemblages as well as functional attributes of the community (e.g., taxa richness, trophic structure, etc.). The reason for this is that the similarity measure we used for classification and ordination (Bray-Curtis similarity) is rather insensitive to loss of rare or uncommon taxa, but taxa loss is reflected in the taxa richness measures used in index development.

3.2.3 Metric Selection

Metrics for biological assessment are characteristics of the biota that change in some predictable way with increased human perturbation (Barbour et al. 1999). For a metric to be useful, it must be: (1) ecologically relevant to the biological assemblage or community; and (2) sensitive to stressors so that a response can be discerned from natural variation (Barbour et al. 1999). All metrics that have ecological relevance and that respond to stressors are potential metrics for use in an index. Since the universe of potential metrics is very large, it is necessary to identify candidate metrics that are informative and warrant further analysis.

Benthic macroinvertebrate metrics were selected based on examination of box-and-whisker plots comparing reference and test lakes within each lake class. Criteria for selecting metrics were:

- consistency of response among lake classes

- At least one response (among lake classes) where the median of the test sites was beyond a quartile of the reference sites. A test median below the reference quartile is equivalent to 50% or more of test observations below the reference quartile, showing a measurable response (below the quartile) in half or better of test observations.

- At least one metric representing each of 4 relevant classes of metrics for macroinvertebrates:
  - -- taxonomic diversity
  - -- community structure
  - -- trophic structure
  - -- indicator groups (tolerance/intolerance)

  The second criterion (test median below reference quartile) was allowed to be relaxed to meet the representational criterion.

- Minimal redundancy with other metrics.

3.2.4 Macroinvertebrate Index Development

The purpose of an index is to provide a means of integrating information from the various measures of biological attributes (or metrics). Metrics vary in their scale—they are integers, percentages, or dimensionless numbers. Prior to developing an integrated index for assessing biological condition, it is necessary to standardize core metrics via transformation to unitless scores. The standardization assumes that each metric has the same value and importance (i.e., they are weighted the same), and that a 50% change in one metric is of equal value to assessment as a 50% change in another.

Where possible, the scoring criterion for each metric was based on the distribution of values in the population of sites, which include reference lakes; for example, the 95th percentile of the data distribution is commonly used (Figure 3-2) to eliminate extreme outliers. From this upper percentile, the range of the metric values can be standardized as a percentage of the 95th percentile value, or other (e.g., trisected or quadrisected), to provide a range of scores. Those values that are closest to the 95th percentile would receive higher scores, and those having a greater deviation from this percentile would have lower scores. For those metrics whose values *increase* in response to perturbation (see Table 3-2 for examples of "reverse" metrics for benthic macroinvertebrates) the 5th percentile is used to remove outliers and to form a basis for scoring.

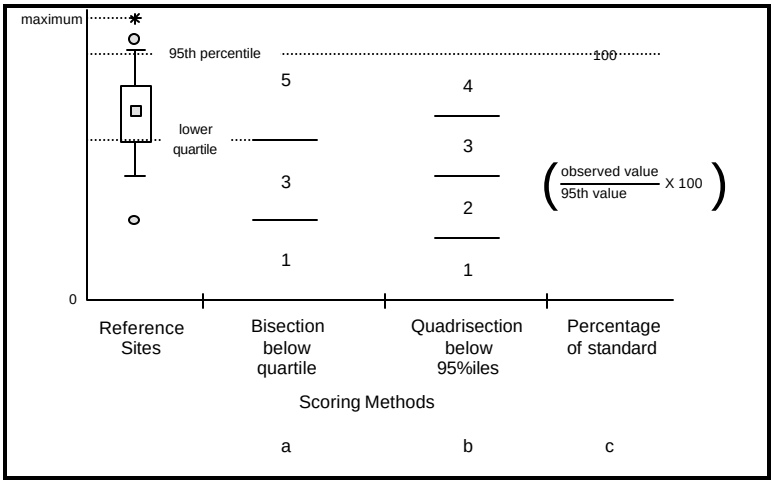


Figure 3-2. Alternative methods of metric scoring. Circles are outliers and asterisks (*) represent extreme values.

Alternative methods for scoring metrics, as illustrated in Figure 3-2, are currently in use in various parts of the US for multimetric indexes (Barbour et al. 1999). A three-part scoring range has been well-documented (Karr et al. 1986, Ohio EPA 1987, Fore et al. 1994, Barbour et al. 1996). A four-part range has been found to be useful for benthic assemblages (DeShon 1995, Maxted et al. 2000). We tried 3 alternatives: (1) 5-3-1 scoring based on the 25th percentile of the reference sites and bisection of the range below the quartile (Fig. 3-2a). This was the initial method for metric scoring in the Florida Stream Condition Index (Barbour et al. 1996). (2) The second alternative was to use a continuous standardization of all metrics as percentages of the 95th percentile value (Fig. 3-2c). This yields a more sensitive index, because information of the component metrics is retained (Hughes et al. 1998). Indexes developed for Idaho, Wyoming, Arizona, and West Virginia, support this alternative for scoring metrics (Barbour et al. 1999). (3) The third alternative was to calibrate each metric to continuous covariates that emerged in the classification analysis. In this approach, instead of fixed classes (e.g., lake pH above or below 6.5), the explanatory variable was used as a continuous variable, and regression models were used to predict metric values for all sites. Predictive variables allowed in the regression were those that emerged from the classification analysis: pH and water color. A multiple

regression was developed for each metric in the reference sites, and metrics were scored using the 95th percentile of the residuals of the regression.

3.2.5 Trophic Indexes

In addition to the benthic macroinvertebrate indexes, we also developed two trophic indexes to distinguish reference lakes from stressed lakes. Since they involve a comparison to reference lakes, they are not absolute measures of lake trophic state, unlike Carlson's trophic state index (TSI; Carlson 1977). The first trophic index used chlorophyll *a* concentration and Secchi depth as the only metrics, and was constructed in the same way as the benthic macroinvertebrate index using the 95th percentile as the "standard best value". This index was calibrated separately for each lake type.

The second trophic index followed the discriminant analysis approach developed by Davies et al. (1993). Using the set of reference and non-reference lakes as a calibration data set, a discriminant function model was developed to distinguish between reference and non-reference. All water quality data were considered in the model development.

3.2.6 Associations Among Metrics, Stressors and Sources

Following examination of potential metrics and development of a preliminary lake benthic and trophic index, we examined associations between the metrics and measures of stressors and sources of stress. The metrics were selected based on comparison of lakes judged to be least stressed by anthropogenic activities to lakes judged to be stressed to some degree. Assessment of anthropogenic stress was by best professional judgment of DEP personnel. This exercise examined the associations between biological metrics and measures of stressors or measures of sources.

We also examined association between biological metrics and land use surrounding the lakes. Land use was a surrogate for sources of stress (polluted runoff and groundwater) for a lake ecosystem. Due to the generally flat topography and Karst landscape of most Florida lake districts, catchments of individual lakes are generally unknown and difficult to delineate without extensive hydrologic investigations of groundwater flow. Instead, we elected to define buffer zones around each lake at 100 m, 500 m, and 1000 m from the lake shore. Seven categories of land use were estimated within each buffer zone from the Florida statewide land-use database. Personnel from DEP delineated buffer zones and characterized land use (see Chapter 7).

4.0 SAMPLING ISSUES

The implementation of bioassessment for lakes by DEP has raised questions on the precision and accuracy of the methods used to sample lakes, and on the best allocation of sampling effort. Three issues are of particular concern:

- How much field sampling effort is necessary to adequately characterize a lake sample unit?
- Subsampling - How much subsampling effort is necessary to adequately characterize a sample?
- What is the best allocation of effort among Florida's more than 7,000 lakes among 47 identified lake regions?

In biological sampling, with spatial heterogeneity occurring at all scales, the most cost- effective way to characterize a sample unit is with a composite sample consisting of several grabs (deployments) of the sampling equipment (e.g., multiple PONARs, Surbers, dip net sweeps), or complete sampling of the sample unit. Because numbers of organisms may be very high (thousands), such composite samples are often subsampled to reduce the overall laboratory analysis effort, becoming a two-stage sample.

4.1 Field Sampling

4.1.1 Field Sampling Effort

A critical step in developing a biological survey is the definition of the sampling unit, that is, the smallest spatial unit that will be considered a separate data point in analysis and interpretation (Ludwig and Reynolds 1989, Hurlbert 1984). Biological variability occurs on all spatial scales: individual rocks in a stream, patches of organic muck on a lake bottom, lake zones, lake basins, among lakes, and among regions. The definition of a sample unit implies that variability within sample units is not of intrinsic interest: we wish to assess a stream reach, or a lake; not individual rocks or riffles in the stream, nor adjacent patches in the lake. Although variability among patches within the sample unit is not of principal interest, it can affect our ability to characterize the sample unit with minimal error. Variability within sample units is then a component of measurement error, i.e., our failure to accurately measure the sample unit.

The field sampling effort to adequately characterize the sample unit depends on:

- The efficiency of the sampling gear and its selectivity
- Spatial heterogeneity of organisms within the sample unit and the distribution of species among sampler-sized patches in the sample unit
- Temporal heterogeneity among sampling times (index periods) within the sample unit

- The cost of sampling alternatives.

Individual species tend to be distributed by the negative binomial distribution, but rare species can be approximated by the Poisson distribution (Green and Young, 1993). Determination of optimal sampling effort requires replicated sample data to determine variability among samples.

Two critical components of variance in bioassessment studies are the measurement error and the population variance, also called sampling error. Measurement error is the variability of repeated measurements of the same thing, and it indicates how well we can characterize a single sample unit (site). For a lake survey, measurement error includes both natural variability as well as true error: natural spatial variability within a lake sampling unit; as well as errors in sampling methodology, incorrect identification, counts, etc. The magnitude of measurement error is assessed by repeated sampling, often from QA replicates done at 10% of the sites. Measurement error is used for determining whether a single site meets reference conditions. Population variance, or sampling error, takes into account natural variability among sites that are considered to be members of the same population. It is the variance when we make observations at several sites within a class of lakes, for example, reference lakes of sandy ridges.

4.1.2 Analysis of Field Sampling Effort

The original Florida sampling protocol called for identifying one to three sublittoral sites and a central site in each lake, and sampling each site with three to six grabs of a petite PONAR or Ekman benthic sampler. If benthic macroinvertebrates were abundant, three grabs were composited into a single sample, and organisms were randomly subsampled until at least 100 had been counted. If organisms were less abundant, then grabs 4 to 6 were added until 100 organisms had been counted.

The 1993-94 data were collected at one to three sites per lake, with three to six sampler grabs at each site. Data were analyzed separately for each grab at several of the lakes sampled in 1993. These data allow us to estimate the variance due to each component of sampling: among grabs within sites; among sites within lakes; and among lakes, using a nested analysis of variance to pool grabs, sites, and lakes (Snedecor and Cochran 1967).

Figure 4-1 shows the variance attributable to grabs, sites, and lakes for four candidate metrics. Taxa richness and HI have variance less than or equal to the mean, and both show the largest spatial component of variance to be sites within lakes (Fig. 4-1). In contrast, for species composition metrics (% dipterans and % head down deposit feeders) grabs within sites have a larger variance than sites within lakes.

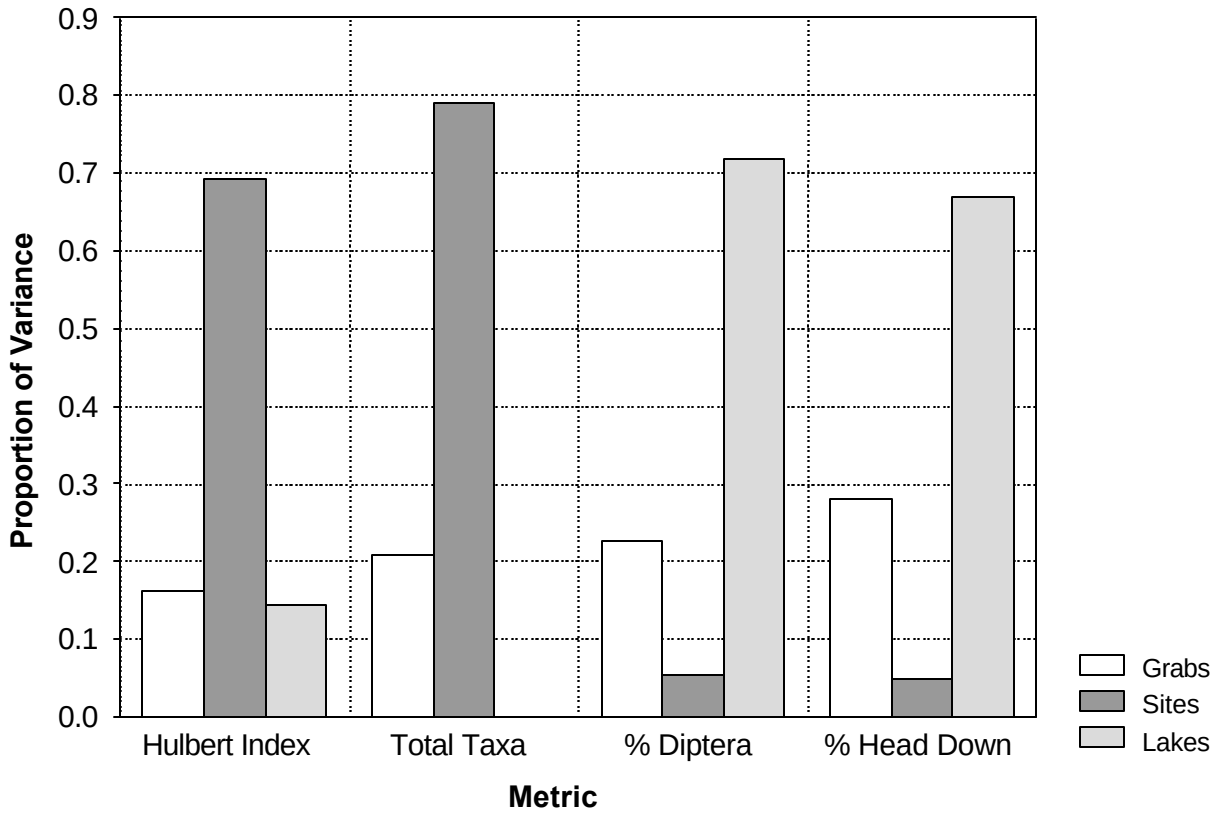


Figure 4-1. Variance of benthic metrics attributable to different sources: multiple PONAR grabs within sites; multiple sites within lakes; and lakes.

The percentage metrics have large variance in individual grabs because they contained few organisms (usually less than 50), so that random variation of few organisms results in relatively large variation in percent composition metrics.

Taxa richness metrics are correlated with organism numbers, especially at low numbers, so the variance of taxa richness will be reduced at low numbers. These data suggest that lakes can be characterized best when sampled with multiple grabs and at multiple sites. Each site requires at least one grab, so that a composite sample of several sites with a single grab at each site comprises both multiple grabs and multiple sites.

Based on the preceding results, DEP modified the lake sampling procedures for 1995 and subsequent sampling (Florida DEP 1996). Twelve grabs of a petite PONAR or Ekman sampler were made in the sublittoral zone (2-4 m) of a lake, spread out over twelve segments of the sublittoral zone. The twelve grabs were composited into a single sample and counted and identified in the usual way. Lakes larger than 1000 acres were divided into two or more subbasins or quadrants (as appropriate), and each subbasin or quadrant was sampled separately, as if it were a separate site. This sampling protocol was initiated in the 1995 sampling, and answered the requirement for multiple grabs and multiple sites at each lake.

4.2 Subsampling Effort

How much subsampling effort is necessary to adequately characterize a sample? Subsampling effort, given a parent sample, is straightforward to estimate as long as subsampling is assumed to be random, because the number of individuals of a species found in a subsample is a binomial sampling problem; therefore, the distributions of metrics from random subsampling can all be derived from the binomial distribution. However, different categories of metrics (i.e., proportions and richness) may require different levels of subsampling.

We illustrate different subsampling approaches with macroinvertebrates collected from nine lakes in 1995 (Barbour and Gerritsen 1996). Following the 1995 protocol, each of the 9 lakes was sampled with twelve petite PONAR grabs (0.02 m^2) distributed approximately equidistant in the sublittoral zone of the lake (2-4 m depth). Instead of compositing, each grab was kept separate in laboratory identification and enumeration. The lakes spanned a wide range in benthic macroinvertebrate diversity and abundance (Table 4-1), from 4 to 54 taxa, and 228 to 3540 organisms in 0.24 m^2 sampled. In seven of the nine lakes, taxa richness continued to increase with sampling effort, and did not reach an asymptote with twelve PONAR samples.

Table 4-1. Number of taxa and individuals in 12 cumulative PONAR samples from 9 Florida lakes.

Lake	Cumulative taxa	Cumulative individuals
Overstreet	54	768
Poston	44	454
Camel	39	3540
Logan	34	1649
Miccosukee	31	2828
Ocheese	28	1849
Delancy	13	228
Pickett	7	370
Adams	4	495

4.2.1 Sample Size and Metrics

Proportional (percentage) metrics

Proportional indicators (metrics expressed as percentages) are described by binomial sampling. For large samples (n>30) the binomial is approximated by the normal or Poisson distributions, with corrections for sampling-without-replacement (SWOR) from the parent sample. In the

normal approximation, the expected sample mean is p, the proportion in the parent sample, and the sample standard deviation is given by:

$$\sqrt{\frac{p(1-p)}{n}\frac{(N-n)}{(N-1)}} \qquad (1)$$

where N is the size of the parent sample and n is the size of the subsample. The term (N-n)/(N-1) is the reduction in variance due to sampling without replacement from a fixed population, in this case the parent sample (Wonnacott and Wonnacott 1972). If the subsample is very small relative to N, then the normal or Poisson approximations are used. In almost all cases, N is unknown, but we can assume a value (say, N=1000), or we may conservatively assume that N is very large and use the uncorrected normal or Poisson approximations. Actual sample variances will then be smaller than predicted by the uncorrected approximations. The Poisson approximation is used for those metrics expected to be 5% or less in a 100-organisms subsample. The sample mean and variance for the Poisson are both given by p.

The data from Lake Miccosukee were randomly subsampled to illustrate the effects of subsampling on percentage metrics. The randomized subsampling from Lake Miccosukee resulted in a slightly smaller standard deviation than predicted by the theoretical distribution. The theoretical distribution of s.d. (N=2828, n=100) is shown in Figure 4-2, with estimates based on randomized subsamples.

Taxa Richness Metrics

The number of individuals of a species in a subsample (given the species' presence in the parent sample) is also binomially distributed as above, corrected for SWOR. For sampling without replacement from a finite parent sample, we can consider each organism in the subsample to be an independent draw from the parent, with the probability of *not* finding the species being:

$$P(h=0) = (N - pN)/N. \qquad (2)$$

The probability of *not* finding the species in the second draw is:

$$P(h=0) = (N - pN - 1)/(N - 1). \qquad (3)$$

The probability of finding the species in *n* draws is then:

$$P(h \geq 1) = 1 - \frac{(N - pN)! / (N - pN - n)!}{N! / (N - n)!} \qquad (4)$$

Equation (4) is the basis for the "species rarefaction" method of estimating taxa richness developed by Hurlbert (1971). Since taxa richness is the sum of many presence/absences, it can be approximated by a normal variate (by the Central Limit Theorem). The variance of taxa

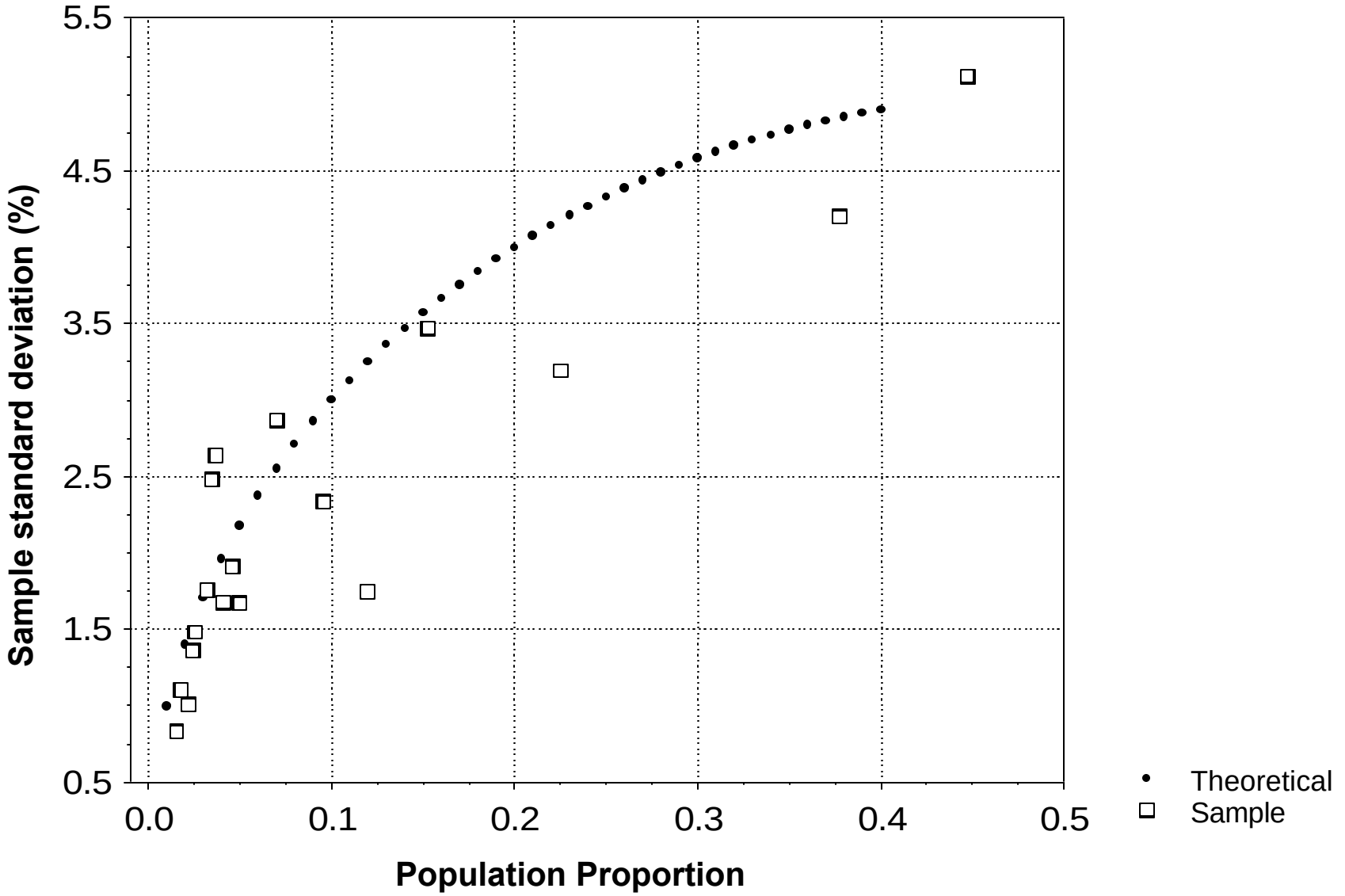


Figure 4-2. Standard deviation of percentage metrics as a function of the true proportion, 100-organism subsample from a composited sample of 2828 organisms (Lake Miccosukee).

richness is then the sum of variances of individual presence/absences. Taxa richness indicators of subsets of taxa (e.g., EPT, EOT, Chironomidae) should also be approximately normal because the component variables are binomial or Poisson, which can themselves be approximated by normal variates. Similarly, other indices (Shannon, Simpson, HBI) can be approximated by normal variates because they are the sums of random variables.

4.2.2 Composite Samples

To illustrate the effects of compositing sample casts, each sample of 12 grabs was composited into 2 replicate samples of 6 grabs, so that each sample consisted of alternate grabs. This yielded two alternative sampling protocols: 12 Ponar replicates for each lake, and two replicates of 6 Ponars each. Four candidate metrics were calculated: number of taxa (cumulative for composited samples), percent dominance, sensitive taxa (Ephemeroptera, Trichoptera, Odonata), and log abundance. Standard deviation of each metric, as measurement error in determining the "true" value for each lake, was estimated with the root mean square error (RMSE) from an analysis of variance (Table 4-2).

Table 4-2. Comparison of two sample processing protocols, Florida lakes.

	mean of 12 Ponars				mean of 2 samples of 6 composited Ponars			
Metric	**Population mean (9 lakes)**	**Range (9 lakes)**	**s.d. (Individual lake)**	**CV (average lake)**	**Population mean (9 lakes)**	**Range (9 lakes)**	**s.d. (Individual lake)**	**CV (average lake)**
No. of taxa	8.85	2-19	3.62	40.9%	25.7	5.5-44.5	4.36	16.95%
% dominance	58.8%	40%-96%	14.8%	25.2%	50.4%	16%-96%	8.9%	17.7%
Sensitive taxa (EOT)	0.39	0-1.7	0.628	161%	1.6	0-5.5	1.27	79.4%
Total Indiv (ln)	4.13	2.78-5.60	0.717	17.4%	6.12	4.68-7.48	0.145	2.4%

All metrics had a lower coefficient of variation (CV) in the composited protocol than in the uncomposited, showing the advantages of compositing multiple deployments of small sample gear such as Ponars. Composited samples reduce costs because fewer jars and records are required, and sampling time is reduced some. Laboratory analysis can be reduced by subsampling a fixed number of organisms (e.g., 100, 200, or 300) from the composite sample for identification.

4.2.3 Subsampling of Taxa Richness

Cumulative taxa distributions of the 9 lakes are shown in Figure 4-3, as relative abundance and taxon rank. The plot reflects both evenness and taxa richness. Based on this plot, we divided these lakes into three groups with similar slopes and taxa richness, or low, intermediate and high "diversity", respectively

(Fig. 4-3). The cumulative distributions of Figure 4-3 were randomly subsampled to estimate the efficacy of different subsampling approaches (Fig. 4-4). From each

distribution, we randomly drew subsamples of 100, 200, and 300 organisms; 1/4 of the sample; and a subsample that was the smallest of either 1/4, 1/2, or all of the sample, and that was at least 300 organisms. Random subsamples were estimated only once for each lake and subsampling method, that is, we did not estimate averages of many random subsamples. These were compared to cumulative taxa richness and the mean richness of 12 grabs from a lake. Analysis of variance followed by Tukey's multiple comparison was used to test each method's ability to discriminate among the groups identified from the rank-abundance plot.

In spite of the small sample size (n=9 lakes), the three organism-based enumerations performed the best in discriminating among groups (Fig. 4-4; Table 4-3). All three could discriminate the three identified groups at $p<0.05$. Methods based on the whole sample or a fraction of the whole sample were poorer in their ability to discriminate among the defined lake types. Poorest of all was mean number of taxa per grab, which could not discriminate any of the groups.

Organism-based subsampling estimates taxa richness, or the number of taxa found per standard number of organisms, not taxa density, or the number of taxa found in a standard area -(Hurlbert 1971). As pointed out by Vinson and Hawkins (1996), expected taxa richness for a given number of organisms can be estimated using the rarefaction formula of Hurlbert (1971), and the variance estimated from the formula in Heck et al. (1976). The example here shows that taxa richness is affected more by evenness than is taxa density: Camel Lake had the same taxa density as Poston Pond (54 taxa in 12 grabs), but Camel Lake had lower evenness and lower taxa richness than Poston Pond (Fig. 4-3). Although there may be interesting reasons to estimate taxa density, taxa richness is more economical to estimate; discriminates well among habitat types, and can be estimated to a constant subsample size to allow comparison of different collections.

4.2.4 Adjustment of Subsample Size

Taxa richness indicators are heavily dependent on sample size and subsample size. This is illustrated in Figure 4-5, showing expected taxa richness of subsamples from a parent sample with 80 taxa and 1000 individuals. Taxa richness based on a subsample of 100 organisms is different from a subsample based on 200 organisms, yet both are from the same parent sample (Fig. 4-5). Clearly, this can introduce serious errors to estimates of taxa richness metrics if subsamples of different sizes are mixed in the analysis. Nevertheless, we cannot be certain that subsamples will always be the same size, and it should be possible to adjust taxa richness metrics for samples that are too large. Subsamples that are too small (for whatever reason) should not be used for estimation of taxa richness.

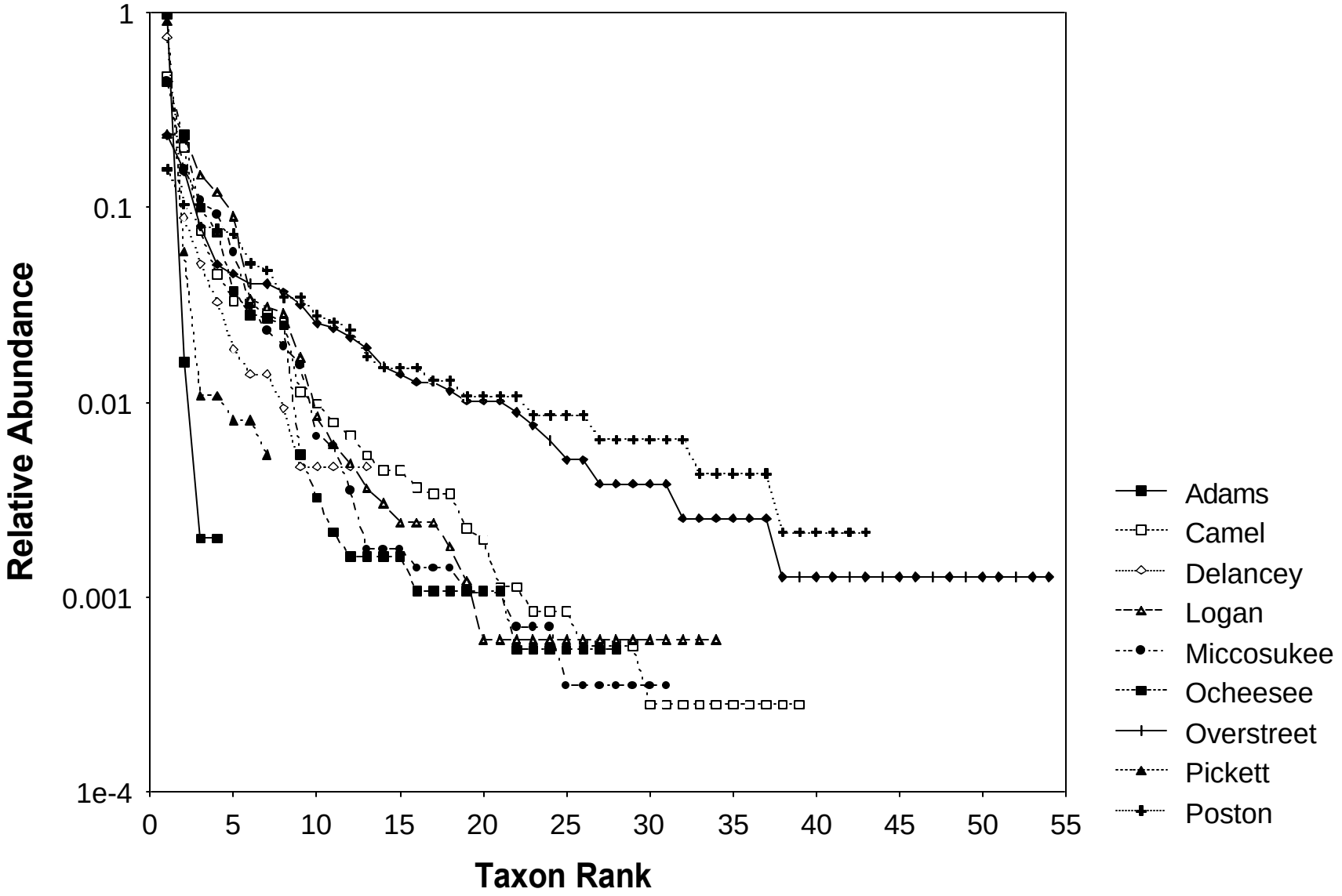


Figure 4-3. Ranked abundances of taxa from 9 lakes

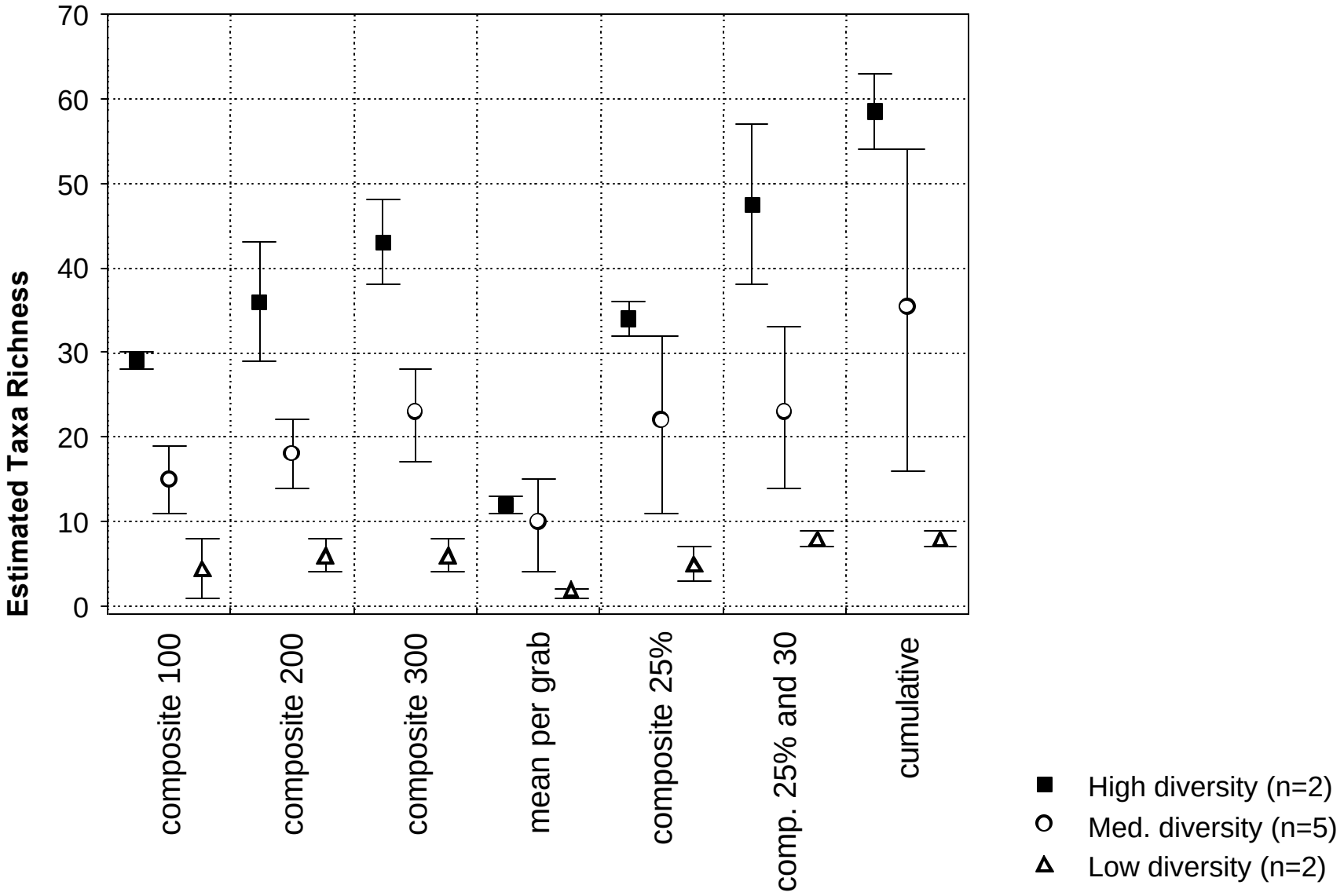


Figure 4-4. Estimated taxa richness or taxa density for seven subsampling alternatives.

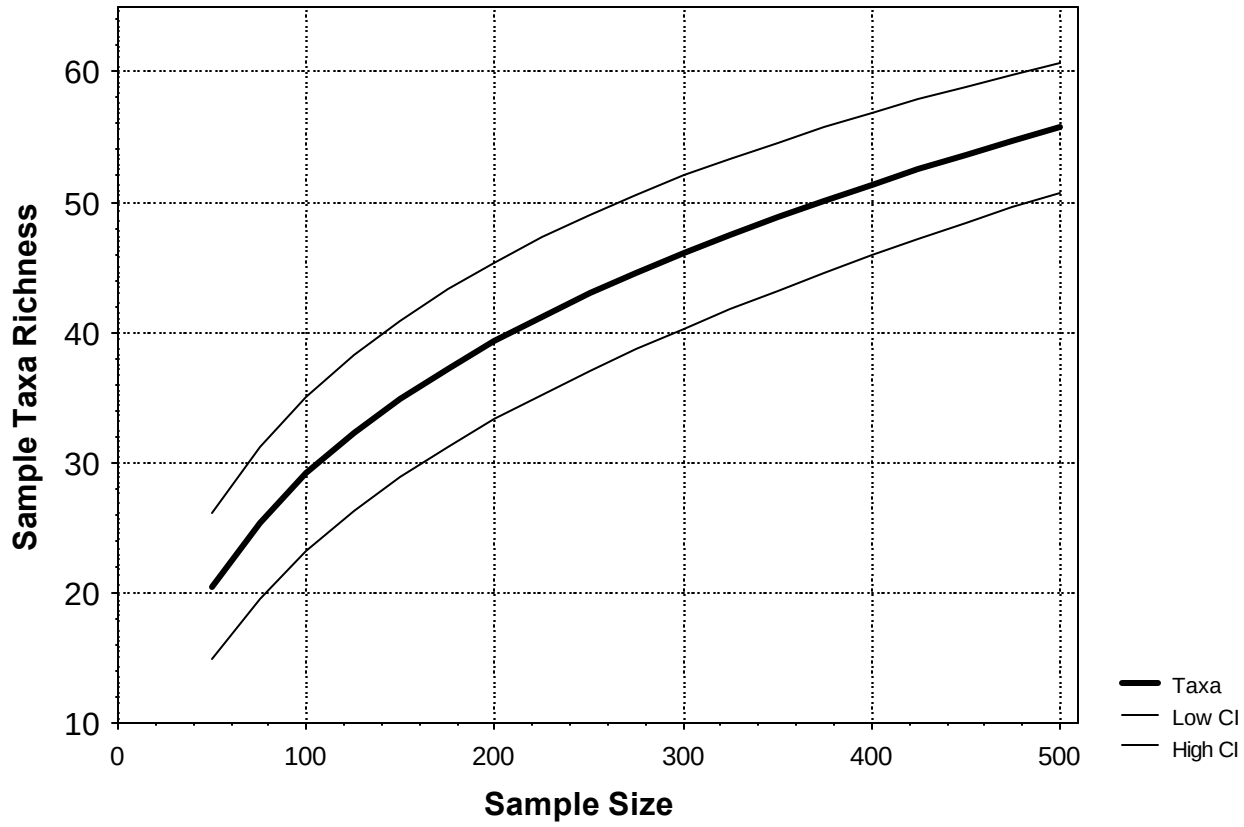


Figure 4-5. Effect of subsample size on mean number of taxa in subsample, taken from total sample of 1000 individuals of 80 taxa, ± 95% CI.

From Figure 4-5, the differences in number of taxa between subsamples that are near the target subsample size are not significantly different, and will not contribute a large amount of the total variance. Therefore, if a subsample is within 80-120% of its target size, no adjustment is necessary. Subsamples smaller than 80% should fail QA, and those larger than 120% should have taxa richness metrics adjusted. For example, if the target subsample is 100 organisms, then subsamples smaller than 80 organisms would not be used for analysis. Samples from reference lakes with fewer than 80 organisms were not used for invertebrate index development.

Subsamples larger than 120% would have the number of taxa recalculated according to equation (4) above, where the probability of finding each taxon is recalculated for the proper subsample size. The adjustment would be an expected value based on randomly re-subsampling the sample at the correct subsample size. Rather than a single random subsample, the adjusted values continue to use all of the information that has been collected. The adjustments would apply only to taxa richness metrics, including total taxa, EPT, EOT, Florida Index, and HI. Percentage metrics are not biased by sample size, and the sample Shannon-Wiener index is only slightly biased, because most of its value is determined by the more abundant taxa.

Table 4-3. Results of ANOVAs testing ability of subsampling methods to discriminate groups identified in Figure 4-4. Significant comparisons from Tukey's multiple comparisons procedure at $p<0.05$.

Subsampling	**F-ratio** (2,6 df)	**Significant comparisons**
100 organisms	26.2	3
200 organisms	24.4	3
300 organisms	30.1	3
Mean per grab	2.97	0
One quarter	5.08	1
At least one quarter and 300 organisms	11.3	2
Cumulative	9.26	1

5.0 CLASSIFICATION OF FLORIDA LAKES

Classifications of Florida lakes have been based primarily on ambient water chemistry, trophic state, and physiography (e.g., Shannon and Brezonik 1972, Canfield 1981, Canfield et al. 1983, Canfield and Hoyer 1990, Hendrickson 1993). An early classification (Shannon and Brezonik 1972) recognized four lake types based on intrinsic water chemistry: colored acid lakes, colored alkaline lakes, clear soft water lakes, and clear alkaline lakes. Most investigations recognized that the clear, soft lakes occur primarily on the sandy ridges of Florida, and are largely oligotrophic (e.g., Canfield et al. 1983, Garren et al. 1989, Hendrickson 1993).

There has been less agreement on the classification of lakes not on the Florida ridges, because these lakes exhibit more varied water chemistry: they may or may not be influenced by groundwater and springs, they may be colored or clear, and many are on large streams (4th order and above). A recent geographic statewide lake classification (Griffith et al. 1997) identified many of these anomalies and developed a more comprehensive geographic classification of Florida lakes. The classification identified 47 lake regions, based on soil and sediment type, lake origin, hydrology, and water chemistry (Griffith et al. 1997).

The objective of this biological classification was to identify lake classes based on benthic macroinvertebrate biota, and to reconcile the biological classification with a classification based on water chemistry and the existing geographic classification. The biological classification at this stage was based entirely on reference lakes to try to identify natural groupings of lakes that are relatively less affected by human activities. The chemical classification was based on all lakes in the chemical data set (Griffith et al. 1997).

5.1 Chemical Ordination Analysis

Principal components analysis of 570 lakes and 8 chemical variables in the Griffith et al. chemical data set revealed 2 principal axes that accounted for 78% of the variance in the data set. The first two eigenvalues were 4.46 and 1.79, respectively. Statistical significance of PCA axes can be determined with the "broken stick" model (Jackson 1993), which compares the eigenvalues obtained with those from a theoretical random data set. For 2 axes to be significant in an 8-variable PCA, the "broken-stick" model requires the second eigenvalue to be 1.72 or greater (Jackson 1993). No other eigenvalues were significant. Factor loadings (Figure 5-1) showed that the first axis was associated strongly with pH, conductivity and alkalinity, and the second axis was associated with color and Secchi transparency. The three trophic variables, total nitrogen, total phosphorus, and chlorophyll *a*, were all strongly correlated with each other, as well as being positively correlated with both the first axis (primarily pH) and the second axis (color and transparency).

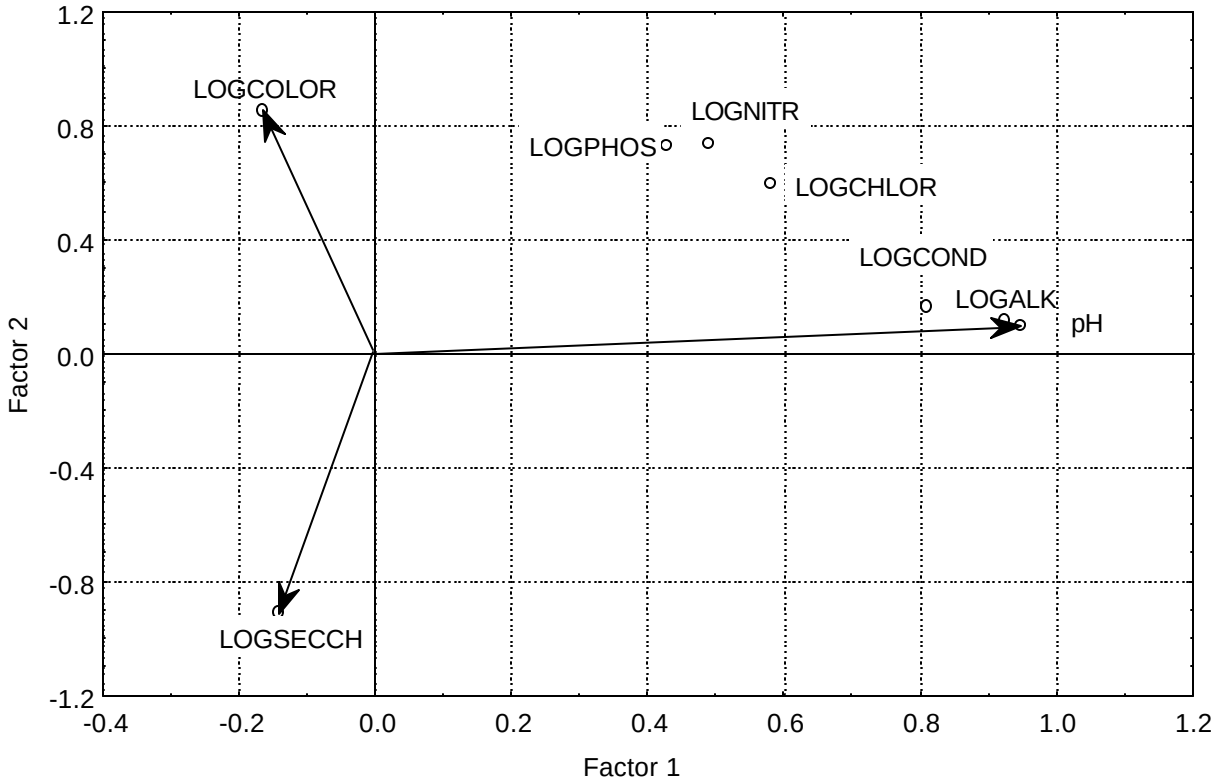


Figure 5-1. Factor loadings of principal components analysis of lake water quality data set. Arrows shown to emphasize loadings of pH, color, and secchi depth.

In accordance with the PCA results, the lakes in the Griffith et al. data set were divided into acid and alkaline groups (at pH 6.5), and colored and clear groups (initially at 40 Platinum-Cobalt units; PCU; later this was modified to 20 PCU based on macroinvertebrate distributions; see below). A scatter plot of the resulting 4 groups in PCA ordination space (Figure 5-2) revealed very little overlap among them. These results confirm the chemical classification of Shannon and Brezonik (1972), of 4 principal groups of lakes in Florida: consisting of acid-clear, acid-colored, alkaline-clear, and alkaline-colored.

Since the Griffith et al. chemical data set covered all lakes of Florida, with no identification of the least or most anthropogenically stressed lakes, these lakes were divided into 3 groups by summertime chlorophyll *a* concentration, at 5 and 30 :g/L chlorophyll a (<=5; 5-30; >30). Plotting the groups in ordination space (Figure 5-3) revealed that the highest chlorophyll concentrations were almost entirely among the alkaline lakes, but included both alkaline clear and alkaline colored. A few acid-colored lakes had high chlorophyll concentrations, but no acid clear lakes had chlorophyll *a* above 10 :g/L.

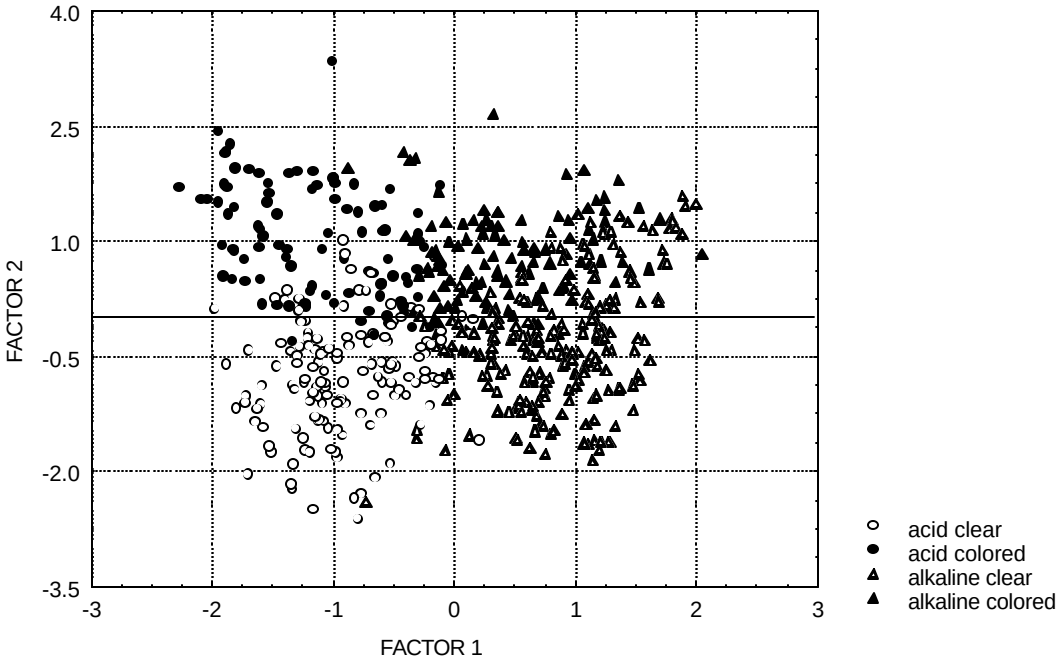


Figure 5-2. Lake water types in PCA ordination space, Griffith et al. (1997) data set. Each point represents a single lake.

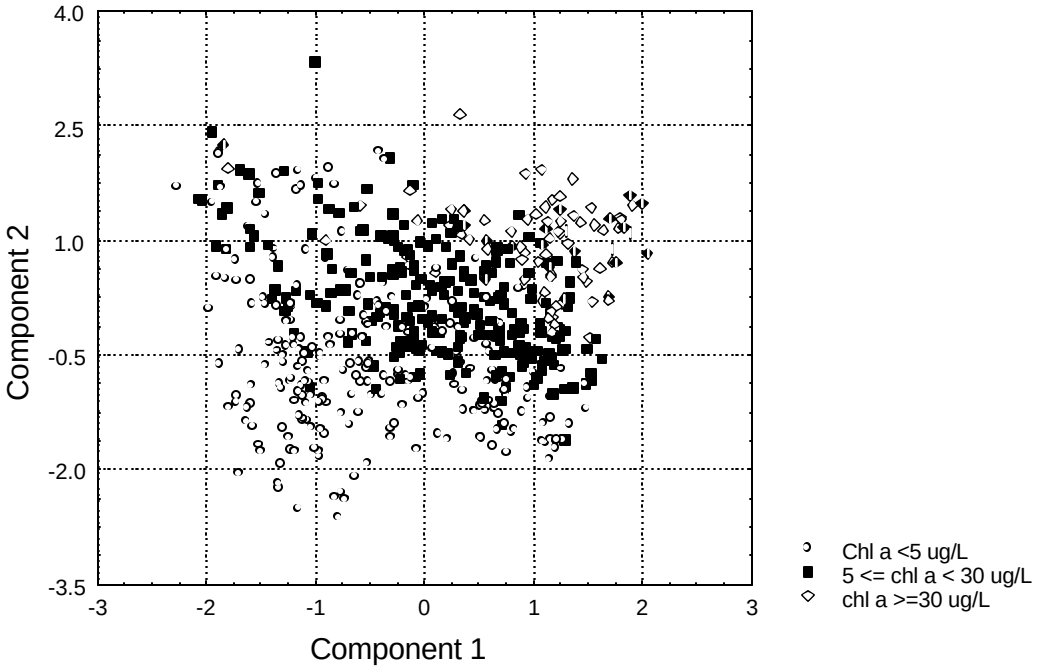


Figure 5-3. Chlorophyll concentration classes of lakes, shown in ordination space.

Of the chemical variables in the data set, TN, TP, chlorophyll, and Secchi transparency are the most affected by cultural eutrophication, therefore, these 4 variables were not used further to develop a classification of relatively unstressed lakes. Conductivity, alkalinity, and pH are relatively stable and are closely related to geology, hydrology, and vegetation of a lake watershed. They are highly correlated with each other and are redundant; therefore, pH was selected as the most commonly available variable to express the first PCA axis, and color as the variable to express the second PCA axis.

5.2 Biological Classification and Ordination

After reduction, the DEP biological data set comprised 315 observations on 206 lakes (Appendix A; Table 5-1, Figure 5-4). Of these, 202 samples were from 122 reference lakes. All lakes were sampled in the summer index period, but only a smaller subset were sampled in winter. The lakes sampled were in 36 lake regions.

Table 5-1. Breakdown of samples in data set.

		Reference	Non-reference	Impoundment	Row Totals
Summer samples					
65 acid colored		15	2	4	21
65 acid clear		24	3	0	27
Total		39	5	4	48
65 alk colored		2	6	5	13
65 alk clear		0	1	1	2
Total		2	7	6	15
75 acid colored		31	18	0	49
75 acid clear		21	0	0	21
Total		52	18	0	70
75 alk colored		41	40	0	81
75 alk clear		12	15	0	27
Total		53	55	0	108

Table 5-1 (continued). Breakdown of samples in data set.

		Reference	Non-reference	Impoundment	Row Totals
Winter samples					
65 acid colored		3	0	0	3
65 acid clear		3	0	0	3
Total		6	0	0	6
65 alk colored		0	0	0	0
65 alk clear		0	0	0	0
Total		0	0	0	0
75 acid colored		22	2	0	24
75 acid clear		14	0	0	14
Total		36	2	0	38
75 alk colored		11	4	0	15
75 alk clear		3	3	0	6
Total		14	7	0	21
Column Total		202	94	10	306*

*n = 306 because of missing pH or color data; total samples = 315

Ordination analysis

Ordination analysis of the invertebrate assemblages included both detrended correspondence analysis (DCA) and non-metric multidimensional scaling (NMDS). Both ordination methods gave substantially the same results, and DCA for 3 axes will be shown in the results. The broken-stick model is not appropriate for DCA because the detrending process breaks the ordination into segments. With a large data set such as this, 3 axes are usually sufficient for the ordination, and more than 3 are difficult to interpret.

Ordination means putting things in order; in ordination analysis the sites are ordered along the principal axes. If an environmental gradient (e.g., pH, color, lake size) influences the species composition, then we would expect that gradient to be reflected by one or more of the ordination axes. This can be examined with correlation analysis of the environmental variables with the ordination axes. A strong correlation would suggest that the environmental gradient may explain changes in species composition. Correlation analysis of the DCA axes with environmental variables showed strong associations of color, Secchi transparency, TKN, and total P with the first DCA axis in both summer and winter observations ($r>0.4$ for all 3 associations; Table 5-2). Dissolved oxygen and conductivity were associated with the

second and third axes (Table 5-2), but the DO and conductivity association were weaker than the association with color, transparency, and nutrients. In spite of the relatively strong association between the invertebrate assemblage composition and nutrient concentrations, there was only a moderate association with chlorophyll *a* concentration, and only in summer. Since the first DCA axis accounts for the greatest proportion of variance in species composition, this suggests that the environmental gradients of water transparency, color, and nutrients exert a large influence on the benthic species composition of lakes. Conductivity, chlorophyll, and pH have a weaker association than the first group.

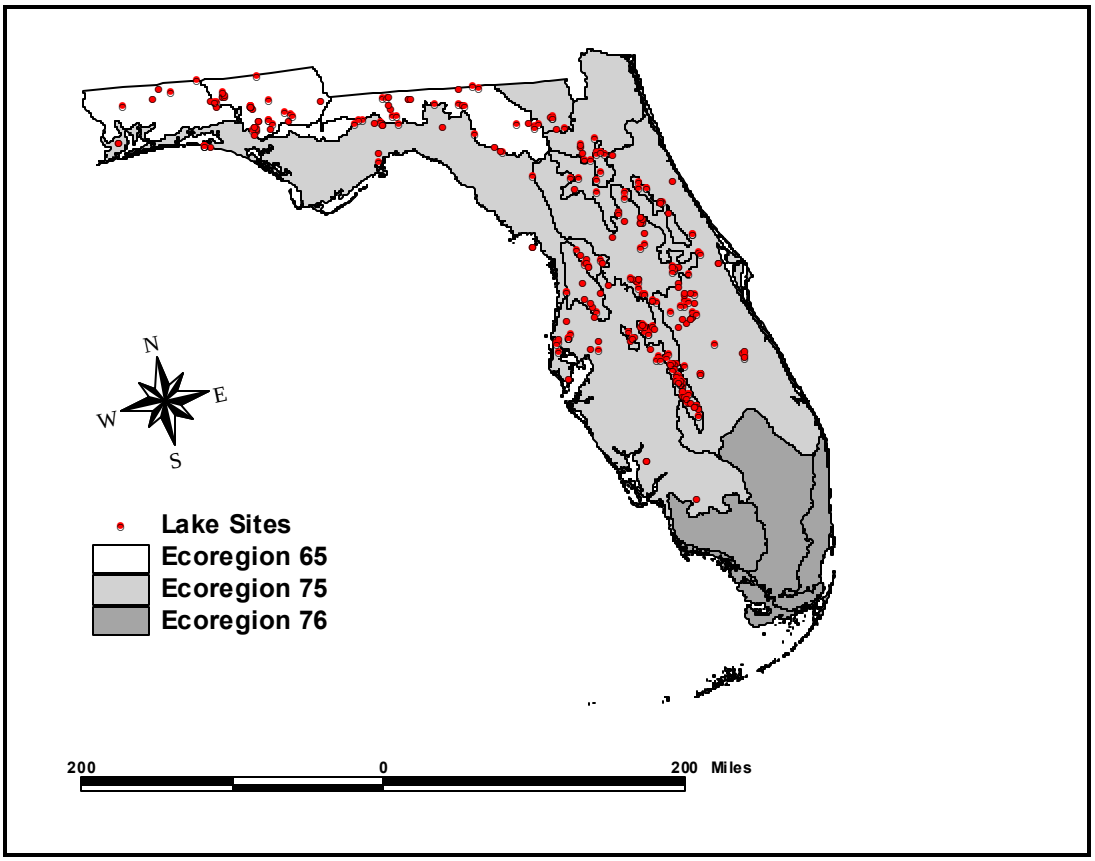


Figure 5-4. Map of lakes used in study and Florida's ecoregions.

The strongest association of species composition was with Secchi transparency and water color (on the first DCA axis), but the association with pH-conductivity was weak at best (Table 5-2). Water color and transparency also comprised one of the principal axes of the chemical analysis (Fig. 5-2), showing the importance of color and transparency in both chemical and biological classification of Florida lakes.

Table 5-2. Correlation coefficients of environmental variables with principal axes of the DCA ordination for reference lakes. Correlations greater than 0.3 shown in bold. Indicated water column concentrations were log-transformed. N varies among measures because of missing data.

a. Summer observations

Environmental Measure	AXIS 1	AXIS 2	AXIS 3	N
Secchi depth	**0.68**	0.02	-0.20	143
Dissolved oxygen	**0.30**	**-0.39**	-0.18	144
pH	-0.13	-0.19	0.05	147
Color (log)	**-0.71**	-0.02	0.11	140
Conductivity (log)	-0.05	**-0.32**	-0.06	143
Kjeldahl nitrogen (log)	**-0.59**	0.02	0.18	145
Total phosphorus (log)	**-0.46**	-0.06	-0.07	145
Chlorophyll *a* (log)	**-0.34**	0.16	0.27	145

b. Winter observations

Environmental Measure	AXIS 1	AXIS 2	AXIS 3	N
Secchi depth	**0.78**	-0.14	-0.12	54
Dissolved oxygen	0.08	**0.41**	-0.04	56
pH	-0.22	-0.25	0.22	57
Color (log)	**-0.69**	-0.11	0.16	57
Conductivity (log)	-0.07	-0.03	**-0.35**	51
Kjeldahl nitrogen (log)	**-0.55**	-0.18	-0.04	51
Total phosphorus (log)	**-0.43**	-0.13	0.11	51
Chlorophyll *a* (log)	-0.05	-0.01	0.13	48

Plots of sites in ordination space confirmed the association with water color (Figure 5-5). Figure 5-5 shows each site sampled in "ordination space"; simply a scatter plot of the first 2 DCA axes. The size of each dot shows the lake's relative color value (log transformed), such that large dots represent deeply colored lakes, and small dots represent clear lakes. The scatter plots below and to the left of the ordination space show lake color with the first and second DCA axes, respectively. Clear lakes are predominantly on the right of Figure 5-5, and colored lakes on the left. Although water color, measured as PCU, is a continuous variable, we found the greatest separation of sites in ordination space at 20 PCU (Figure 5-6). Although the invertebrate assemblages separated well on the basis of water color (Fig. 5-5, 5-6), effects of pH and conductivity were not apparent.

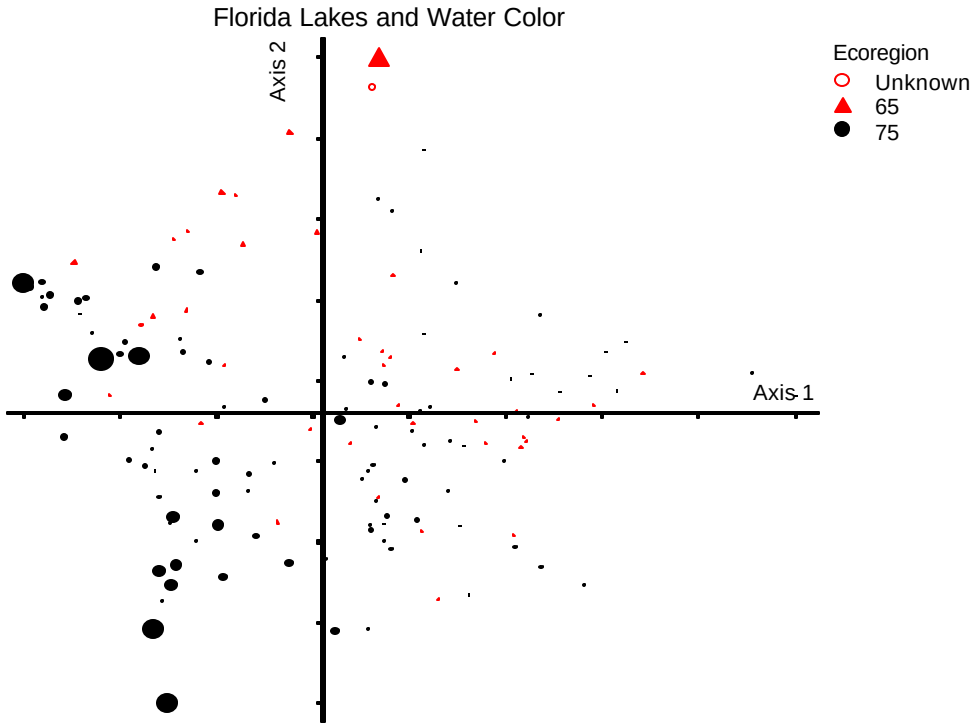


Figure 5-5. Scatter plot of site ordination scores ("ordination space"). Triangles are in ecoregion 65, and closed circles are lakes in ecoregion 75. Size of symbol is proportional to water color; largest symbols are the darkest lakes.

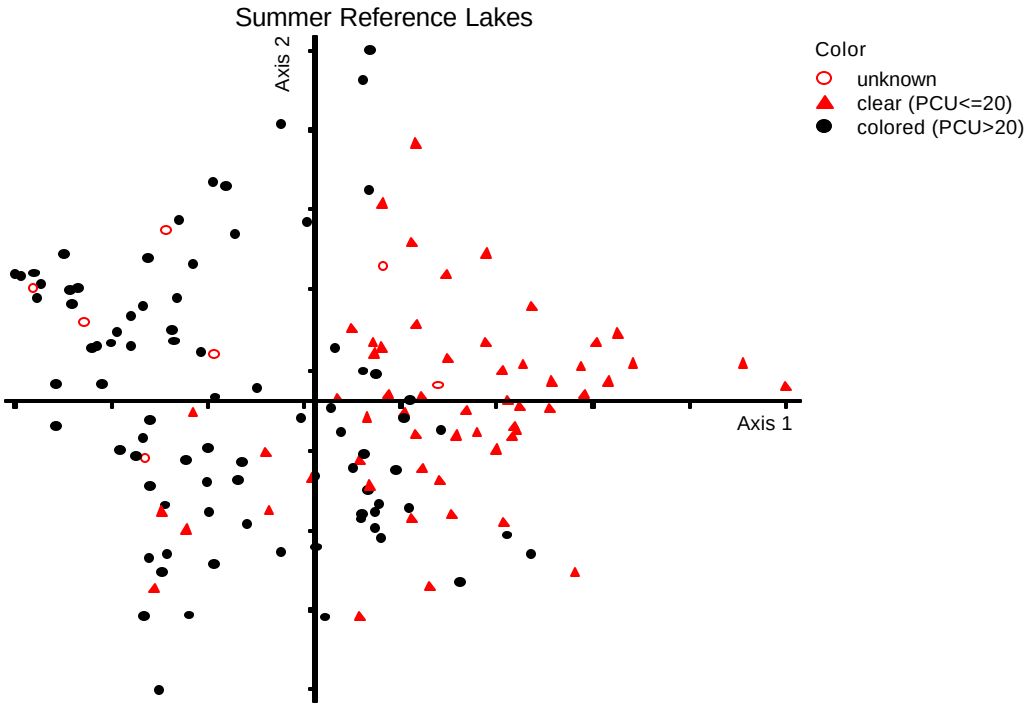


Figure 5-6. Scatter plot of ordination scores with samples identified by water color group.

Taxa distributions

The graphic approach of Figure 5-5 can be used to visualize the distributions of dominant taxa. Figure 5-7 shows the relative abundance of phantom midges, *Chaoborus*, in the summer reference lake data set. The size of the dots represents the relative abundance of *Chaoborus*, and the scatter plots below and to the left of the ordination scatter show the correlation of *Chaoborus* with the first and second ordination axes. *Chaoborus* are dominant in the most heavily colored lakes; often, there are few other taxa present. It was the most abundant and the most common taxon in the summer data, and its distribution therefore dominates the ordination.

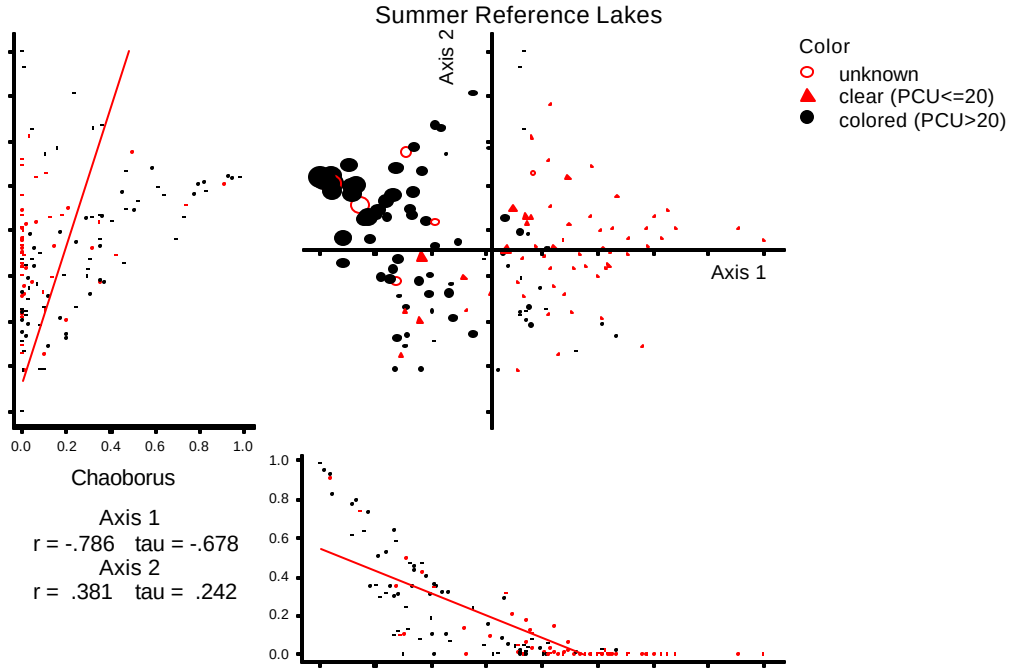


Figure 5-7. Scatter plot in ordination space showing relative abundance of *Chaoborus* spp. Main scatter plot shows *Chaoborus* relative abundance by symbol size, as in Figure 5-4. Side plots show correlation of *Chaoborus* relative abundance with DCA axis 1 and axis 2 of the main plot.

Most species of *Chaoborus* are demersal, feeding on zooplankton in the water column at night, and resting on the sediment during the day. Their abundance and dominance in highly colored lakes is consistent with observations that *Chaoborus* may be reduced or extirpated by fish predation in clear waters (von Ende 1979, Stenson 1981).

In addition to water color, individual taxa also were associated with both water pH and ecoregion. Figure 5-8 shows the distribution of *Oxyethira* with respect to water pH; and Figure 5-9 shows the distribution of *Coelotanypus* with respect to ecoregion. *Oxyethira* is more common and more abundant in acidic lakes (Figure 5-8), and *Coelotanypus* is more common in ecoregion 75, the Coastal Flatwoods (Figure 5-9). The relative "preference" of taxa for different lake types (acid, alkaline, clear, colored) and the two Level 3 ecoregions (65 - Southeast Plains; 75 - Coastal Flatwoods) was

calculated (Table 5-3) as the ratio of occurrence (presence) in one lake type to the complementary lake type. The preferences showed that of the 56 most common genera (including 1 family), 45 showed associations with lake type or ecoregion at greater than 67% (taxon found in preferred lake type 67% more frequently than in non-preferred type, controlled for abundance of different lake types). The preferences are ratios of relative frequencies of occurrence; they are not meant to be predictive nor do they take into account collinearities among lake types. For example, of 41 reference samples from Region 65, only 2 were from alkaline lakes (Table 5-1).

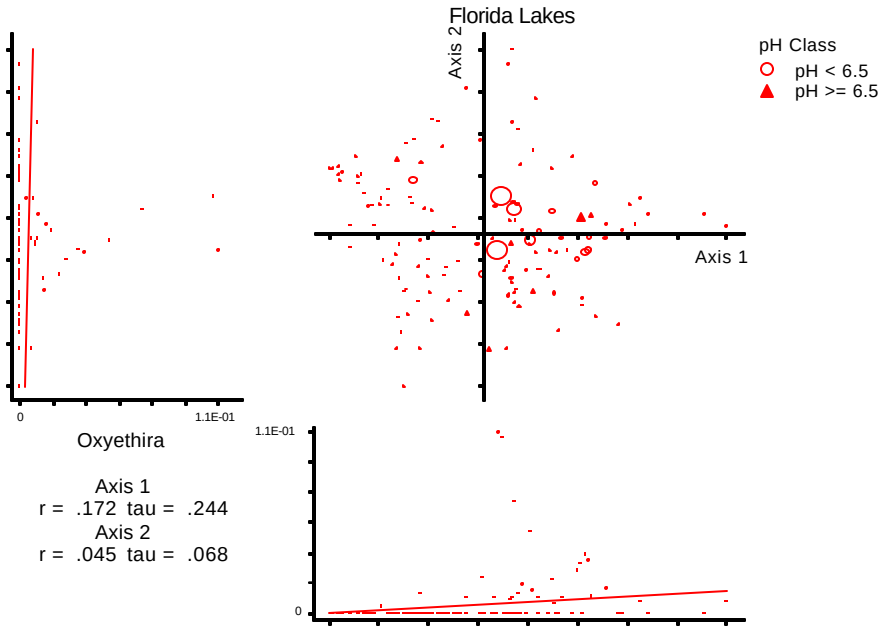


Figure 5-8. Scatter plot of samples in ordination space and *Oxyethira* abundance, with sites identified by pH class (see Fig. 5-6 for explanation). *Oxyethira* is found principally in acid lakes.

We conclude from Table 5-3 that taxa were associated most frequently with water color (35 taxa; 9 with color only), somewhat less frequently with ecoregion (30 taxa; 6 with ecoregion only, and less still with water pH [20 taxa; 1 with pH only]). The pH association especially is not reflected in the ordination (Table 5-2) because the most dominant taxa (e.g., *Chaoborus, Tanytarsus, Polypedilum*) are associated with water color, and the ordination is driven by the most abundant taxa. The associations of Table 5-3 were developed from presence-absence information only.

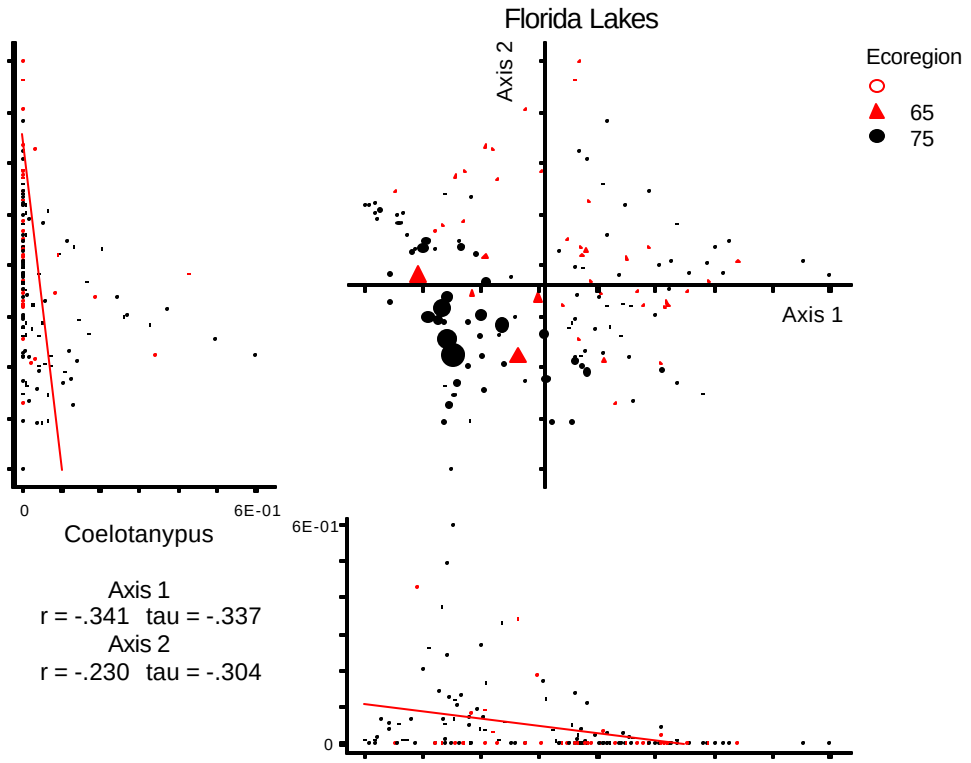


Figure 5-9. Scatter plot of samples in ordination space and *Coelotanypus* abundance, with sites identified by ecoregion. *Coelotanypus* is found principally in Ecoregion 75.

Seasonal comparison

Life histories of benthic invertebrates, especially the insects, leads to a seasonal phenology of species. This was confirmed with ordination of the combined summer and winter samples, which showed slight differences in assemblage composition between the two seasons (Figure 5-10). Summer and winter samples separated on the second ordination axis of the DCA. Several taxa were more abundant in one season or another (Table 5-4).

Lake sediment

An earlier classification with the 1993-94 data (Gerritsen and White 1997) suggested that sediment composition was strongly associated with benthic species composition; in particular, lakes could be divided by the percent of fine sediment.

Lake regions sampled in 1993-94 were characterized by predominant bottom sediment: clean sand ("sandy") or fine sediment ("mud-muck"). There were two potential problems with basing the classification only on the fine sediment fraction:

! Only the 1993-94 samples were analyzed for sediment size distribution; there was no sediment information for the 1995-97 lakes, which included several districts that had not been sampled in 1993-94.

Table 5-3. Habitat preferences (as ratios) of genera found in more than 10 samples, summer reference sites. Selectivities indicating 67% preference or greater (<0.6; >1.67) are shown in bold; asterisks (*) indicate significant log-linear model (p<0.05; maximum likelihood chi-square) for taxa found in 15 or more samples.

Genus	**Samples with taxon (n=146)**	**Selectivity**			**Description of preference**
		acid/alk	**col/clr**	**75/65**	
Chaoborus	112	0.96	**1.68***	1.04	colored water
Limnodrilus	108	0.87	0.95	1.04	
Tanytarsus	100	1.16	**0.58***	0.83	clear water
Polypedilum	90	1.18	0.63*	0.72	
Hyalella	85	0.81	0.80	0.97	
Oecetis	72	1.51*	**0.49***	0.76	clear water
Ablabesmyia	71	1.14	0.81	1.03	
Palpomyia/bezzia grp.	70	0.99	1.03	0.87	
Procladius	68	1.14	0.85	0.71	
Dero	67	1.05	0.82	**0.54***	Region 65
Cladotanytarsus	64	1.04	**0.43***	0.85	clear water
Coelotanypus	62	0.81	**2.96***	**2.80***	colored water, Region 75
Hexagenia	58	0.88	0.94	1.52	
Glyptotendipes	56	0.77	0.82	1.12	
Dicrotendipes	54	1.48	**0.30***	0.65	clear water
Cladopelma	52	1.18	**0.56***	0.61	clear water
Cernotina	48	1.37	**0.32***	0.76	clear water
Cryptochironomus	45	0.93	0.69	**2.25***	Region 75
Chironomus	43	0.86	0.95	**0.52***	Region 65
Pseudochironomus	41	1.20	**0.42***	0.62	clear water
Lauterborniella	35	1.19	1.10	**0.43***	Region 65

Table 5-3 (continued). Habitat preferences (as ratios) of genera found in more than 10 samples, summer reference sites. Selectivities indicating 67% preference or greater (<0.6; >1.67) are shown in bold; asterisks (*) indicate significant log-linear model (p<0.05; maximum likelihood chi-square) for taxa found in 15 or more samples.

Genus	Samples with taxon (n=146)	Selectivity			Description of preference
		acid/alk	col/clr	75/65	
Eclipidrilus	34	**1.73**	**0.50**	**0.31***	Region 65; clear, acid water
Oxyethira	34	**2.02**	**0.31***	**0.40***	Region 65; clear, acid water
Paratanytarsus	31	1.13	**0.46***	**0.36***	Region 65; clear water
Helobdella	29	**0.51**	**2.52***	1.64	colored, alkaline water
Parachironomus	29	0.77	**0.56**	0.79	clear water
Caenis	28	1.56	**0.16***	**0.44***	clear water, Region 65
Aulodrilus	28	**0.29***	**4.61***	1.57	colored, alkaline water
Pagastiella	28	**2.86***	**0.31***	**0.49**	clear, acid water; Region 65
Ceratopogonidae	27	**3.58***	**0.20***	**0.42***	clear, acid water; Region 65
Nilothauma	27	0.91	0.61	**0.57**	Region 65
Stictochironomus	26	**2.61***	**0.13***	**0.42***	clear, acid water; Region 65
Einfeldia	25	1.32	**1.87**	**0.50**	colored water, Region 65
Corbicula	25	**0.29***	**2.37**	**1/0***	Region 75; alkaline, colored water
Labrundinia	25	**1.97**	**0.35***	1.07	clear, acid water
Piona	25	1.11	1.41	**0.36***	Region 65
Cryptotendipes	24	1.04	**0.46**	1.01	
Djalmabatista	22	**2.80**	**0.26***	**2.13**	clear, acid water; Region 75
Unionicola	22	**6.22***	0.91	**0.43**	acid water; Region 65
Arrenurus	20	**5.60***	**0.33***	**0.36**	clear, acid water; Region 65
Haber	19	**0.29***	0.82	1.00	alkaline water

Table 5-3 (continued). Habitat preferences (as ratios) of genera found in more than 10 samples, summer reference sites. Selectivities indicating 67% preference or greater (<0.6; >1.67) are shown in bold; asterisks (*) indicate significant log-linear model (p<0.05; maximum likelihood chi-square) for taxa found in 15 or more samples.

Genus	Samples with taxon (n=146)	Selectivity			Description of preference
		acid/alk	col/clr	75/65	
Palaemonetes	19	0.86	**0.38***	**0.28***	Region 65, clear water
Pristina	17	**0.44**	**0.36***	0.65	clear, alkaline water
Koenikea	16	1.04	0.99	1.07	
Pisidiidae	15	**0.31***	**7.24***	0.71	colored, alkaline water
Elliptio	15	**0.23***	**2.41**	**1/0***	Region 75; alkaline, colored water
Clinotanypus	14	1.56	**0.22**	**0.57**	clear water, Region 65
Parakiefferiella	14	1.12	**0.49**	**4.63**	Region 75; clear water
Larsia	13	1.40	0.66	**0.09**	Region 65
Bratislavia	13	**0.39**	0.66	**0.57**	Region 65; alkaline water
Physella	12	**0.06**	0.79	**1.78**	alkaline water, Region 75
Limnesia	12	0.87	**0.07**	1.60	clear water
Haemonais	11	1.09	**2.63**	**0.13**	Region 65, colored water
Tanypus	11	1.66	1.15	1.25	
Planorbella	11	**0.14**	**0.28**	**3.56**	alkaline, clear water; Region 75
Stenelmis	11	**6.22**	**0.00**	**0.36**	clear, acid water; Region 65
Enallagma	11	1.66	**0.15**	**0.13**	Region 65; clear water

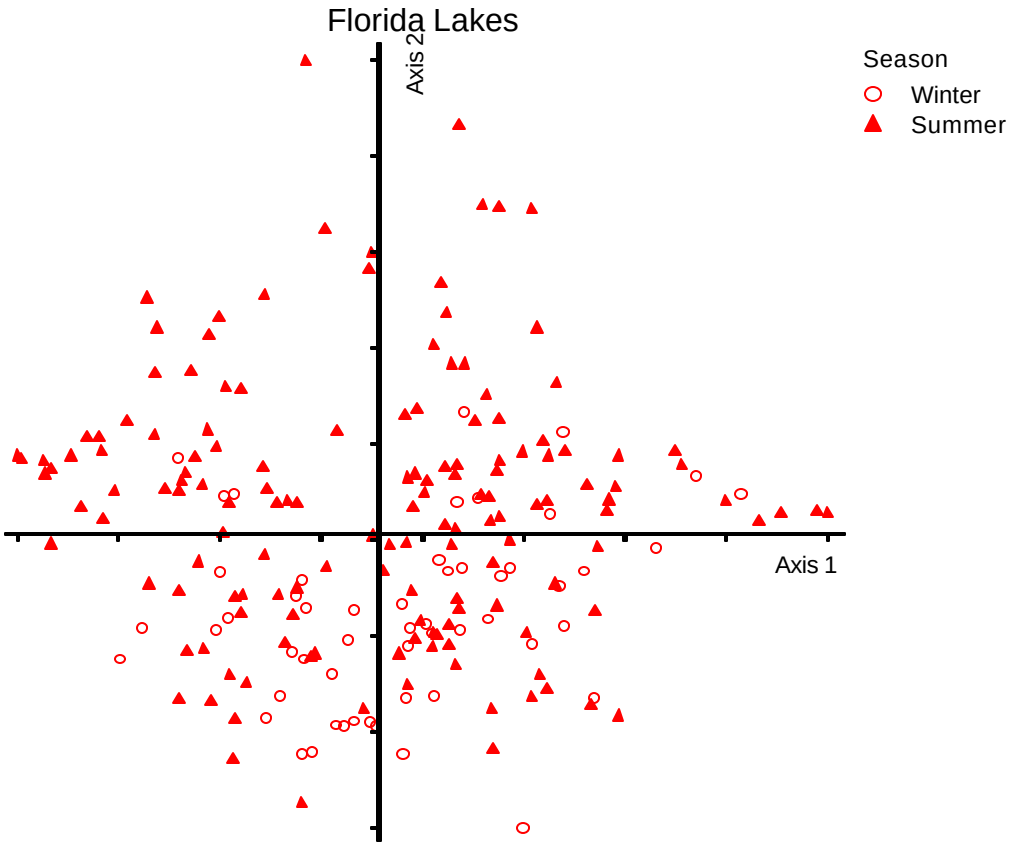


Figure 5-10. Samples in ordination space identified by sampling season. Most winter samples.

- **!** The fine sediment fraction includes both fine organic muck and mineral silt-clay, because the analytical methods do not distinguish fine organic muck from silt-clay (DEP SOP EA-13, EA-14, EA-15; 1993). At a test lake, organic muck (and hence, % fines) may be increased by anthropogenic eutrophication, making the % fines a poor variable for classifying test lakes.

The correlation of the earlier ordination with fine sediment was r = -0.47 (n=62 samples). Water color data were not available for the earlier analysis, but color had a stronger association with the current ordination than did sediment composition (Table 5-2). Sediment analysis was dropped from the field methods in 1995 because multiple grabs were composited, and lake sediment type may vary among areas of the sublittoral. Water color measurement was included in the standard protocols, and color information was added to the data set from the Griffith et al. (1997) data for lakes that had been sampled in both programs.

Table 5-4. Taxa showing seasonal phenology of 50% greater frequency of occurrence in either summer or winter samples. Only those taxa occurring in 25 or more samples shown. Asterisks (*) indicate statistical significance at $p < 0.05$ (see Table 5-3).

Genus	Total ocurrence (203 samples)	Summer occurrence (146 samples)	Winter occurrence (57 samples)	S/W ratio	
Hexagenia	102	58	44	0.51	winter*
Palpomyia/bezzia gr.	88	70	18	1.52	summer
Cryptochironomus	81	45	36	0.49	winter*
Ceratopogonidae	57	27	30	0.35	winter*
Chironomus	53	43	10	1.68	summer
Pagastiella	45	28	17	0.64	winter
Nilothauma	45	27	18	0.59	winter
Lauterborniella	40	35	5	2.73	summer*
Djalmabatista	40	22	18	0.48	winter*
Unionicola	39	22	17	0.51	winter*
Helobdella	33	29	4	2.83	summer*
Einfeldia	29	25	4	2.44	summer
Piona	26	25	1	9.76	summer*
Tubificidae	25	12	13	0.36	winter*
Apedilum	25	9	16	0.22	winter*

5.3 Practical Classifications

Categorical Classification

We conclude from the above analyses that a practical classification for Florida lakes, based on the benthic macroinvertebrate assemblage, is 6 lake types:

- Acid-clear lakes of the Southeast Plains (Ecoregion 65)
- Acid-colored lakes of the Southeast Plains
- Acid-clear lakes of the Southeast Coastal Plain (Ecoregion 75)
- Acid-colored lakes of the Southeast Coastal Plain
- Alkaline-clear lakes
- Alkaline-colored lakes

We could not determine whether alkaline lakes separated between ecoregions 65 and 75 because there were too few alkaline lakes in region 65.

Non-categorical (continuous) Classification

Both pH and color are continuous variables, without a true "break" in their distributions to separate acid and alkaline; clear and colored (Fig. 5-2). The pH break point of 6.5 gave the best separation in water chemistry ordination (Fig. 5-2), and the color break point of 20 PCU gave the best separation of macroinvertebrate species composition (Fig. 5-6). Instead of categorization of pH and color, they could also be treated as continuous variables in a working classification.

Because of demonstrated faunal differences between the two ecoregions, this classification would consist of ecoregion (65 or 75) and the measured values of water color and pH. Having these, it should be possible to develop a regression model to predict metric values in reference lakes, based on pH and water color, in each ecoregion. Indexes using both alternative classifications (categorical and continuous) are developed in Chapter 6.

6.0 METRIC SELECTION AND INDEX DEVELOPMENT

Development of a multimetric index requires identification of metrics that respond to anthropogenic stresses, and calibration of scoring criteria for each responsive metric. Responsive metrics were identified by comparing their distribution in reference and test lakes, separately for each of the lake types identified. The best metrics are combined into an additive lake condition index. We examined two index types, one for benthic macroinvertebrates and one for trophic condition. We also examined alternative index scoring methods (see 3.2.4), and the effect of alternative lake classification systems on the indexes.

As shown in Chapter 5, Florida lakes are best classified by water color and pH. Since these are continuous variables, this leaves open the option of treating them as continuous variables, or of breaking each into the categories of "clear", "colored", "acid", and "alkaline." Since we also observed biological differences between the two ecoregions, independent of water chemistry, the results of the classification suggested that two alternatives are possible for Florida lakes:

- a categorical classification consisting of at least 6 (and up to 8) classes:
  - 2 ecoregions
  - 2 pH classes
  - 2 color classes; and

- a continuous classification consisting of 2 ecoregions and the measured values of pH and water color.

Lake condition indexes were developed for each of the classification systems. Indexes included a benthic macroinvertebrate index as well as a trophic index. In all cases, the approach was to compare the reference and non-reference lakes, and select metrics and the index to enable discrimination between reference and non-reference. Metrics and indexes were evaluated with summer data only because there were insufficient non-reference lakes sampled in winter.

6.1 Benthic Macroinvertebrate Indexes

6.1.1 Metrics

In the lake classification step (Chapter 5) we identified 6 lake types: acid clear lakes of Ecoregion 65, acid-colored lakes of Ecoregion 65, acid-clear lakes of Ecoregion 75, acid-colored lakes of Ecoregion 75, alkaline-clear lakes (of both ecoregions), and alkaline-colored lakes. There were not enough test lakes to adequately test metrics in acid-colored lakes of both ecoregions; for metrics and index development, these groups were combined into the single group of acid-colored lakes.

Macroinvertebrate metrics examined are listed in Table 3-2. Of the 33 metrics examined, nine were selected as candidate metrics for an invertebrate index for Florida lakes. Responsive metrics, and metrics that were thought beforehand to be good candidates, are shown in Appendix B. Many metrics had different values among the lake types.

Several metrics were correlated with each other (Table 6-1). The Shannon index was strongly correlated with both total taxa and with dominance. Graphic examination of the relationships among the

metrics showed that the Shannon-total taxa relationship was not entirely linear, and the Shannon-dominance relationship had large and asymmetric variance in the middle of the range (Fig. 6-1). Because of the variance and nonlinearity of the relationships, all of the candidate metrics were retained for inclusion in a potential index.

For selection of final metrics, candidate metrics were given ordinal scores for the strength of apparent responses shown in Appendix B, ranging from 0 (no detectable difference between reference and non-reference) to 3 (little or no overlap between reference and non-reference distributions) (Table 6-2). Six metrics were selected for the macroinvertebrate indexes, based on the comparisons of Appendix B, correlation in Table 6-1, and responsiveness in Table 6-2:

- Total taxa: strong and consistent in clear lakes; weak and less consistent in colored lakes
- EOT taxa: strong in acid clear lakes
- % EOT: strong in acid-clear and alkaline colored
- Hulbert Index: strong and most consistent throughout
- Shannon-Wiener diversity: strong in clear lakes, consistent throughout
- % Diptera: weak, but consistent throughout.

6.1.2 Metric Scoring and Candidate Lake Indexes

Two scoring systems were examined for the categorical index: 5-3-1 ordinal scoring, and percentage scores of a "standard best value" (see Figure 3-2). The "standard best value" was the 95th percentile of the distribution of values, to eliminate effects of extreme outliers on performance of the index. Use of the 95th percentile as the standard best value means that 5% of sites will score higher than 100%. These were set to 100%, so that no metric score could be greater than 100%. The average value of the 6 metrics was used as the index value; the index could thus range from 1 to 5 for the 5-3-1 scoring, or from 0-100% for the percentage scoring.

For Total Taxa, EOT Taxa, Percent EOT, Shannon-Weiner, and Hulbert index, the formula for metric scores based on the 95th percentiles is as follows:

$$\text{Score} = \text{minimum } \{100,\ 100\ (\text{metric value} / 95^{th}\ \text{percentile})\},$$

where the 95th percentile is from Table 6-3. For Percent Diptera, the formula is:

$$\text{Diptera Score} = \text{minimum } \{100,\ 100\ (100 - \%\text{Diptera}) / (100 - 13.6)\}.$$

Table 6-1. Correlation matrix of candidate metrics, summer data. Correlation coefficients greater than 0.8 shown in bold.

	Total Taxa	EOT Taxa	% EOT	% Diptera	Shannon-Wiener Index	% Dominance	Hulbert Index	% Head-up Obligate Collectors	% Filterers	Log Secchi Depth
EOT Taxa	.66									
% EOT	.18	.57								
% Diptera	-.19	-.13	-.32							
Shannon-Wiener Index	**.91**	.60	.22	-.23						
% Dominance	-.71	-.47	-.23	.21	**-.92**					
Hulbert Index	.74	**.82**	.45	-.06	.69	-.54				
% Head-up Obligate Collectors	.08	.27	.52	-.10	.12	-.16	.31			
% Filterers	-.06	.02	.25	-.32	.06	-.13	.09	.23		
Log Secchi Depth	.57	.48	.10	-.07	.54	-.40	.52	.10	-.05	
Log Chlorophyll Conc.	-.38	-.42	-.20	.07	-.39	.31	-.47	-.19	-.16	-.67

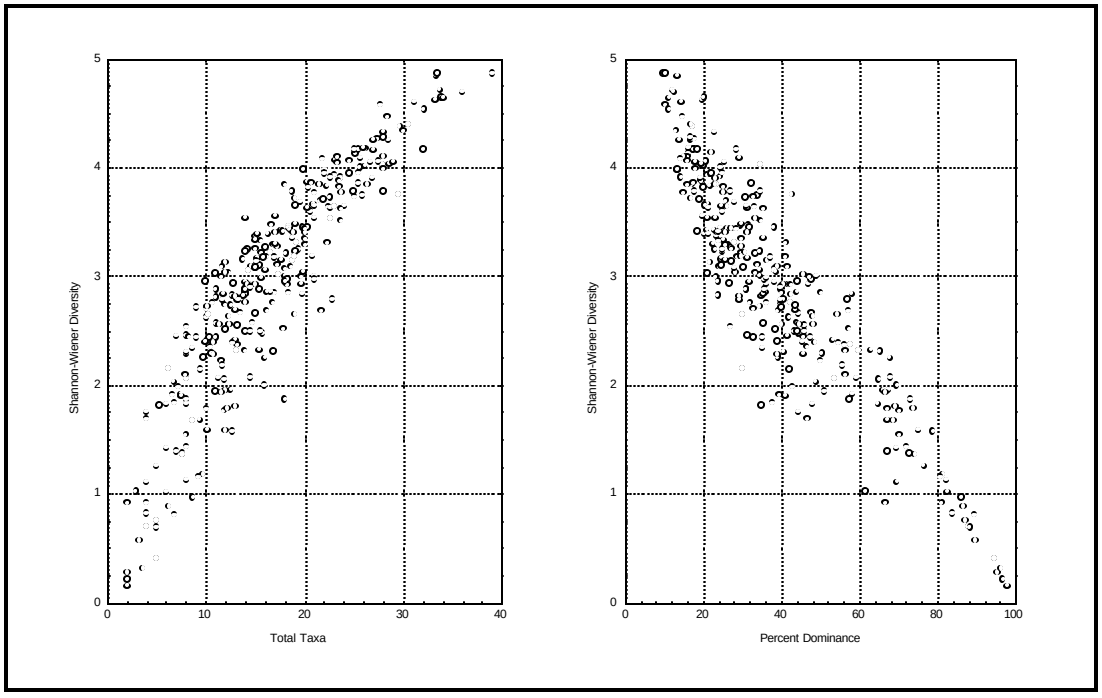


Figure 6-1. Association of Shannon-Wiener diversity with total taxa and percent dominance.

Table 6-2. Strength and consistency of metric responses from Appendix B. Numbers are strength of the response (0 = no response). Signs indicate increase (+) or decrease (-) of metric values in non-reference sites.

Metric	65 Acid Clear	75 Acid Clear*	Acid Colored	Alkaline Clear	Alkaline Colored
Hulbert Index (HI)	-3	-	-½	-2	-2
% dominance	+3	-	0	+½	+1
% filterers	0	-	0	0	-1
% surface deposit feeder	0	-	0	-3	0
% EOT	-3	-	0	-1	-2
% Diptera	+1	-	+½	+½	+1
Total taxa	-3	-	0	-2	-½
Shannon index	-3	-	-½	-3	-1
EOT taxa	-3	-	0	-½	-1

*There were no non-reference lakes in Ecoregion 75 acid-clear.

Table 6-3. Scoring criteria for benthic indexes for categorical lake classes.

		Percent Scoring	Ordinal Scoring					
Metric	**Response**	**95th percentile (reference)**	**Score**	**65 Acid-Clear**	**75 Acid-Clear**	**Acid-Colored**	**Alkaline-Clear**	**Alkaline-Colored**
Total Taxa	Decrease	30.5	5	30.3	23.7	20.9	26.5	20.3
			3	15.1	11.8	10.4	13.3	10.1
EOT Taxa	Decrease	5.2	5	5.8	3.7	2.9	3.1	2.5
			3	2.9	1.9	1.4	1.5	1.3
Percent EOT	Decrease	34.4	5	19.2	10.3	13.2	11.0	9.9
			3	9.6	5.1	6.6	5.5	4.9
Percent Diptera	Increase	13.6[1]	5	57.2[2]	49.0[2]	51.4[2]	21.9[2]	40.5[2]
			3	78.6	74.5	75.7	60.9	70.3
Shannon-Weiner Index	Decrease	4.39	5	4.37	3.87	3.54	4.06	3.45
			3	2.18	1.93	1.77	2.03	1.73
Hulbert Index	Decrease	17.4	5	15.9	12.3	10.0	12.3	10.2
			3	8.0	6.1	5.0	6.2	5.1

[1] 5th percentile
[2] 75th percentile

Indexes for Categorical Lake Classes

Scoring criteria and scoring thresholds for the two alternative scoring methods are shown in Table 6-3. The index values and performance are compared in Figures 6-2 - 6-3. Both indexes showed good separation of reference and non-reference sites for clear lakes, but neither index showed any separation in acid-colored lakes (Fig. 6-2). The index based on 95th percentile scoring had lower variability in the reference sites, and was able to discriminate better in the alkaline-clear lakes (Figs. 6-2, 6-3).

Index for Continuous Lake Classification (Covariate Index)

The approach for index development using continuous values of pH and water color was to develop a regression model to predict the value of each metric as a function of color and pH. Thus, for any combination of pH and water color, a metric value could be predicted as the "standard best value." The standard best value was taken as the 95% line of the residuals, or the value within which 95% of the observations occurred for the given pH and color (Fig. 6-4).

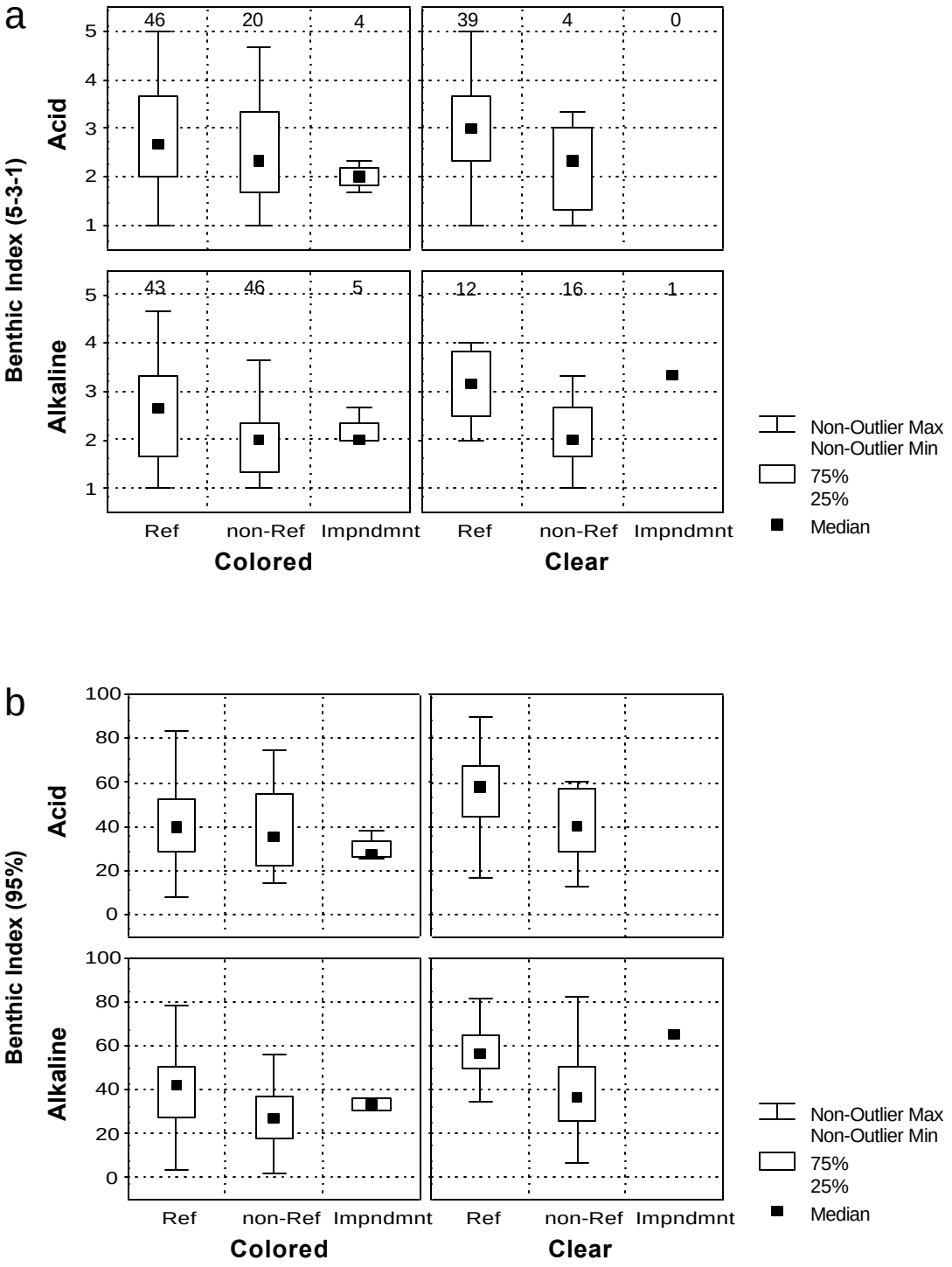


Figure 6-2. Performance of categorical benthic indexes in lake classes, combined ecoregions.

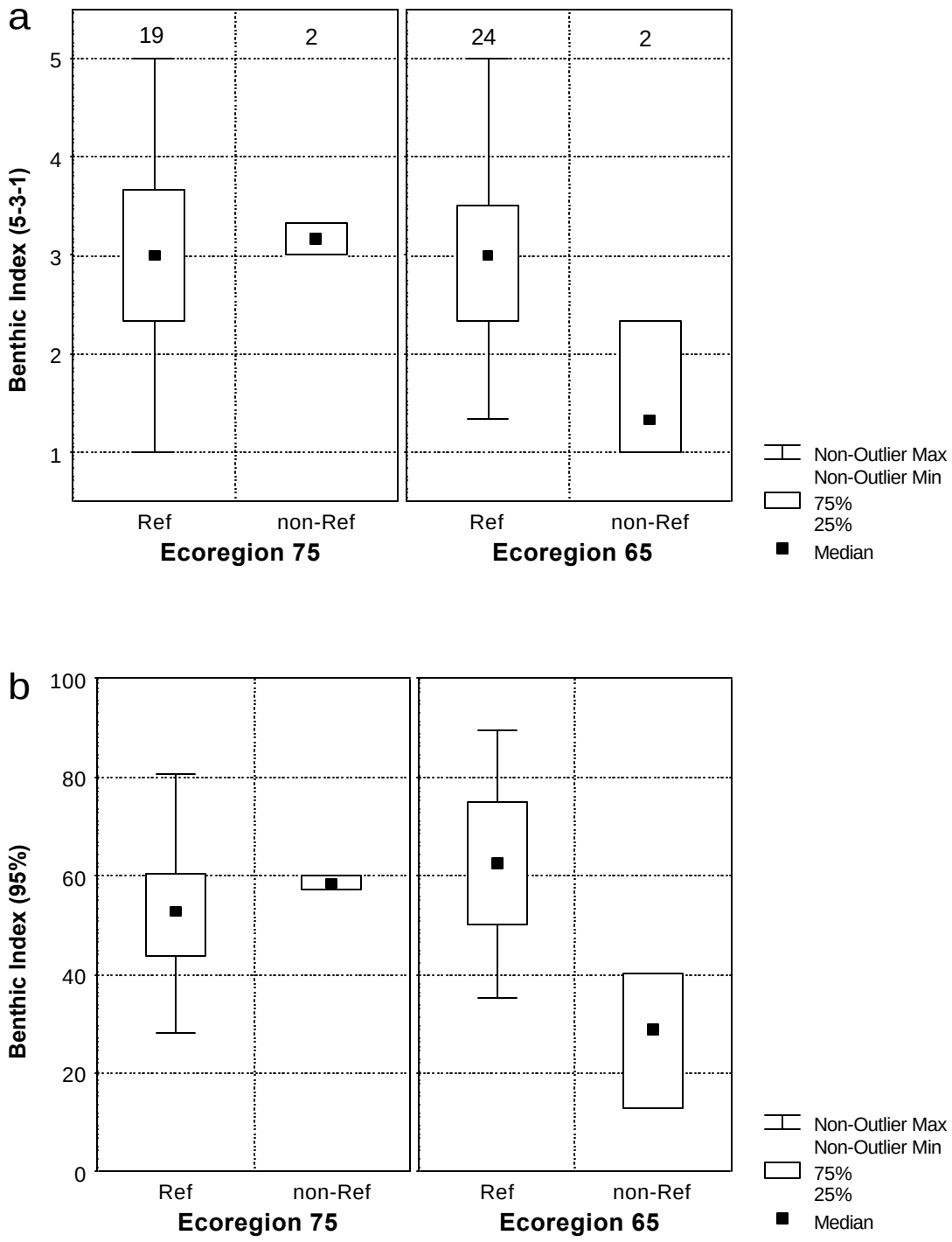


Figure 6-3. Performance of categorical benthic indexes in acid-clear lakes within Ecoregions 65 and 75.

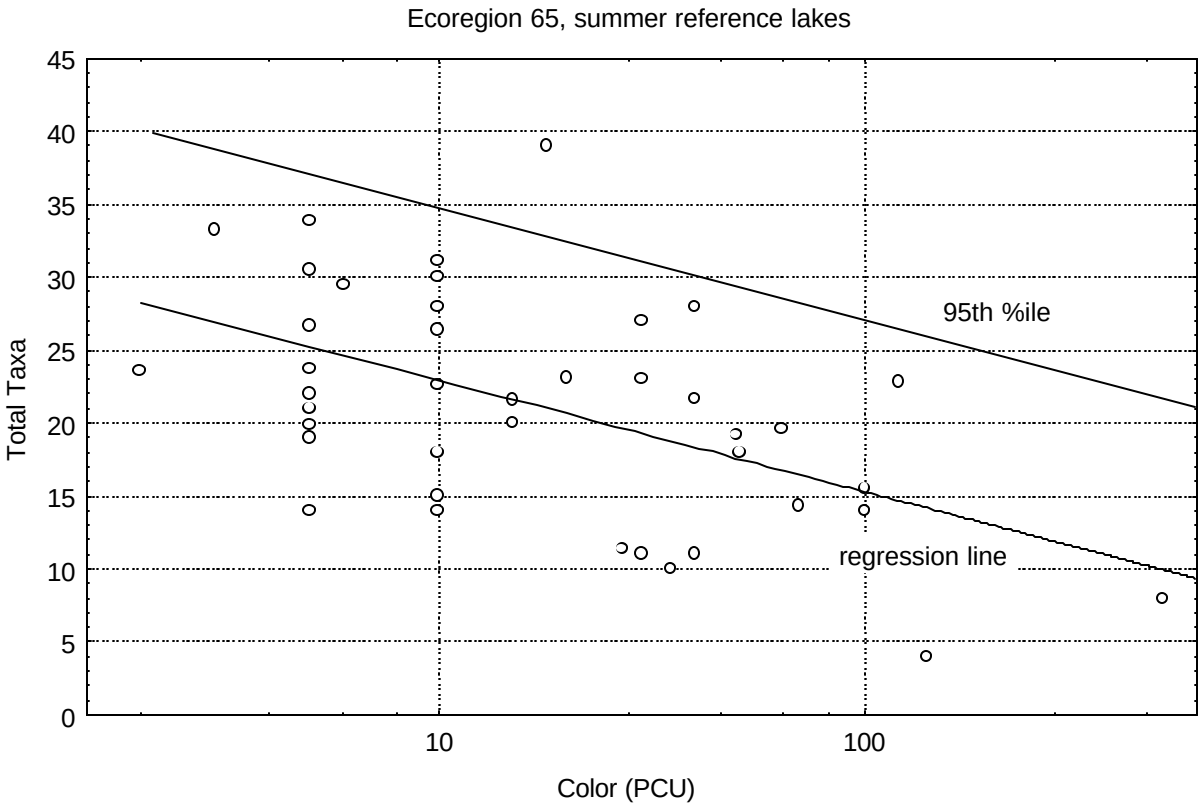


Figure 6-4. Regression of total taxa versus color. The regression line plus the 95th percentile was used as the standard best value.

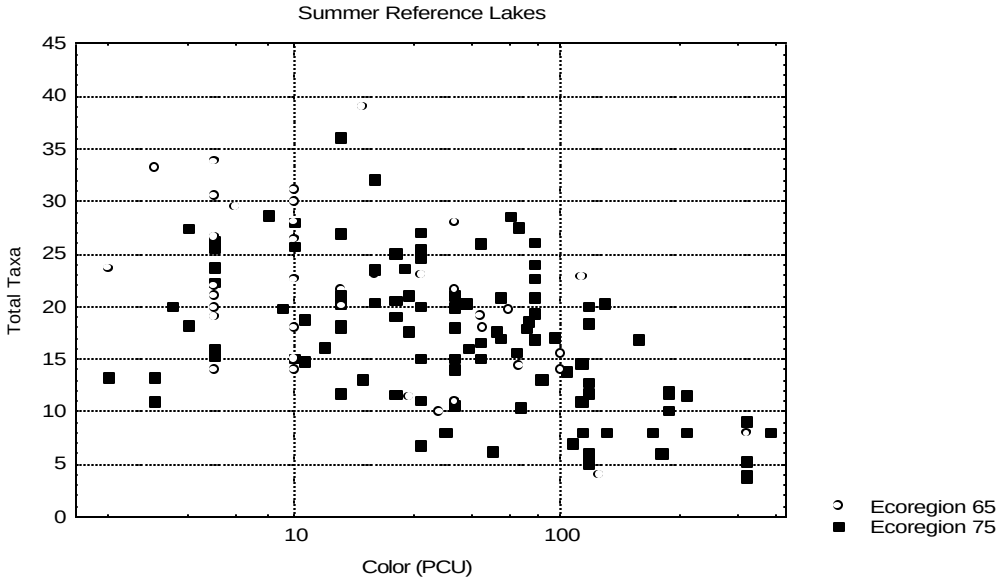


Figure 6-5. Scatterplot of total taxa and water color, showing different slopes of Ecoregions 65 and 75.

Table 6-4. Regression equations and regression statistics for scoring metrics for covariate index.

Metric	Regression equation	+ residual 95%ile	Regression Statistics		
			R^2	F	p
Region 65 (n=35)					
Total Taxa	31.27 - 8.47(Log_{10}[Color])	11.78	0.28	14.5	<0.001
EOT Taxa	5.73 - 2.35(Log_{10}[Color])	4.39	0.26	13.1	<0.001
% EOT	38.28 - 5.61(pH)	17.44	0.19	8.88	<0.01
% Diptera	--	28.79	---	---	n.s.
Shannon-Wiener index	4.78 - 1.096(Log_{10}[Color])	1.164	0.40	23.9	<0.0001
Hulbert Index	17.68 - 6.887(Log_{10}[Color])	8.49	0.39	23.2	<0.0001
Region 75 (n=102)					
Total Taxa	19.9 - 6.21(Log_{10}[Color]) + 1.1(pH)	8.74	0.26	18.3	<0.00001
EOT Taxa	3.36 - 0.907(Log_{10}[Color])	2.68	0.11	14.0	<0.001
% EOT	--	37.8	---	---	n.s.
% Diptera	98.8 - 5.87(pH)	-45.75	0.065	8.01	<0.01
Shannon-Wiener index	4.02 - 0.64(Log_{10}[Color])	1.21	0.16	20.4	<0.0001
Hulbert Index	12.97 - 2.89(Log_{10}[Color])	8.32	0.11	14.1	<0.001

Preliminary analysis of covariance (ANCOVA) revealed that some metrics exhibited significantly different slopes between the two ecoregions (Figure 6-5); therefore, a separate regression model was developed for each metric and each ecoregion. Regression models used only reference sites and are shown in Table 6-4. Residuals were calculated for all reference sites, and the 95th percentile of the residuals was estimated.

The "standard best value" was the predicted metric value from the regression (y-hat) plus the 95th percentile value of the residuals (Figure 6-4). Metric scores were simply the observed value divided by the standard best value, as a percentage:

$$S = \left(\frac{y_{obs}}{\hat{y} + resid_{95}} \right) 100$$

As before, scores > 100% were set to 100%. The continuous class index was calculated as the average of the 6 metric scores, for each of the two ecoregions. The covariate index performed slightly better than the two categorical indexes (Fig. 6-6; broken down categorically for comparison).

6.2 Trophic Indexes

Reference and non-reference lakes differed in water column measurements (Appendix B). Non-reference lakes as a group had higher chlorophyll concentrations and reduced Secchi transparency, and higher total phosphorus than corresponding reference lakes, showing increased trophic state in the test lakes. Two trophic indexes were developed: a categorical index for the four water quality lake classes, and an index based on continuous lake classes.

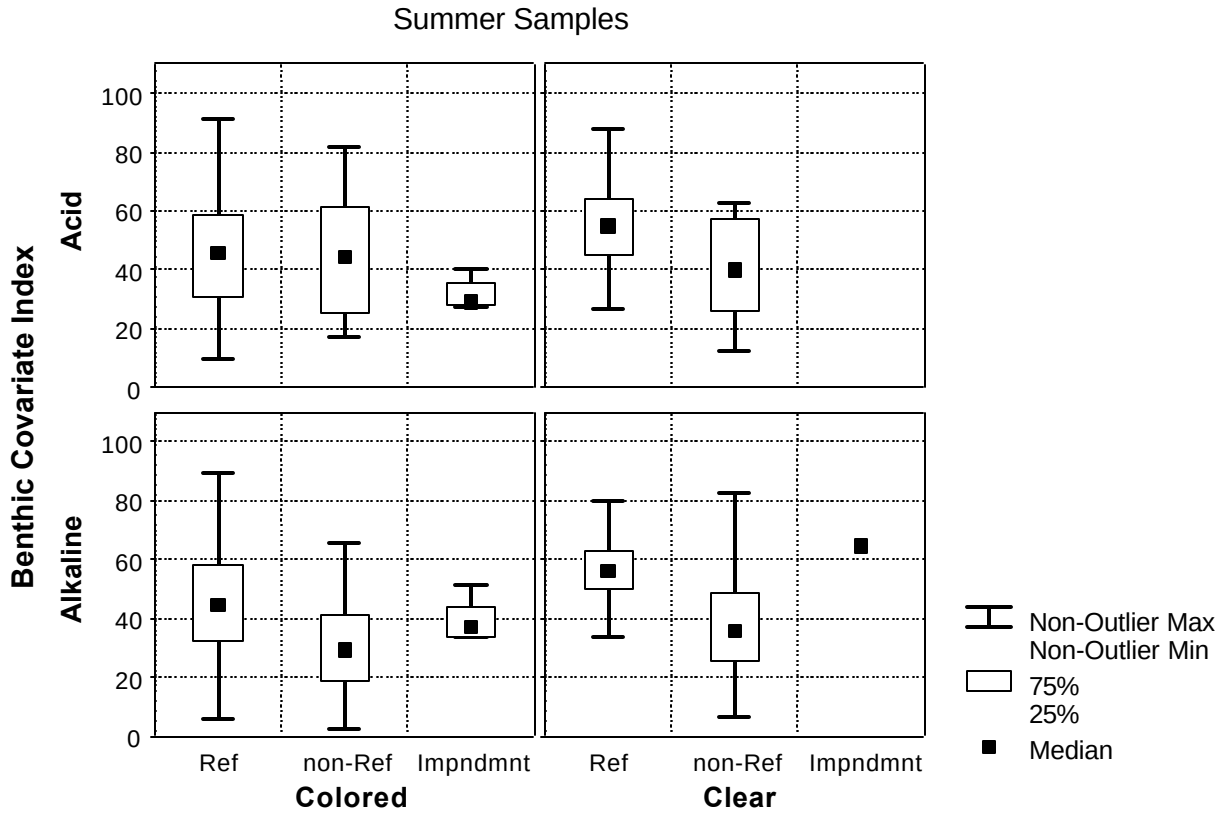


Figure 6-6. Performance of benthic covariate index; broken into site classes for comparison (c.f. Figures 6-2, 6-3).

6.2.1 Categorical Trophic Index

The categorical trophic index used only Secchi depth and chlorophyll concentration, and was constructed in the same way as the benthic index using the 95th percentile of reference as the standard best value. Formulas for calculating the two metric scores were:

Chlorophyll score = maximum {0, 100 (1 - log chlorophyll) / 2.7}

Secchi score = minimum {100, 100 (log Secchi depth + 1.222) / 1.602}, for Secchi depth > 0.06m.

Secchi depth is measured in meters and chlorophyll *a* is measured in :g/L. The standard best value for Secchi depth (95th %ile) was 2.4m, and for chlorophyll *a* (5th %ile) was 1 :g/L. Although a single standard best value was used, the index was compared to reference lakes in each of the four water classes (Fig. 6-7). The trophic index was more responsive in colored lakes than in clear lakes (Fig. 6-7).

6.2.2 Discriminant Function Trophic Index

The water quality variables are closely related, as was demonstrated by the PCA analysis of the water chemistry data (Figures 5-1, 5-2, 5-3). Rather than develop an additive index for the continuous classification, using only Secchi depth and chlorophyll as metrics, we used discriminant function analysis (DFA) to develop a model to predict whether a site is more like a reference lake or not.

The discriminant analysis was 2-way, with reference and non-reference the only classes. A stepwise forward model-building procedure was used, and variables permitted into the model were ecoregion, Secchi depth, chlorophyll *a* concentration, color, pH, total Kjeldahl nitrogen, and total phosphorus. All but ecoregion and pH were log-transformed. Variables that contributed to the model were pH, Secchi depth, color, and total phosphorus.

Results of the DFA are shown in Table 6-5. The classification functions in Table 6-5 are used to predict class membership (reference or non-reference) of a given site as a linear equation:

$$\text{Score} = b_0 + b_1x_1 + b_2x_2 + b_3x_3 + b_4x_4$$

where x_i, x_2... are the measured values of the predictive variables in the table (Secchi depth, pH, color, total P), and the b_i, b_2..., are the coefficients in the table. The constant is b_0. A score is calculated for each equation (reference and non-reference), and the site is assigned to the group for which it has the highest score.

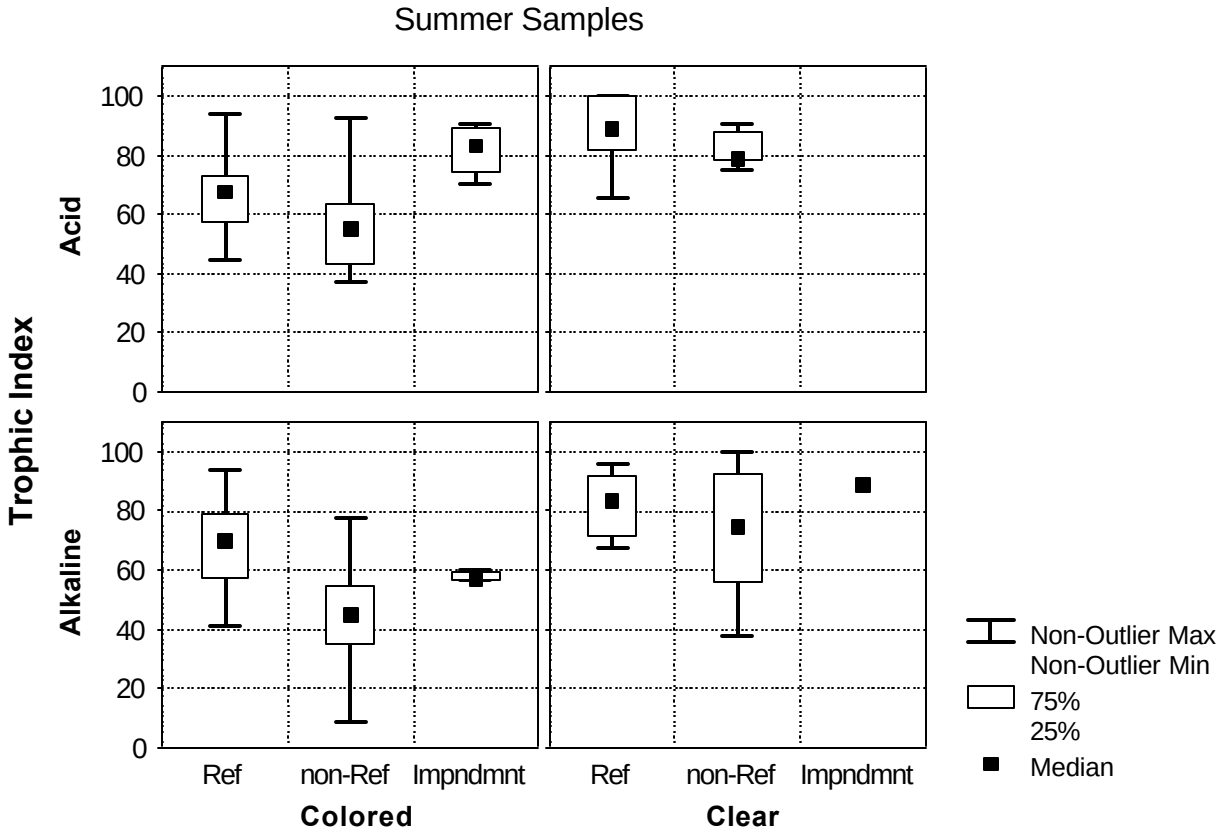


Figure 6-7. Performance of categorical trophic index in lake classes.

Table 6-5. DFA classification functions. F (4, 211) = 24.11; p < 0.000001.

	Coefficient	
Variable	**Reference**	**Non-reference**
pH (b_1)	5.0264	7.409
$\log_{10}$ (Secchi Depth, m) (b_2)	5.5636	2.7753
$\log_{10}$ (Color [PCU]) (b_3)	15.8413	14.5524
$\log_{10}$ (TP, mg/L) (b_4)	-22.7176	-21.8134
Constant (b_0)	-47.9945	-49.1482

The discriminant model correctly classified 80% of the natural lake sites. Since these were the summer data that were used to develop the models, they are not an independent test of model performance. We used the winter samples as an independent test, which resulted in 74% correct classification for 62 winter samples. This independent test was 73% correct overall for reference samples, and 78% correct for non-reference samples.

Although the discriminant model had good performance overall, its performance within each of the lake types was variable. For example, it identified all acid-clear lakes as reference, even the three non-reference lakes identified as such by the benthic indexes (Figs. 6-2, 6-3). Among acid-colored lakes, the discriminant model correctly classified only 44% of the non-reference lakes, and among alkaline-colored lakes, the model identified only 63% of reference lakes.

6.2.3 Index Performance

Performance of all the indexes is summarized in Tables 6-6 and 6-7, showing their abilities to discriminate *a priori* reference from non-reference samples. Table 6-6 summarizes the performance of the five indexes for reference and non-reference sites, and Table 6-7 breaks down the discrimination of non-reference lakes among the regions and lake types. It should be noted that some of the non-reference lakes were identified as non-reference merely because it was not known whether they met criteria for reference lakes. A better measure of index performance would be obtained by including only stressed lakes (from known stressors or sources of stress) in the non-reference group.

Index performance was not uniform across lake types. Although the two trophic indexes performed better overall than the invertebrate indexes (Fig. 6-6, Table 6-6), performance of each index was variable among lake types (Table 6-7). In general, the benthic invertebrate indexes performed better in clear lakes, and the trophic indexes performed better in colored lakes. Within clear lakes, the benthic index using 95th percentile scoring was best at discriminating non-reference lakes (Table 6-7).

Table 6-6. Summary of performance of indexes at classifying sites.

	Index	*A priori* Class	Index Assignment	
			Reference	Impaired
Benthic Indexes	5-3-1 scoring	Reference (148)	80.4%	19.6%
		Non-Reference (91)	62.6%	37.4%
		Impoundment (10)	80%	20%
	95%ile scoring	Reference (148)	72.3%	27.7%
		Non-Reference (91)	45.1%	54.9%
		Impoundment (10)	60%	40%
	Covariate index	Reference (148)	75%	25%
		Non-Reference (91)	50%	50%
		Impoundment (10)	50%	50%
Trophic Indexes	Chlorophyll-Secchi index	Reference (148)	71.6%	28.4%
		Non-Reference (91)	28.6%	71.4%
		Impoundment (10)	70%	30%
	Trophic discriminant function analysis	Reference (141)	83.5%	16.5%
		Non-Reference (80)	26.3%	73.7%
		Impoundment (10)	45%	55%

Table 6-7. Thresholds for assessing impairment and discrimination efficiencies (DE) of non-reference lakes only by 5 alternative lake indexes.

Index	Region 65 Acid-clear (3)		Region 75 Acid-clear (2)		Acid-col (20)		Alk-clr (16)		Alk-col (46)		Region 65 Total (12)	Region 75 Total (78)	Clear lakes (21)	Colored lakes (66)
	Thresh	DE	Thresh	DE	Thresh	DE	Thresh	DE	Thresh	DE	DE	DE	DE	DE
Benthic 5-3-1	2.33	67%	2.33	0	2.0	25%	2.5	69%	1.67	26%	50%	35%	62%	26%
Benthic 95	50.1	100%	43.7	0	28.7	35%	49.5	75%	27.6	52%	75%	51%	71%	47%
Benthic covariate[1]	35.3	100%	41.0	0	NA	45%	NA	63%	NA	72%	67%	63%	62%	64%
Trophic chl-Secchi	82.0	67%	75.0	0	57.8	65%	71.8	50%	57.5	83%	75%	71%	48%	77%
Trophic discriminant[2] **(n)**	NA	0(2)	NA	0(2)	NA	44%(18)	NA	80%(15)	NA	91%(43)	60%	76%	63%	77%

[1] Covariate index threshold applies only to regions

[2] Discriminant function model has no threshold

7.0 ASSOCIATION OF INDEX VALUES AND LAKE AND WATERSHED CONDITIONS

Conditions of lakes, and dynamics of lake processes, are strongly influenced by their catchment. Human activities directly affect the water flow, sediment, and loadings of various substances into lakes. Although most point-source discharges to lakes have been eliminated or reduced, non-point source (NPS) pollution, resulting from human activities in the watershed, may contribute substantial sediments, nutrients, and contaminants to a lake ecosystem. Non-point source pollution is primarily associated with land use: urban runoff, agricultural runoff, construction activity, suburban runoff, etc. The objective of the analyses reported in this chapter was to examine potential associations between land use in a lake catchment, and the condition of the biota in the lake.

Because of flat topography and extensive groundwater hydrologic connections in the Karst landscape of Florida, surface watersheds are not likely to reflect an actual catchment of a lake. Instead of delineating watersheds, we used a "proximity" approach, and defined buffer zones at 100 m, 500 m, and 1,000 m inland from the lake shore. Within each buffer, land use was characterized according to standard land use classes used by Florida DEP.

Correlation analysis of macroinvertebrate and water quality metrics with the percent of different land uses in buffers showed that the 500 m buffer was most likely to be associated with some measure of lake condition. For the analyses discussed in this chapter, we used only the 500 m buffer. Land use classes were aggregated into 4 major classes:

- Percent urban (including residential, commercial and industrial)
- Percent agriculture (row crops and feedlots, not including orchard and rangeland)
- Percent orchard (mostly citrus)
- Percent natural vegetation and silviculture and range.

Neither silviculture nor range had initial associations different from natural vegetation, and therefore they were aggregated with natural vegetation.

7.1 Benthic Macroinvertebrate Index

The associations between land use categories and lake condition were inconsistent among lake types (Figures 7-1, 7-2, 7-3, 7-4, 7-5). The only lake type with fairly strong associations with land use was acid clear lakes of ecoregion 65 (Figure 7-1). The LCI score increased with percent natural land use and decreased with agricultural and urban land use. No associations were apparent among the acid-clear lakes of Ecoregion 75, however, all lakes with complete data in this class were reference lakes (Figure 7-2). Neither acid colored nor alkaline clear

lakes exhibited any association between macroinvertebrate index value and land uses (Figures 7-3, 7-4). The LCI in alkaline-colored lakes showed a weak association with urban land use (Figure 7-5).

It should be noted that the percent agriculture in the 500 m buffers was quite low for most lakes (< 20%), and our analysis did not consider direct drainage from cropped areas to a lake, as has been noted for Lake Apopka (Lowe et al. 1999). This is especially true for the alkaline colored lakes, which are in heavily agricultural areas of Florida. We conclude that the question of effect of land use on lake condition cannot be resolved with fixed buffer zones, but must include more comprehensive watershed delineation for each lake. Examination of association between the trophic index (Fig. 6-5) and land use in the 500 m buffers also gave the same results: few visible associations (not shown).

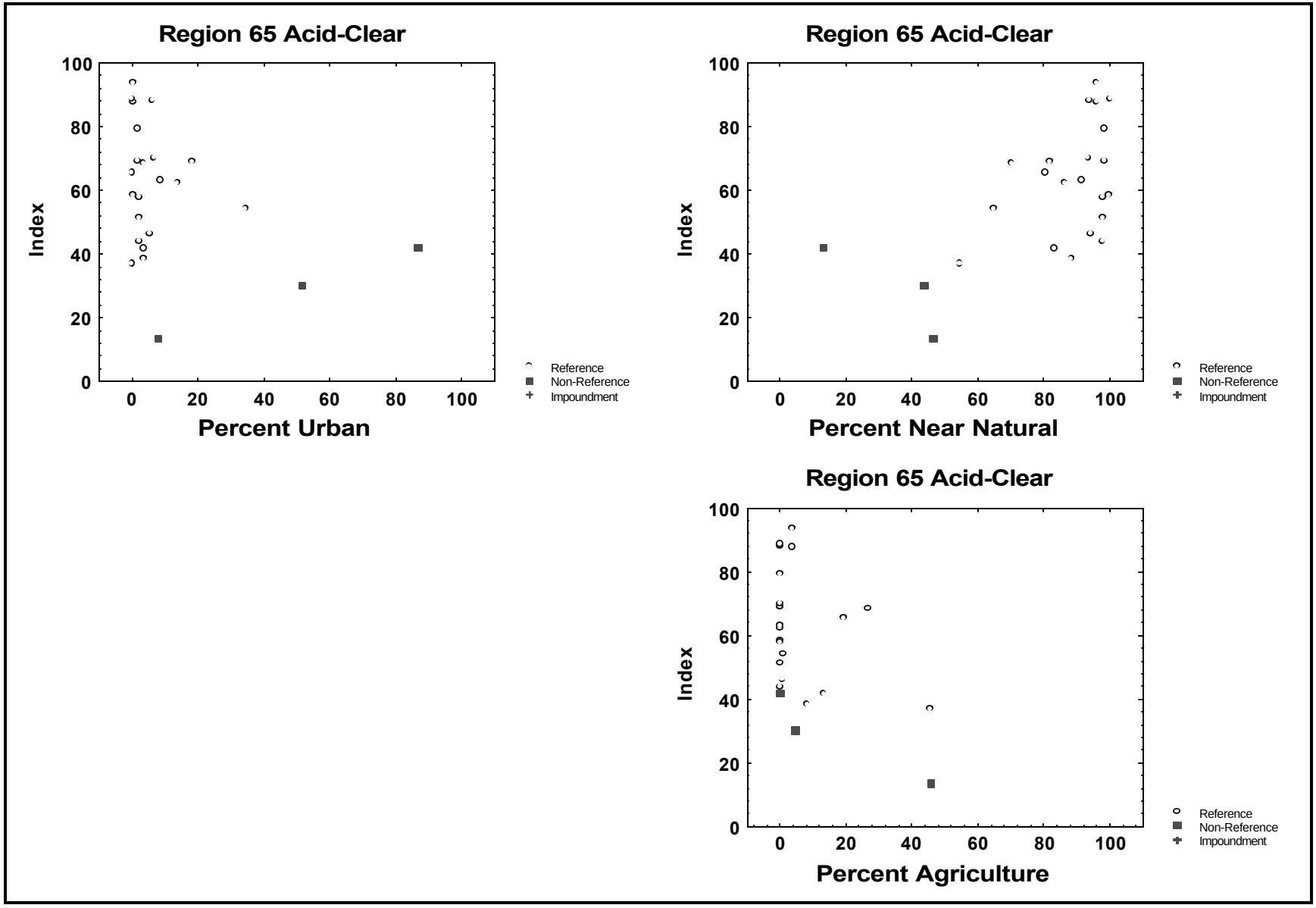


Figure 7-1. Benthic LCI scores and land use within 500 m of acid-clear lakes of Ecoregion 65.

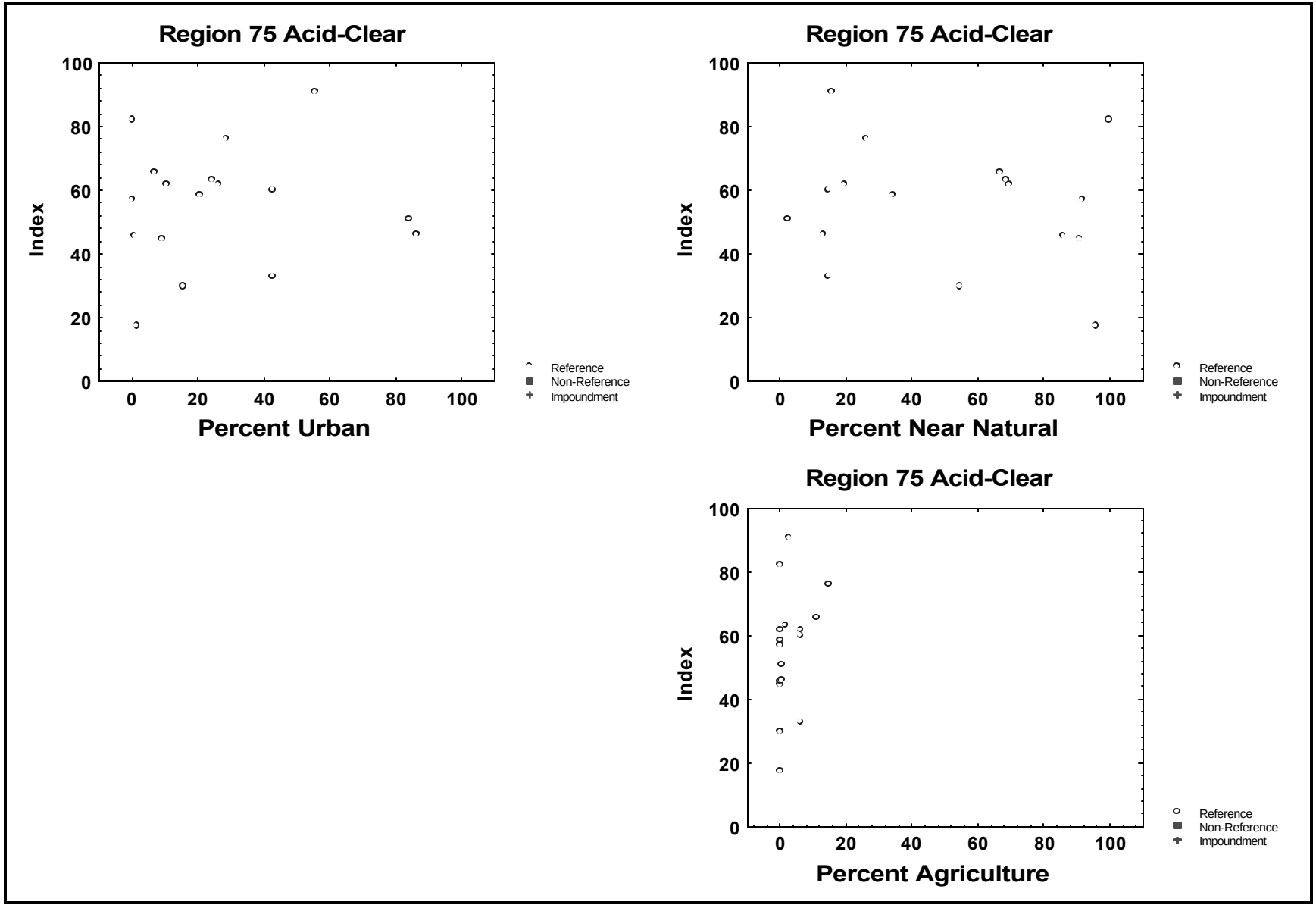


Figure 7-2. Benthic LCI scores and land use within 500 m acid-clear lakes of Ecoregion 75.

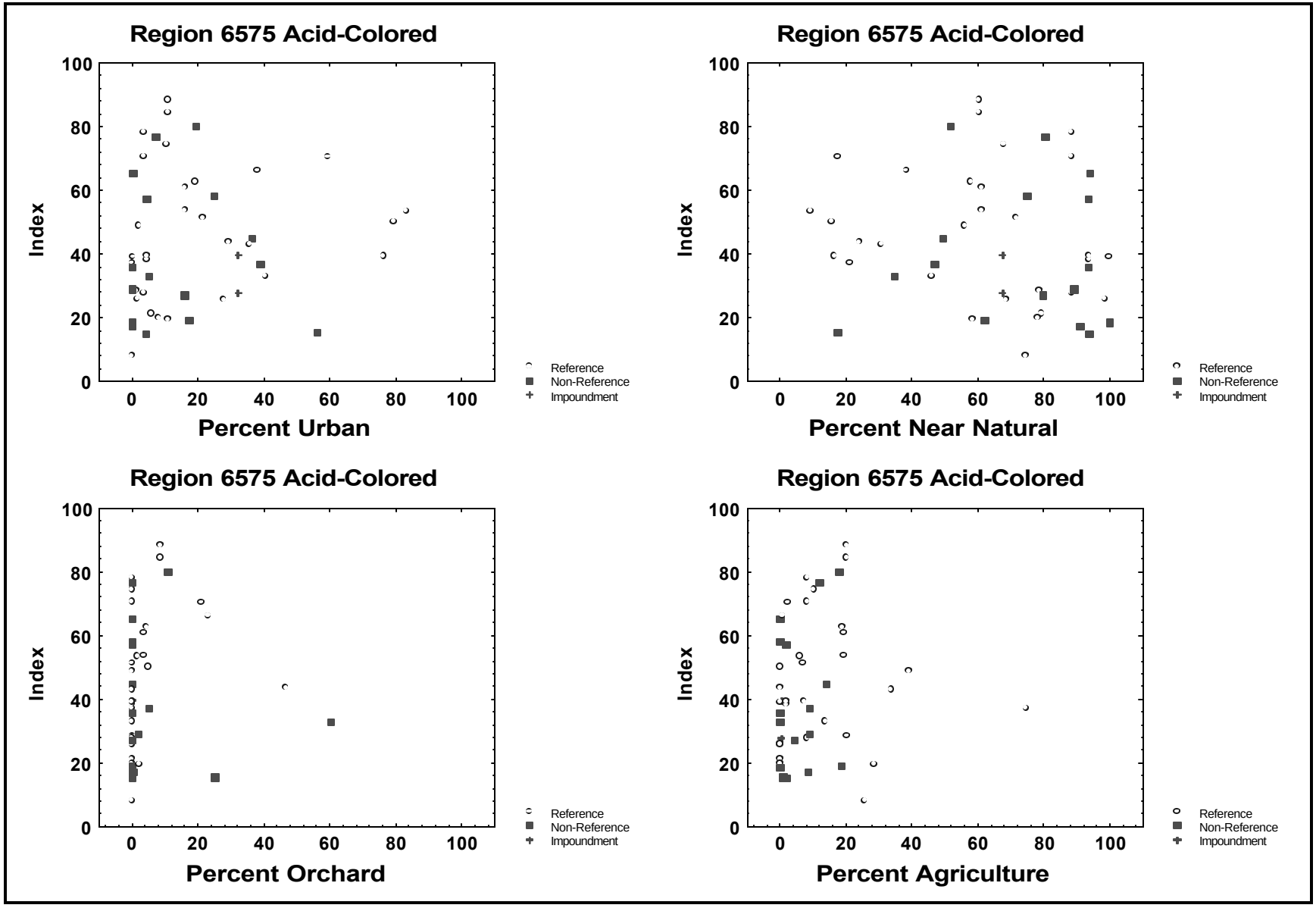


Figure 7-3. Benthic LCI scores and land use within 500 m of acid-colored lakes (both ecoregions).

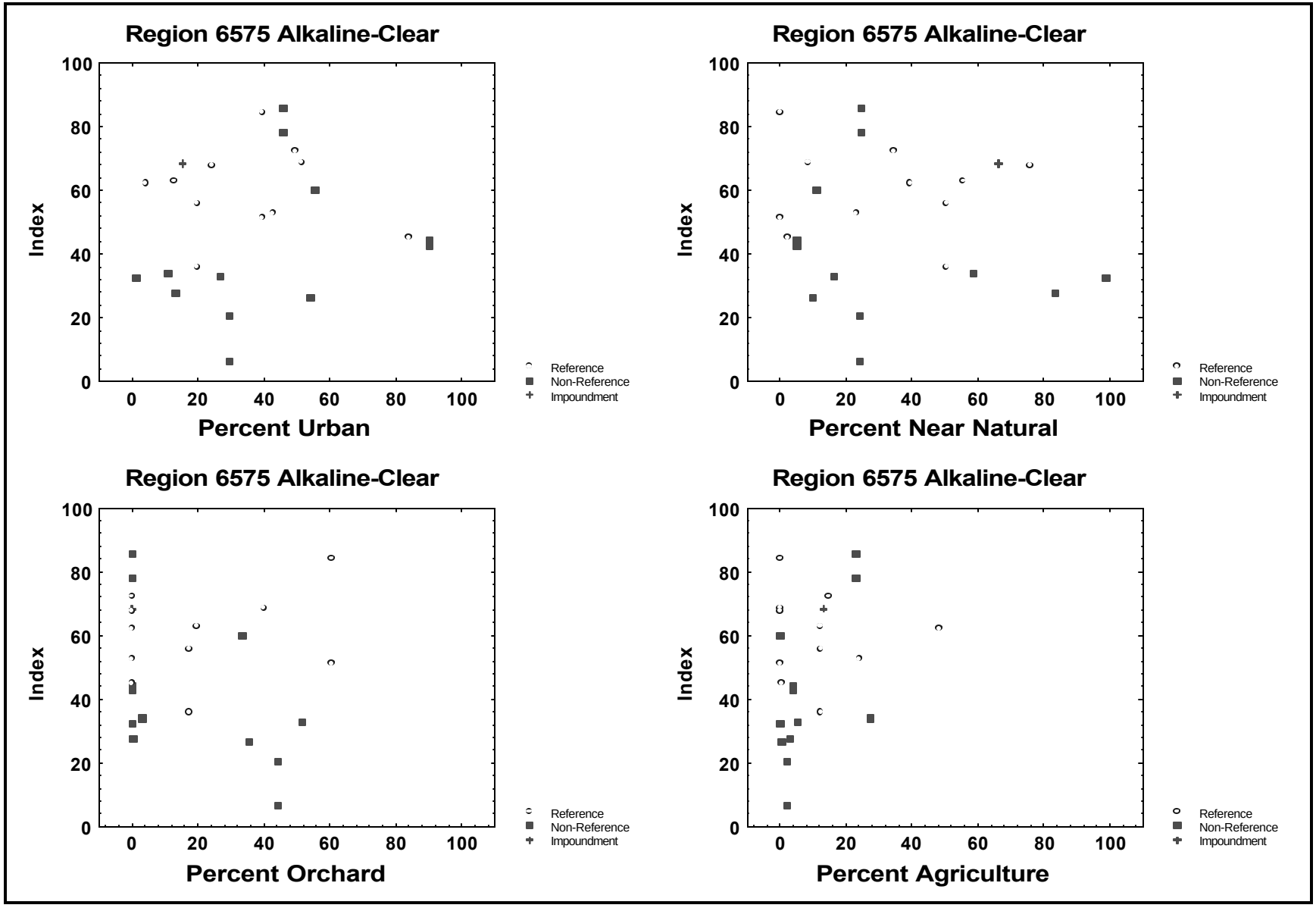


Figure 7-4. Benthic LCI scores and land use within 500 m of alkaline-clear lakes (both ecoregions).

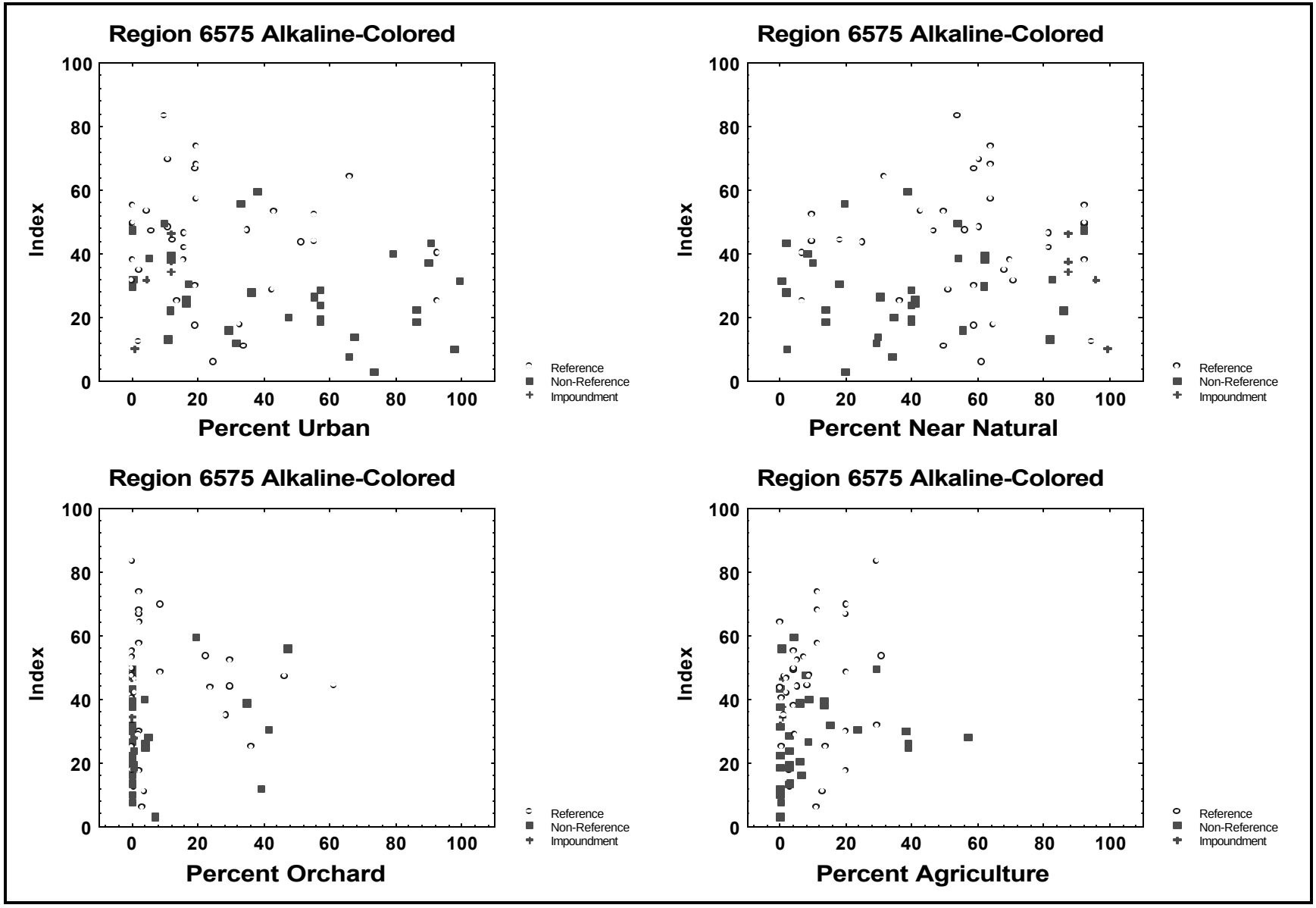


Figure 7-5. Benthic LCI scores and land use within 500 m of alkaline-colored lakes (both ecoregions).

8.0 CONCLUSIONS AND RECOMMENDATIONS

8.1 Lake Classification

The lake classification in this report was intended primarily for biological index development, however, it should apply equally well to eventual nutrient criteria development, and for other issues in lake management. The classification consists of 3 independent factors: water color, pH, and ecoregion. Water color and pH may be used as continuous covariates, or the lakes may be divided into colored and clear classes; and acid and alkaline classes, depending on convenience. The ecoregions are the Level 3 ecoregions (Omernik 1987) of the Southeast Plains (Ecoregion 65) and the Atlantic Coastal Plain (Ecoregion 75). The subtropical south Florida ecoregion was not considered in this report. On a practical basis, determined by the data set obtained by DEP, we have identified five lake types: acid-clear lakes of ecoregion 65, acid-clear lakes of ecoregion 75, acid-colored lakes, alkaline-clear lakes, and alkaline-colored lakes.

8.2 Lake Condition Indexes For Florida

This effort developed three benthic macroinvertebrate indexes of lake biological condition, and two water quality indexes of lake trophic condition. No single index was consistently able to discriminate reference lakes from non-reference lakes among all five lake types. However, the macroinvertebrate indexes were generally effective in clear lakes but not in highly colored lakes, and the trophic indexes were more reliable in the colored lakes, but not in the clear lakes.

We therefore recommend the use of two indexes to assess Florida lakes: the benthic macroinvertebrate index (categorical, using 95% metric scoring) for clear lakes throughout the state, and the chlorophyll-Secchi trophic index for colored lakes throughout the state. These two indexes were the most consistent and reliable within their respective lake types (Table 6-7), and each is relatively simple to apply. Discrimination efficiency is predicted to be 75% overall, and 75% within lake types (Table 6-7).

Nutrient enrichment and eutrophication remain the most widespread and most severe impairments of Florida lakes. Contamination by toxic substances is relatively less common. Development and calibration of a biotic index requires a data set that includes impaired sites, and the resultant index can only be a reliable indicator of the stressors present in the data set. Only one lake in the data set was known or suspected to have toxic contamination (Submarine Lake). The indexes developed are therefore only responsive to eutrophication, which was a common stressor in the data set.

As was shown in Chapter 5, lake benthic macroinvertebrate assemblages are strongly associated with water color and tranparency, with fewer taxa occurring in colored or turbid waters. The relatively depauperate assemblage in highly colored lakes may be the result of bottom habitat (organic muck), low DO in the sediment, or potential food sources (reduced abundance of algae among organic detritus). The practical consequence of a depauperate community for index development and assessment is that further reductions in taxa richness are difficult to distinguish from natural variability;

furthermore, many of the taxa in the organic highly colored lakes are tolerant (see Chapter 5).

Although benthic macroinvertebrates were not responsive to increased eutrophication in highly colored lakes, both chlorophyll and Secchi transparency were associated with non-reference status in the colored lakes. That they were less responsive in clear lakes suggests that their measurement is not precise enough in clear lakes; or that increased trophic state may manifest itself as changes in the benthic assemblage before it is detectable as increased chlorophyll or reduced transparency. For example, if an increase in production is rapidly cropped (as may occur in an oligotrophic lake), it may be observed as changes in the animal community, but the standing crop of algae (measured in chlorophyll or Secchi transparency) could remain unchanged.

The separation of "clear" from "colored" lakes is at 20 PCU. This demarcation is based on an optimal separation of macroinvertebrate species composition in the classification analysis (chapter 5). The division is somewhat arbitrary, first because measured color of lake water is not constant, and secondly because lakes near the 20 PCU color division may respond adequately for both benthic macroinvertebrate and trophic indexes. Thus, there may be an intermediate color range where either index would work well.

For mandated assessment purposes (i.e., 305(b), 303(d)), DEP could assign ordinal ratings to LCI scores. Four ordinal ratings, corresponding to very good, good, poor, and very poor, are shown for each of the two recommended LCIs (Figures 8-1, 8-2; Table 8-1). Lake types were kept separate for the benthic LCI for clear lakes, because metric values differed among the lake types (Fig. 8-1, Appendix B). Lake types were combined for the trophic LCI for colored lakes, because the two metrics (chlorophyll *a*, Secchi transparency) were not associated with water pH.

8.3 Recommendations

! Based on results outlined in this report, we recommend a trial adoption of two Lake Condition Indexes (LCIs) for Florida:
  - a benthic macroinvertebrate LCI for clear lakes (# 20 PCU), rated separately for the three clear lake types: acid-clear of ecoregion 65, acid-clear of ecoregion 75, and alkaline clear.
  - a trophic LCI for colored lakes (> 20 PCU).

  Each LCI is sensitive to anthropogenic stress (primarily eutrophication) in lake types in which the other index is not sensitive.

! The benthic LCI has not been calibrated adequately in the acid-clear lake types. Although there were sufficient reference sites, only five non-reference lakes were identified in the acid-clear category (both ecoregions). Response of the benthic LCI is uncertain, and needs to be examined with a larger set of stressed lakes. We recommend sampling a minimum of ten acid-clear, stressed lakes in each of ecoregions 65 and 75, and testing the benthic LCI with these.

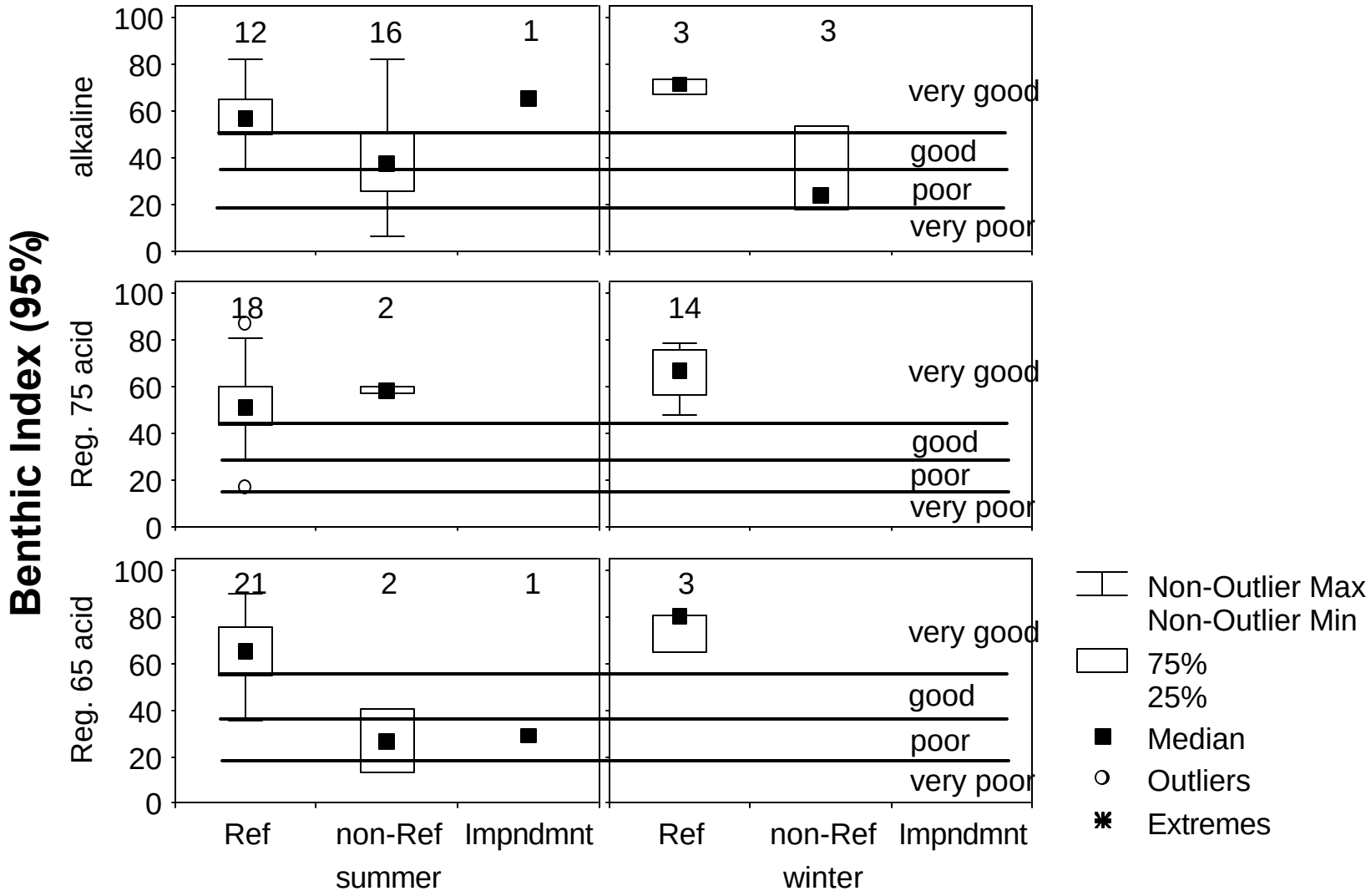


Figure 8-1. Recommended benthic index and lake ratings in clear lakes (# 20 PCU).

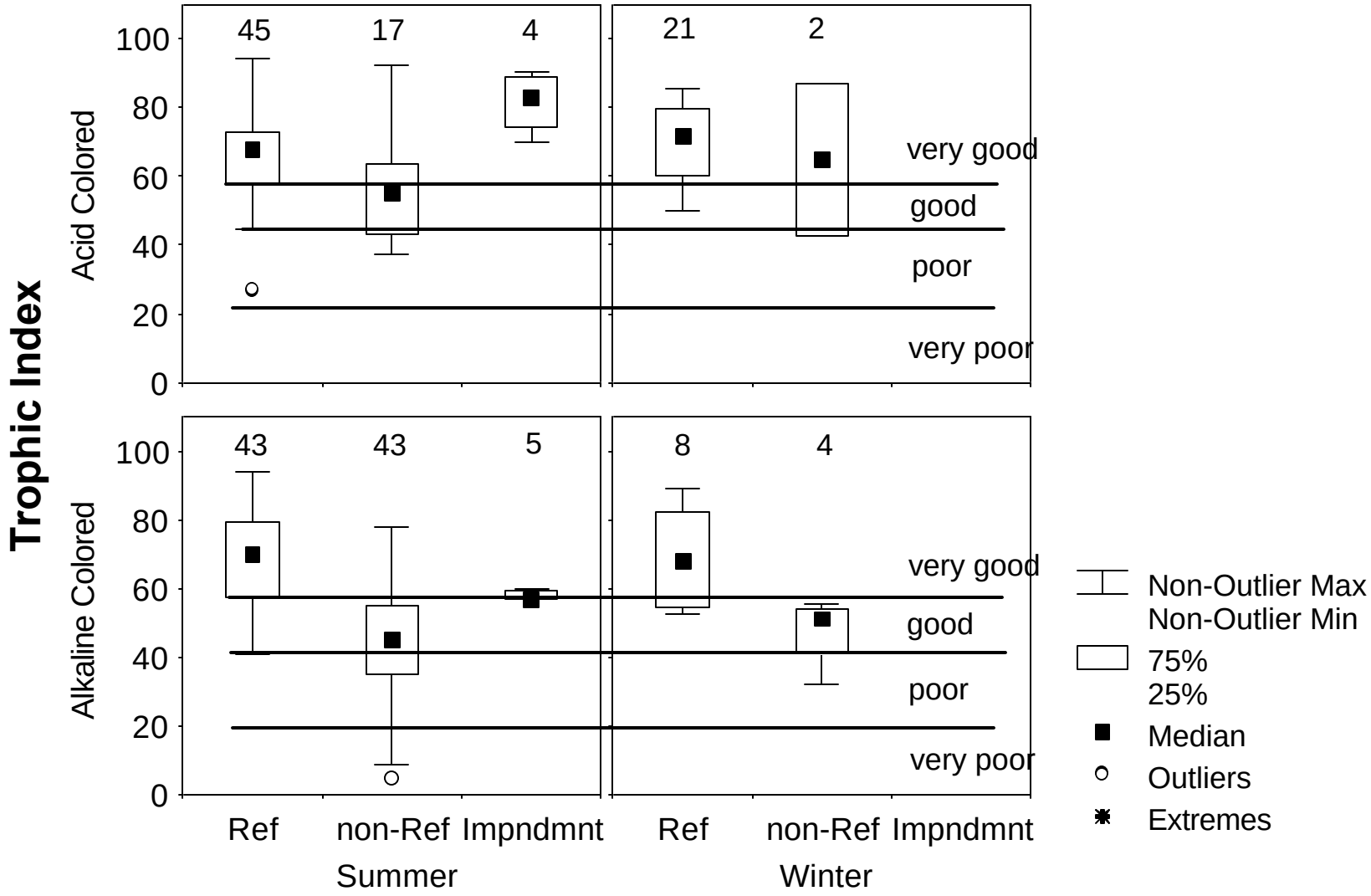


Figure 8-2. Recommended trophic index and lake ratings in colored lakes (> 20 PCU).

- The color division of 20 PCU was based on species composition and not on response of the LCI's. We recommend re-examination of the benthic LCI up to 80 PCU and the trophic LCI to 10 PCU to determine whether both indexes should be monitored in intermediate lakes; to examine the reliability of the indexes at the extremes of their effective ranges, and to determine the best way of assessing intermediate or variable lakes.

- Because the primary stress on Florida lakes is nutrient enrichment and eutrophication, the two LCIs can be used as primary response variables for determining nutrient criteria for Florida lakes. Traditional water quality measures, especially chlorophyll, are less sensitive than the benthic macroinvertebrates for detecting early changes due to nutrient enrichment in clear lakes. We recommend development of nutrient criteria using the two LCIs as the primary response variables to stress.

Table 8-1. Proposed LCI thresholds for 4 lake ratings.

	Benthic LCI Clear Lakes			**Trophic LCI Colored Lakes**	
	Region 65 Acid	**Region 75 Acid**	**Alkaline**	**Acid**	**Alkaline**
Very Good	$ 55	$ 44	$ 50	$ 58	$ 58
Good	$ 35	$ 30	$ 35	$ 44	$ 41
Poor	$ 18	$15	$ 18	$ 22	$ 20
Very Poor	< 18	< 15	< 18	< 22	< 20

9.0 REFERENCES

Barbour, M.T., and J. Gerritsen. 1996. Subsampling of benthic samples: A defense of the fixed-count method. J. N. Am. Benthol. Soc. 15:386-392.

Barbour, M.T., J. Gerritsen, B.D. Snyder, and J.B. Stribling. 1999. Rapid bioassessment protocols for use in streams and wadeable rivers: Periphyton, benthic macroinvertebrates and fish. Second Edition. EPA/841-B-99-002. U.S. EPA, Office of Water, Washington, D.C.

Barbour, M.T., J. Gerritsen, G.E. Griffith, R. Frydenborg, E. McCarron, J.S. White, and M.L. Bastian. 1996. A framework for biological criteria for Florida streams using benthic macroinvertebrates. J. N. Am. Benthol. Soc. 15:185-211.

Barbour, M.T., J. Gerritsen, and J.S. White. 1996. Development of the Stream Condition Index (SCI) for Florida. Prepared for Florida Dept. of Environmental Protection.

Canfield, D. E., and M. V. Hoyer. 1990. Managing lake eutrophication: The need for careful lake classification and assessment. 17-25. Enhancing States Lake Management Programs, 1989. North American Lake Management Society

Canfield, D.E., Jr. 1981. Chemical and trophic state characteristics of Florida lakes in relation to regional geology. Florida Cooperative Fish and Wildlife Unit.

Canfield, D.E., Jr., M.J. Maceina, L.M. Hodgson, and K.A. Langeland. 1983. Limnological features of some northwestern Florida lakes. J. Freshw. Ecol. 2: 67-79.

Davies, S.P., L. Tsomides, D.L. Courtemanch, and F. Drummond. 1993. Maine Biological Monitoring and Biocriteria Development Program. Maine Department of Environmental Protection, Bureau of Water Quality Control, Division of Environmental Evaluation and Lake Studies. Augusta, Maine.

DeShon, J. E. 1995. Development and application of the invertebrate community index (ICI). Pages 217-244 in W. S. Davis and T. P. Simon (editors). Biological assessment and criteria. Tools for water resource planning and decision making. Lewis Publishers, Boca Raton, Florida.

Duarte, C.M., S. Agusti and D.E. Canfield Jr. 1992. Patterns in phytoplankton community structure in Florida lakes. Limnol. Oceanogr. 37: 155-161.

Florida DER. 1993. SOP #EA-13. Sediment grain size - wet sieve method. Florida Department of Environmental Regulation, Biology Section, Tallahassee, FL.

Florida DEP. 1995. SOP #BA-28. Benthic macroinvertebrate composite grab sample collection for lakes. Florida Department of Environmental Protection, Biology Section, Tallahassee, FL.

Fore, L. S., J. R. Karr, and L. L. Conquest. 1994. Statistical properties of an index of biological integrity used to evaluate water resources. Can. J. Fish. Aquat. Sci. 51: 1077-1087.

Frydenborg, R. 1994. Lake bioassessments for the determination of non-point source impairment in Florida. Florida Department of Environmental Protection, Tallahassee, FL.

Garren, R.A., C. L. Clarkson, and T. L. Crisman. 1989. Chemistry of lakes in the Ocala National Forest, Florida. pp. 12-1 - 12-19 in D.F. Charles and D. R. Whitehead (eds.) Paleoecological Investigation of Recent Lake Acidification (PIRLA): 1983-85. EPRI EN-6526, Electric Power Research Institute, Palo Alto, CA.

Gerritsen, J. 1995. Additive biological indices for resource management. J. N. Am. Benthol. Soc. 14:451-457.

Gerritsen, J., M.T. Barbour, G.R. Gibson, R.H. Kennedy, E.H. Liu, S.A. Markowitz, P.J. Proctor, B.D. Snyder, and J.B. Stribling. 1998. Lake and reservoir bioassessment and biocriteria. Technical guidance document. Office of Water, USEPA, Washington, DC.

Gibson, G.R. (ed.), M.T. Barbour, J.B. Stribling, J. Gerritsen, and J.R. Karr. 1996. Biological criteria: Technical guidance for streams and small rivers. USEPA, Office of Science and Technology, Washington, DC. EPA 822-B-96-001.

Green, R. H., and R. C. Young. 1993. Sampling to detect rare species. Ecol. Applic. 3: 351-356.

Griffith, G. E., D. E. Canfield, Jr., C. A. Horsburgh, J. M. Omernik, and S. H. Azevedo. 1997. Florida lake regions. U. S. EPA, Corvallis, OR

Heck, K.L., Jr., G. van Belle, and D. Simberloff. 1975. Explicit calculation of the rarefaction diversity measurement and the determination of sufficient sample size. Ecology 56:1459-1461

Hendrickson, J. C. 1993. Development of a surface waters classification for evaluating ambient water quality in Northeast Florida. St. Johns River Water Mgmt. District

Hoyer, M.V., and D. E. Canfield, Jr. 1990. Limnological factors influencing bird abundance and species richness on Florida lakes. Lake Reserv. Manage. 6: 133-141.

Hughes, R.M., P. R. Kaufmann, A. T. Herlihy, T. M. Kincaid, L. Reynolds, and D. P. Larsen. 1998. A process for developing and evaluating indices of fish assemblage integrity. Can. J. Fish. Aquat. Sci. 55:1618-1631.

Hulbert, J.L. 1989. Benthic macroinvertebrates as water quality indication in Florida lakes. Florida Lake Management Conference, 1989.

Hulbert, J. L. 1990. A proposed lake condition index for Florida. 38th NABS mtg.

Hurlbert, S.H. 1971. The nonconcept of species diversity: A critique and alternative parameters. Ecology. 52: 577-586.

Hurlbert, S.H. 1984. Pseudoreplication and the design of ecological field experiments. Ecol. Monogr. 54: 187-211.

Jackson, D.A. 1993. Stopping rules in principal components analysis: A comparison of heuristical and statistical approaches. Ecology 74(8):2204-2214.

Jongman, R. H., C. J. F. terBraak, and O. F. R. vanTongeren. 1987. Data analysis in community and landscape ecology. Pudoc Wageningen Publishing, Netherlands.

Karr, J. R., K. D. Fausch, P. L. Angermeier, P. R. Yant, and I. J. Schlosser. 1986. Assessment of biological integrity in running waters: A method and its rationale. Illinois Natural History Survey, Champaign, Illinois. Special Publication 5.

Karr., J., E. Chu. 1999. Restoring life in running waters: Better biological monitoring, Island Press.

Lowe, E.F., L.E. Battoe, M. Coveney, and D. Stites. 1999. Setting water quality goals for restoration of Lake Apopka: Inferring past conditions. Lake Res. Manage. 15:103-120.

Ludwig, J.A., and J.F. Reynolds. 1988. Statistical ecology. John Wiley & Sons, New York, NY.

Maxted, J.R., M.T. Barbour, J. Gerritsen, V. Poretti, N. Primrose, A. Silvia, D. Penrose and R. Renfrow. 2000. A framework for assessing mid-Atlantic coastal plain streams using benthic macroinvertebrates. *Journal of the North American Benthological Society.* 19:128-144.

McCune, B., and M.J. Mefford. 1997. PC-ORD. Multivariate analysis of ecological data, Version 3.0. MjM Software Design, Gleneden Beach, Oregon, USA.

Ohio Environmental Protection Agency (Ohio EPA). 1987. Biological criteria for the protection of aquatic life: Volumes I-III. Ohio Environmental Protection Agency, Columbus, Ohio.

Payne, R.G. 1994. Biological Assessment of three northwest Florida lakes.

Payne, R. G. 1995. Biological assessment of Morris Lake, Topsail State Preserve, Walton County, Florida. Florida Department of Environmental Protection, Northwest District.

Payne, R.G. 1997. Report on the biological condition of nine northwest Florida lakes sampled summer 1996.

Payne, R.G. 1998. Report on the biological and chemical condition of four northwest Florida lakes sampled summer 1997 and winter 1998.

Rutter, R. P. 1995. A bioassessment of nine lakes in Highlands County, Florida. Florida Department of Environmental Protection.

Rutter, R. P. 1996. A bioassessment of five lakes in Highlands County and one lake in Collier County, Florida. Florida Department of Environmental Protection.

Rutter, R.P. 1997. A bioassessment of six lakes in Highlands County, Florida with emphasis on the macroinvertebrate fauna.

Rutter, R.P. 1998. A bioassessment of five lakes in Charlotte County, Florida with emphasis on the macroinvertebrate fauna.

Schulze, S. 1996. Biological assessments of four subtropical Florida lakes in different lake ecoregions. Florida Department of Environmental Protection, Southwest District, Tampa, FL

Shannon, E.E., and P. L. Brezonik. 1972. Limnological characteristics of north and central Florida lakes. Limnol. Oceanogr. 17: 97-110.

Snedecor, G. W., and W. G. Cochran. 1967. Statistical methods. Iowa State University Press, Ames, Iowa.

Statsoft, Inc. 1995. STATISTICA for Windows 5.0. Statsoft, Inc., Tulsa, OK.

Stenson, J.A.E. 1981. The role of predation in the evolution of morphology, behaviour and life history of two species of *Chaoborus*. Oikos 37:323-327.

Vinson, M. R., and C. P. Hawkins. 1996. Effects of sampling area and subsampling procedure on comparisons of taxa richness among streams. J. N. Am Benthol. Soc. 15: 392-399.

von Ende, C.N. 1979. Fish predation, interspecific predation, and the distribution of two *Chaoborus* species. Ecology 60(1):119-128.

Wonacott, T.H. and R.J. Wonacott. 1972. Introductory statistics. Second edition. John Wiley & Sons, New York.

APPENDIX A

Lakes sampled 1993-1997

Lake Name	Status	County	Region	Sites
ALLIGATOR LAKE	Non-Reference	Osceola		1
Big Blue Lake	Reference	Washington		1
BRICK LAKE	Non-Reference	Osceola		1
CENTER LAKE	Non-Reference	Osceola		1
CHERRY LAKE LAKE	Reference	Lake		1
County Camp Pond	Non-Reference	Madison		1
Court Martial Lake	Reference	Bay		1
Dixie Lake	Reference	Lake		2
EMMA LAKE	Non-Reference	Lake		1
Hammond Lake	Reference	Lake		2
HOWELL LAKE	Non-Reference	Seminole		1
LAFAYETTE LAKE	Reference	Leon		1
LAKE BONNET	Non-Reference	Highlands	75	2
LAKE BONNET	Reference	Polk		1
Lake Buffum	Reference	Polk		2
Lake Gifford	Non-Reference	Orange		1
Lake Juliana	Reference	Polk		1
Lake Lelia	Reference	Highlands	75	2
Lake McBride	Reference	Leon		1
Lake Rexford	Reference	Orange		1
Lake Submarine	Non-Reference	Highlands	75	2
Lake Webb	Non-Reference	Charlotte		2
Little Lake	Reference	Highlands		2
Little Orange Lake	Non-Reference	Alachua		1
Open Lake	Reference	Washington		1
Piney Z Lake	Non-Reference	Leon		1
Rattlesnake Lake	Reference	Washington		1
Red Beach Lake	Reference	Highlands		2
Sand Hammock Pond	Non-Reference	Holmes		1
TAMPA BAY	Non-Reference	Pinellas		1
WILSON LAKE	Non-Reference	Osceola		1
BEAR LAKE	Impoundment	Santa Rosa	6501	1
CRESCENT LAKE	Impoundment	Escambia	6501	2
CRESCENT LAKE	Impoundment	Lake	6501	1
FORTY ACRE POND	Reference	Santa Rosa	6501	1
Hurricane Lake	Impoundment	Okaloosa	6501	1
KARICK LAKE	Non-Reference	Okaloosa	6501	1
BLUE LAKE	Reference	Washington	6502	1
BLUE POND	Reference	Walton	6502	1
DOUBLE POND	Reference	Holmes	6502	1

Lake Name	Status	County	Region	Sites
JACKSON LAKE	Non-Reference	Walton	6502	1
JUNIPER LAKE	Impoundment	Walton	6502	2
KINGS LAKE	Impoundment	Walton	6502	1
LAKE CASSIDY	Reference	Holmes	6502	3
Lake Defuniak	Non-Reference	Walton	6502	1
OCHEESEE POND	Reference	Jackson	6502	1
PATE POND	Reference	Washington	6502	7
COMPASS LAKE	Reference	Jackson	6503	1
CRYSTAL LAKE	Reference	Washington	6503	1
DUNFORD LAKE	Reference	Washington	6503	2
GAP LAKE	Reference	Washington	6503	2
MAJOR LAKE	Reference	Washington	6503	2
MULEHEAD POND	Reference	Calhoun	6503	1
OWENS Lake	Reference	Washington	6503	1
PORTER LAKE	Reference	Washington	6503	1
SEVENTEEN MILE POND	Reference	Jackson	6503	1
TURKEY PEN LAKE	Reference	Calhoun	6503	1
A.J. HENRY LAKE	Non-Reference	Leon	6504	1
Cherry Lake	Reference	Madison	6504	1
LAKE ERIE	Reference	Leon	6504	1
Lake Iammonia	Non-Reference	Leon	6504	1
Lake Iammonia	Reference	Leon	6504	2
LAKE LOGAN	Reference	Madison	6504	1
LAKE MYSTIC	Reference	Madison	6504	1
MICCOSUKEE LAKE	Reference	Jefferson	6504	3
TALQUIN LAKE	Impoundment	Gadsden	6504	3
SILVER LAKE	Reference	Leon	6505	1
ALLIGATOR LAKE	Non-Reference	Columbia	6506	1
BIVENS ARM	Non-Reference	Alachua	6506	1
LAKE ALCYON	Reference	Hamilton	6506	1
LAKE HAMBURG	Non-Reference	Columbia	6506	1
LAKE JEFFERY	Reference	Columbia	6506	1
LAKE OCTAHATCHEE	Reference	Hamilton	6506	1
LOW LAKE	Reference	Suwannee	6506	1
WATERTOWN LAKE	Non-Reference	Columbia	6506	1
ANDREWS LAKE	Reference	Taylor	7501	1
CAMPBELL POND	Reference	Walton	7501	1
LAKE ADAMS	Reference	Lafayette	7501	1
LAKE BRADFORD	Reference	Leon	7501	1
LAKE ELLEN	Reference	Wakulla	7501	1

Lake Name	Status	County	Region	Sites
LAKE FORT ATKINSON	Reference	Lafayette	7501	1
Lake MUNSON	Non-Reference	Leon	7501	2
MORRIS LAKE	Reference	Walton	7501	2
OTTER LAKE	Reference	Wakulla	7501	1
OYSTER POND	Non-Reference	Walton	7501	1
PICKETT LAKE	Reference	Lafayette	7501	1
TOWNSEND POND	Non-Reference	Lafayette	7501	1
WATERS LAKE	Reference	Gilchrist	7501	1
LAKE PALESTINE	Reference	Union	7502	1
OCEAN POND	Reference	Baker	7502	4
SWIFT CREEK POND	Reference	Union	7502	1
ALTHO LAKE	Reference	Alachua	7503	1
CROSBY LAKE	Reference	Bradford	7503	1
HAMPTON LAKE	Reference	Bradford	7503	1
LITTLE LAKE SANTA FE	Non-Reference	Alachua	7503	1
LITTLE LAKE WEIR	Reference	Marion	7503	1
ROWELL LAKE	Non-Reference	Bradford	7503	1
GEORGES LAKE	Reference	Putnam	7504	1
KINGSLEY LAKE	Non-Reference	Clay	7504	1
LAKE JOHNSON	Reference	Clay	7504	1
LAKE LOWERY	Reference	Clay	7504	1
LAKE LOWERY	Reference	Polk	7504	1
LAKE LOYAL	Reference	Putnam	7504	1
MAGNOLIA LAKE	Reference	Clay	7504	1
SHEELAR LAKE	Reference	Clay	7504	1
EATON LAKE	Reference	Marion	7508	1
HALFMOON LAKE	Non-Reference	Marion	7508	1
Lake Jumper	Non-Reference	Marion	7508	1
Lake Panasofkee	Reference	Sumter	7508	2
LAKE WAUBERG	Non-Reference	Alachua	7508	1
LOU LAKE	Non-Reference	Marion	7508	1
NEWNANS LAKE	Non-Reference	Alachua	7508	1
TROUT LAKE	Non-Reference	Lake	7508	1
GRASSHOPPER LAKE	Reference	Lake	7509	1
LAKE DELANCY	Reference	Marion	7509	1
Lake Kerr	Reference	Marion	7509	1
LAKE SELLERS	Reference	Lake	7509	2
LAKE SELLERS	Reference	Marion	7509	3
WILDCAT LAKE	Reference	Lake	7509	1
ASHBY LAKE	Non-Reference	Volusia	7510	1

Lake Name	Status	County	Region	Sites
ASHBY LAKE	Reference	Volusia	7510	1
BLUE CYPRESS LAKE	Reference	Indian River	7510	5
DISSTON LAKE	Reference	Flagler	7510	3
DORR LAKE	Reference	Lake	7510	1
GORE LAKE	Reference	Flagler	7510	1
HARNEY LAKE	Non-Reference	Volusia	7510	2
Lake Dias	Non-Reference	Volusia	7510	1
LAKE MARGARET	Reference	Putnam	7510	1
SOUTH LAKE	Non-Reference	Brevard	7510	2
LAKE BROWARD	Reference	Putnam	7511	1
LAKE STELLA	Non-Reference	Putnam	7511	2
LAKE FORT COOPER	Non-Reference	Citrus	7512	1
TSALA APOPKA LAKE	Reference	Citrus	7512	6
CLEAR LAKE	Non-Reference	Pasco	7513	1
GENEVA LAKE	Reference	Hernando	7513	1
LAKE IOLA	Reference	Pasco	7513	1
LAKE KING	Reference	Pasco	7513	1
LAKE LINDSEY	Reference	Hernando	7513	1
LAKE MIDDLE	Non-Reference	Pasco	7513	1
SPARKMAN LAKE	Reference	Hernando	7513	1
LAKE DALHOUSIE	Reference	Lake	7515	1
LAKE SEMINARY	Reference	Seminole	7516	2
LAKE TOOKE	Reference	Hernando	7517	2
MOON LAKE	Non-Reference	Pasco	7517	1
BIG GANT LAKE	Non-Reference	Sumter	7518	1
LAKE MINNEOLA	Reference	Lake	7519	6
LAKE BUTLER	Non-Reference	Orange	7520	1
CONWAY LAKE	Non-Reference	Orange	7521	2
LAKE HOWELL	Non-Reference	Seminole	7521	1
Lake Kilarney	Non-Reference	Orange	7521	1
LAKE MAITLAND	Reference	Orange	7521	2
LAKE ORIENTA	Non-Reference	Seminole	7521	2
TARPON LAKE	Non-Reference	Pinellas	7522	4
Keystone Lake/Tampa Bay Basin	Reference	Hillsborough	7523	1
LAKE ALICE	Reference	Hillsborough	7523	3
LAKE HIAWATHA	Reference	Hillsborough	7523	1
BELLOWS LAKE	Non-Reference	Hillsborough	7525	1
LAKE MANGO	Non-Reference	Hillsborough	7525	1
THONOTOSASSA LAKE	Non-Reference	Hillsborough	7525	1

Lake Name	Status	County	Region	Sites
EAST TOHOPEKALIGA LAKE	Reference	Osceola	7527	3
GENTRY LAKE	Reference	Osceola	7527	4
HART LAKE	Reference	Orange	7527	2
LAKE NONA	Reference	Orange	7527	1
MARY JANE LAKE	Non-Reference	Orange	7527	1
MUD LAKE	Non-Reference	Orange	7527	1
LAKE CHAUTAUQUA	Reference	Pinellas	7528	1
HOLLINGSWORTH LAKE	Non-Reference	Polk	7530	1
LAKE BONNY	Non-Reference	Polk	7530	1
LAKE CONINE	Non-Reference	Polk	7531	1
LAKE HARTRIDGE	Reference	Polk	7531	1
LAKE MARIANNA	Non-Reference	Polk	7531	1
CROOKED LAKE	Reference	Polk	7532	2
LAKE CLINCH	Reference	Polk	7532	3
REEDY LAKE	Non-Reference	Polk	7532	2
ANNIE LAKE	Reference	Highlands	7533	2
CLAY LAKE	Reference	Highlands	7533	2
DINNER LAKE	Reference	Highlands	7533	2
FRANCIS LAKE	Non-Reference	Madison	7533	2
FRANCIS LAKE	Reference	Highlands	7533	1
GRASSY LAKE	Reference	Highlands	7533	1
HUNTLEY LAKE	Reference	Highlands	7533	2
Lake Denton	Non-Reference	Highlands	7533	2
Lake Rachard	Non-Reference	Highlands	7533	2
LAKE VIOLA	Reference	Highlands	7533	3
LOTELA LAKE	Reference	Highlands	7533	2
CHARLOTTE LAKE	Reference	Highlands	7534	2
JOSEPHINE LAKE	Non-Reference	Highlands	7534	2
Lake Apthorpe	Reference	Highlands	7534	2
LAKE CARRIE	Non-Reference	Highlands	7534	1
Lake Glenada	Non-Reference	Highlands	7534	2
LAKE HILL	Reference	Highlands	7534	1
LETTA LAKE	Reference	Highlands	7534	2
LITTLE REDWATER LAKE	Reference	Highlands	7534	2
LTL JACKSON LAKE	Non-Reference	Highlands	7534	2
WOLF LAKE	Reference	Highlands	7534	2
ARBUCKLE LAKE	Reference	Polk	7535	1
CYPRESS LAKE	Non-Reference	Osceola	7535	2
FISH LAKE	Non-Reference	Osceola	7535	1

Lake Name	Status	County	Region	Sites
MARION LAKE	Non-Reference	Osceola	7535	1
TOHOPEKALIGA LAKE	Non-Reference	Osceola	7535	2
GIBSON LAKE	Reference	Polk	7536	1
LAKE GARFIELD	Reference	Polk	7536	1
LAKE HAINES	Non-Reference	Polk	7536	1
Lake Henry	Non-Reference	Polk	7536	1
LAKE LIVINGSTON	Non-Reference	Polk	7536	2
LAKE MATTIE	Reference	Polk	7536	4
LIZZIE LAKE	Reference	Polk	7536	1
TRAFFORD LAKE	Non-Reference	Collier	7537	1

APPENDIX B

Discriminatory Ability of Metrics from Florida Lakes

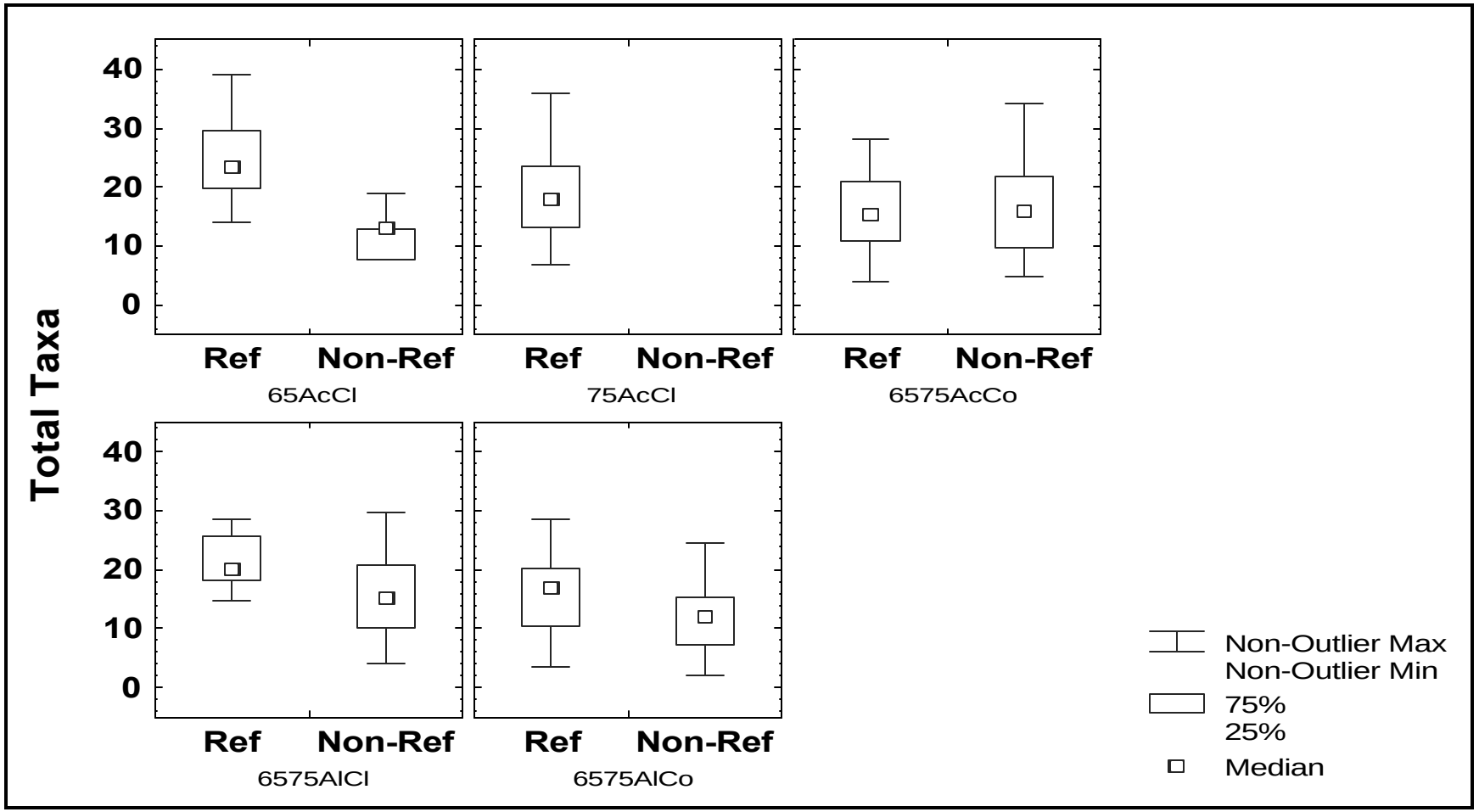


Figure B-1. Metric responses by ecoregion and lake type. AcCl = Acid-Clear; AcCo = Acid-Colored; AlCl = Alkaline-Clear; AlCo = Alkaline-Colored.

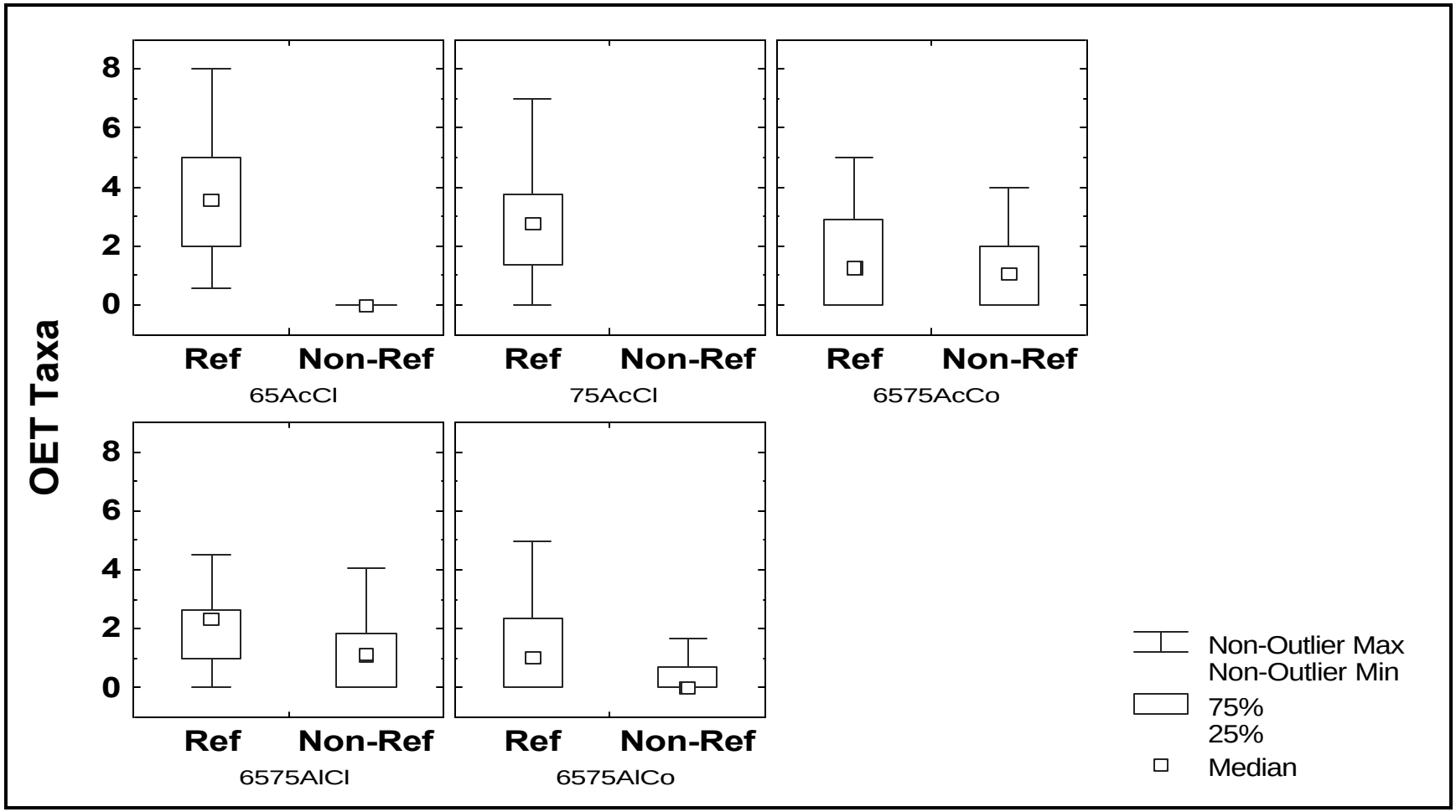


Figure B-1 (continued). Metric responses by ecoregion and lake type. AcCl = Acid-Clear; AcCo = Acid-Colored; AlCl = Alkaline-Clear; AlCo = Alkaline-Colored.

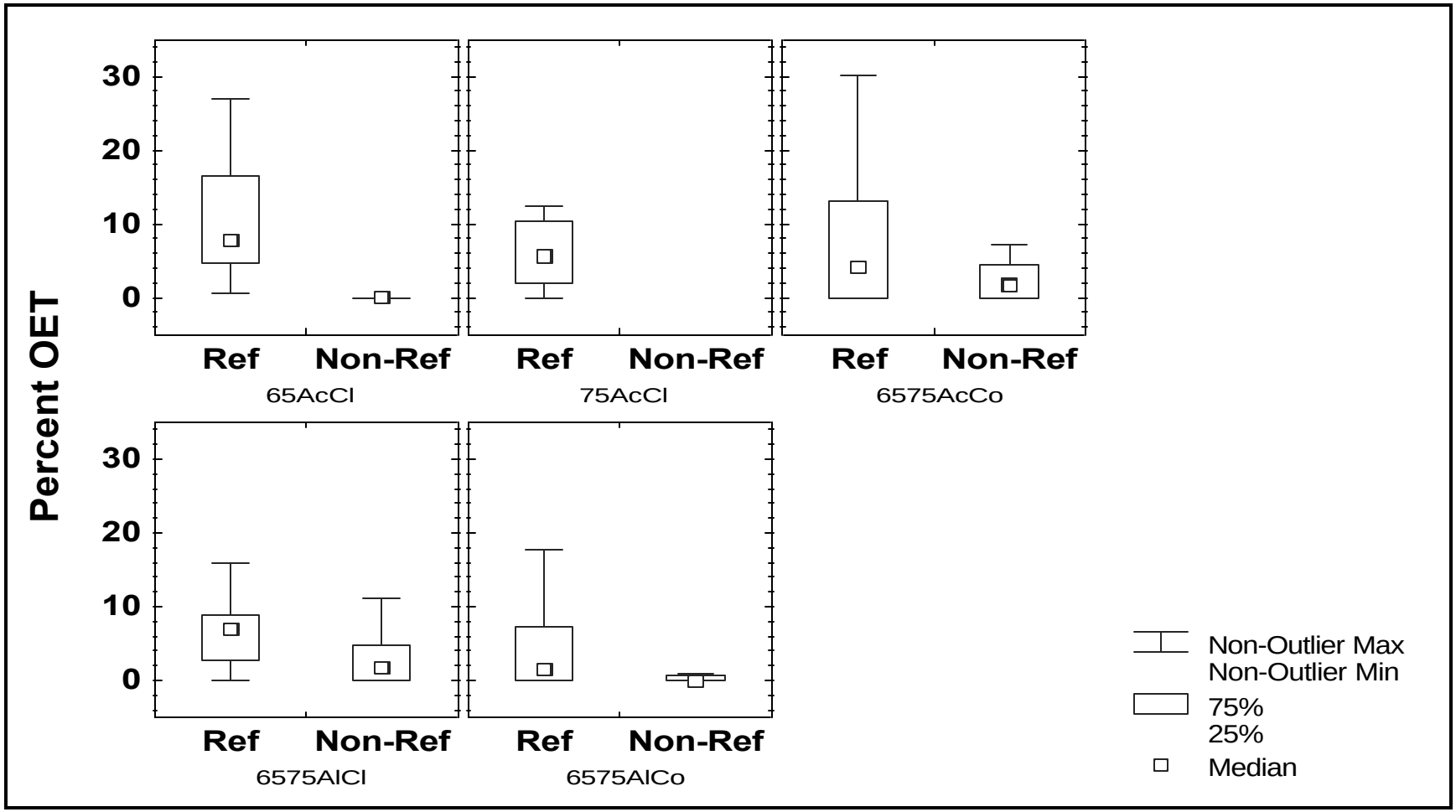


Figure B-1 (continued). Metric responses by ecoregion and lake type. AcCl = Acid-Clear; AcCo = Acid-Colored; AlCl = Alkaline-Clear; AlCo = Alkaline-Colored.

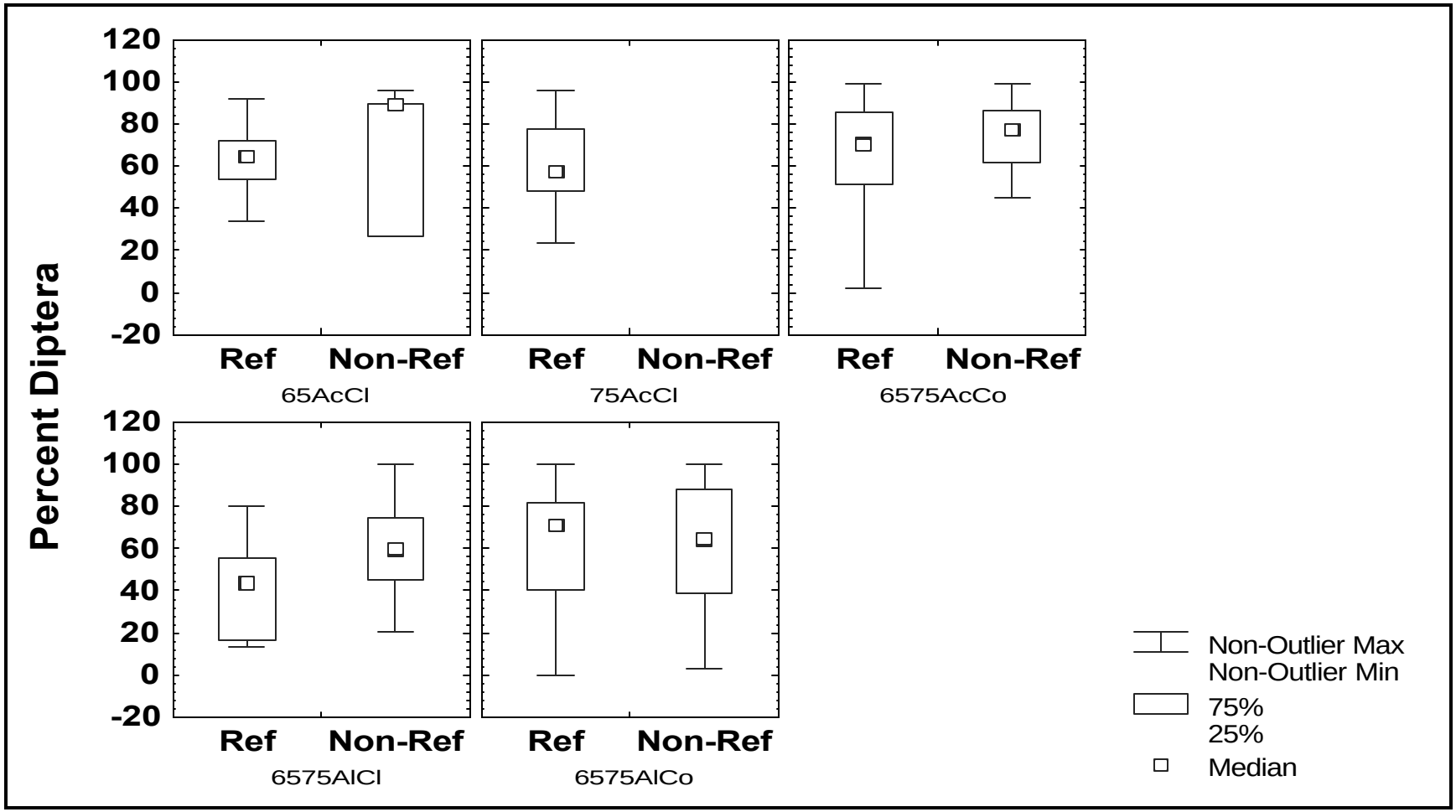


Figure B-1 (continued). Metric responses by ecoregion and lake type. AcCl = Acid-Clear; AcCo = Acid-Colored; AlCl = Alkaline-Clear; AlCo = Alkaline-Colored.

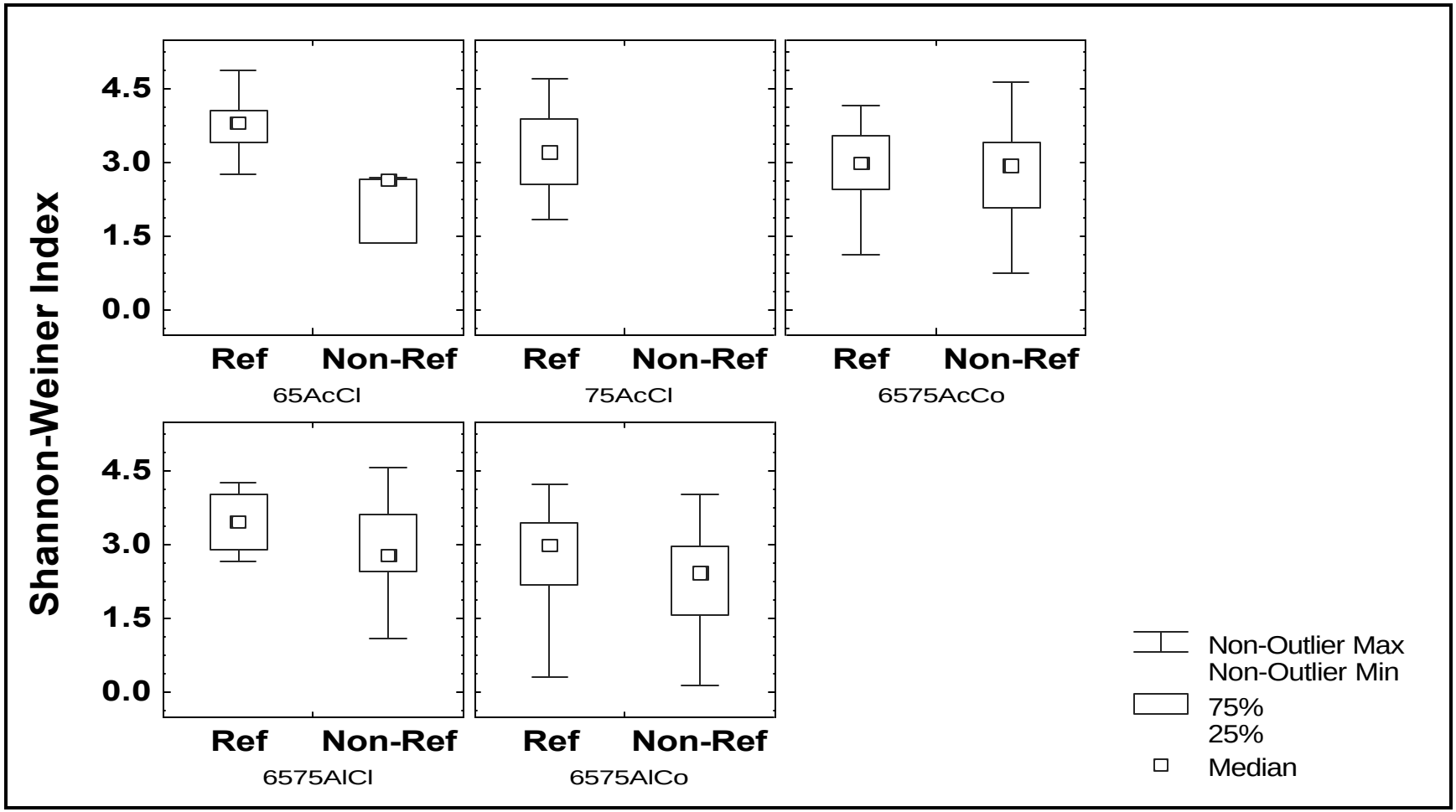


Figure B-1 (continued). Metric responses by ecoregion and lake type. AcCl = Acid-Clear; AcCo = Acid-Colored; AlCl = Alkaline-Clear; AlCo = Alkaline-Colored.

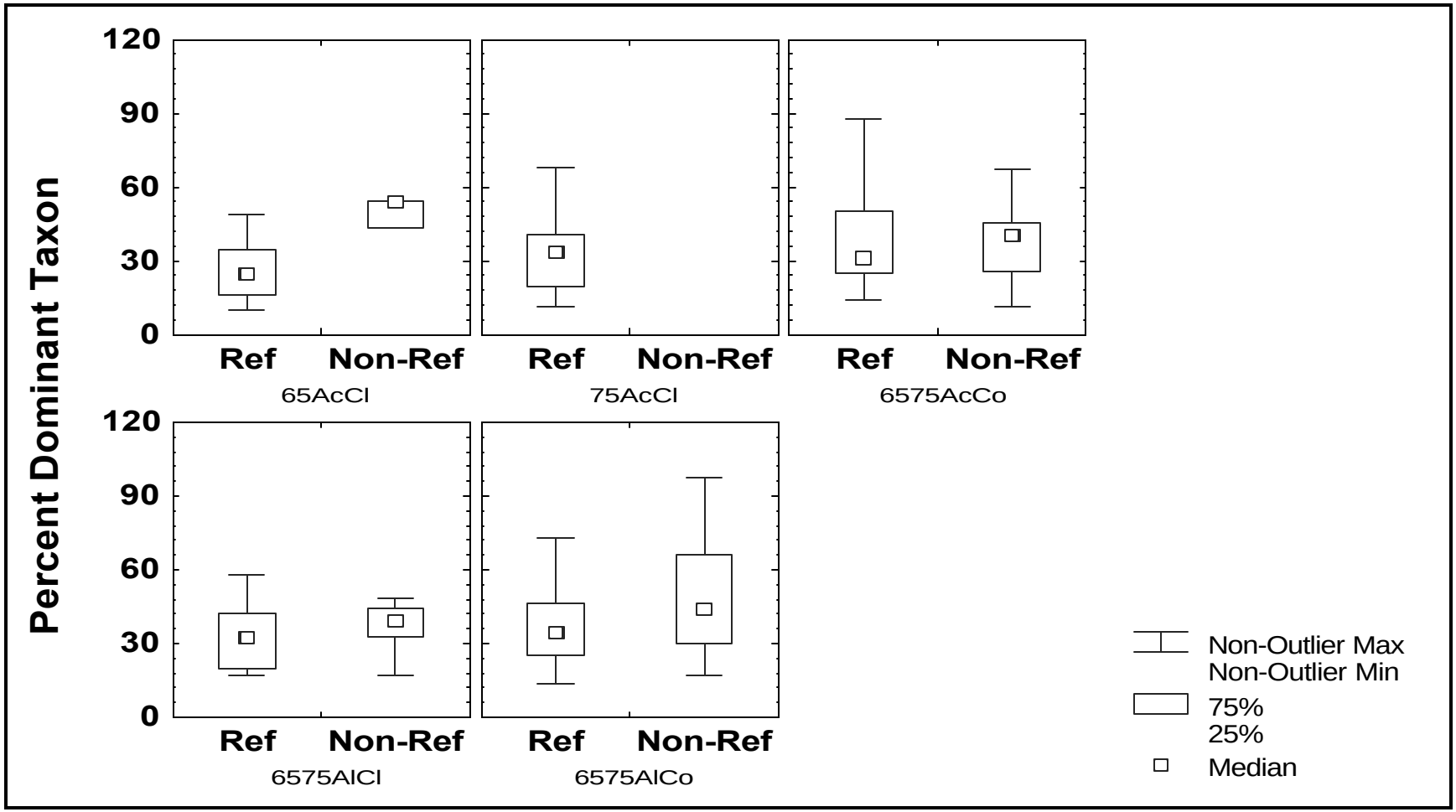


Figure B-1 (continued). Metric responses by ecoregion and lake type. AcCl = Acid-Clear; AcCo = Acid-Colored; AlCl = Alkaline-Clear; AlCo = Alkaline-Colored.

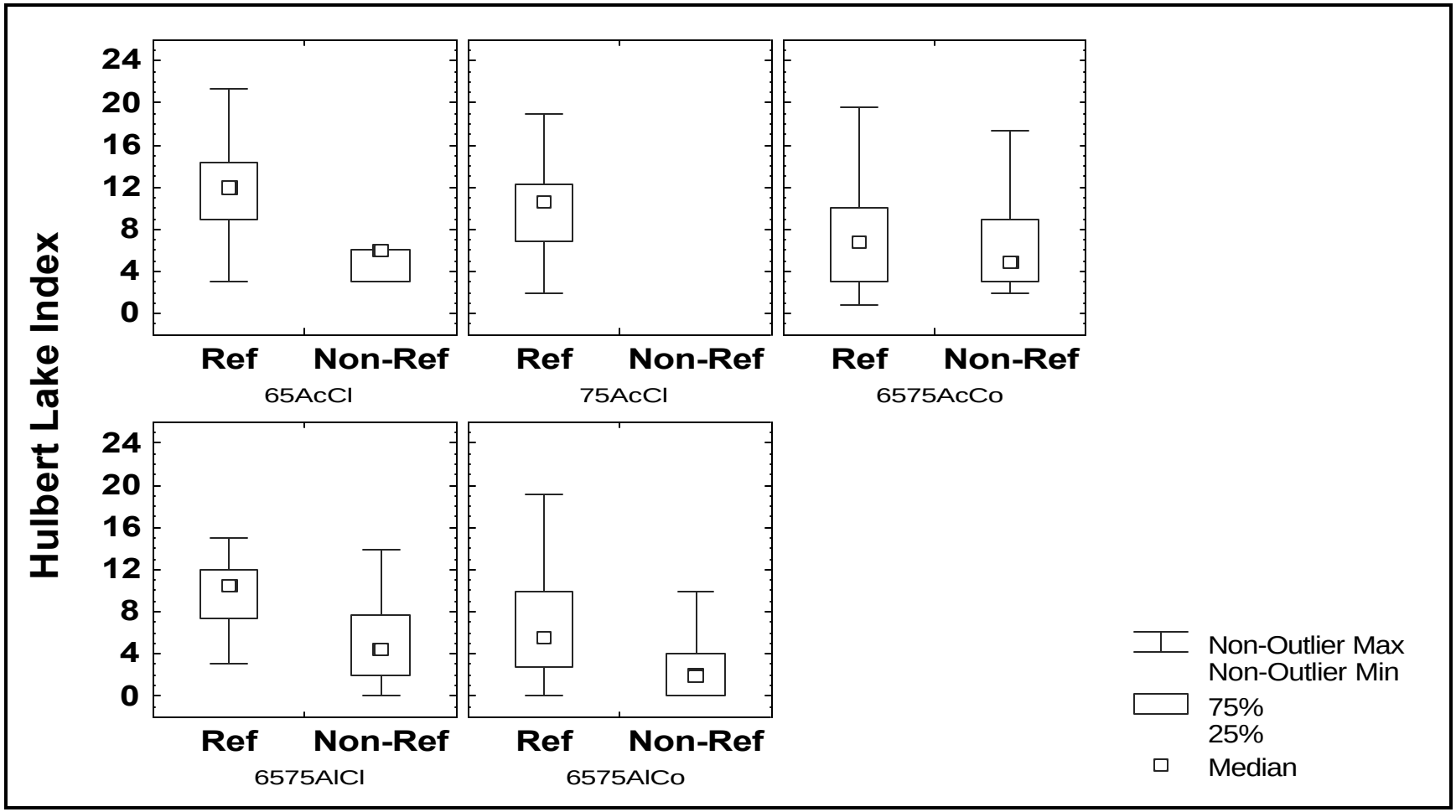


Figure B-1 (continued). Metric responses by ecoregion and lake type. AcCl = Acid-Clear; AcCo = Acid-Colored; AlCl = Alkaline-Clear; AlCo = Alkaline-Colored.

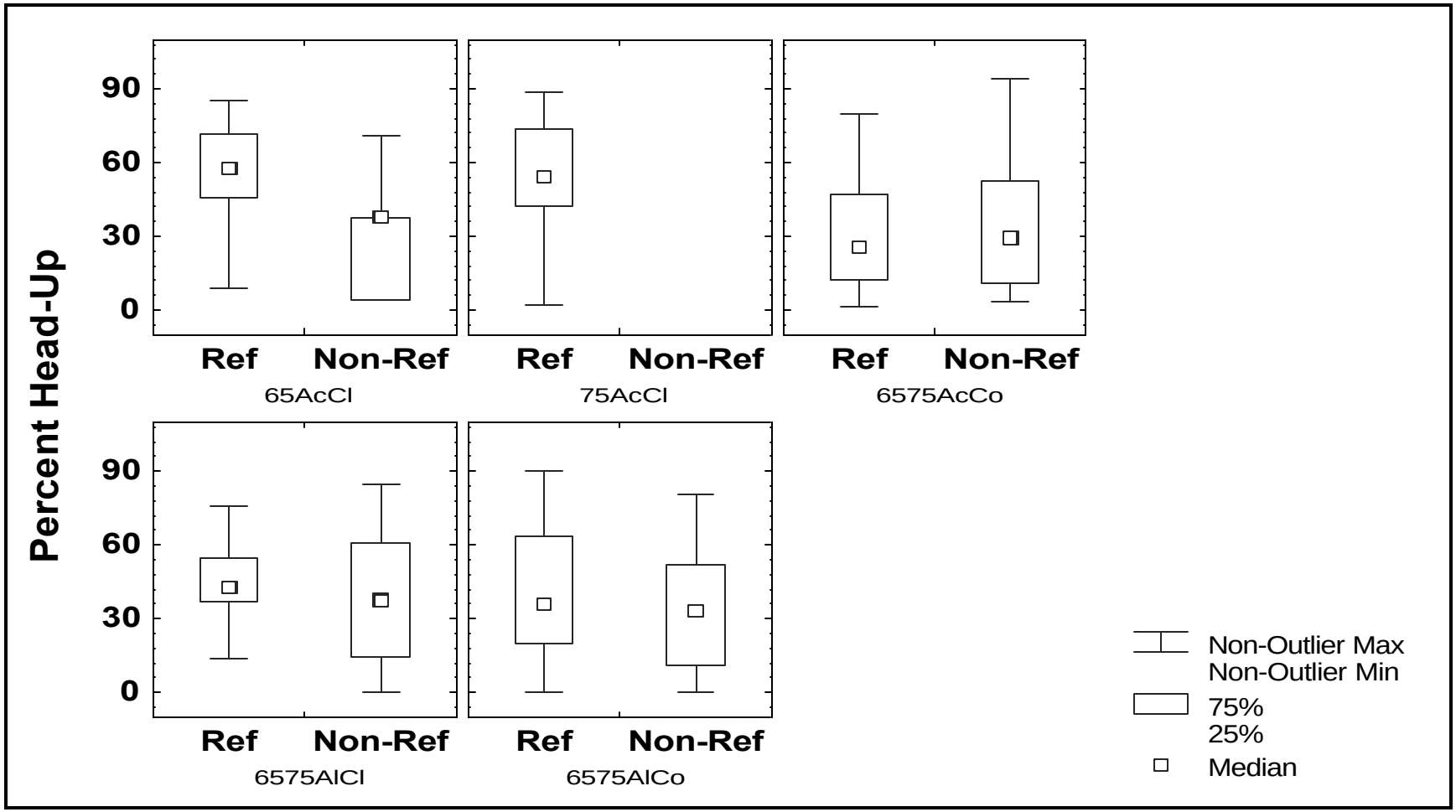


Figure B-1 (continued). Metric responses by ecoregion and lake type. AcCl = Acid-Clear; AcCo = Acid-Colored; AlCl = Alkaline-Clear; AlCo = Alkaline-Colored.

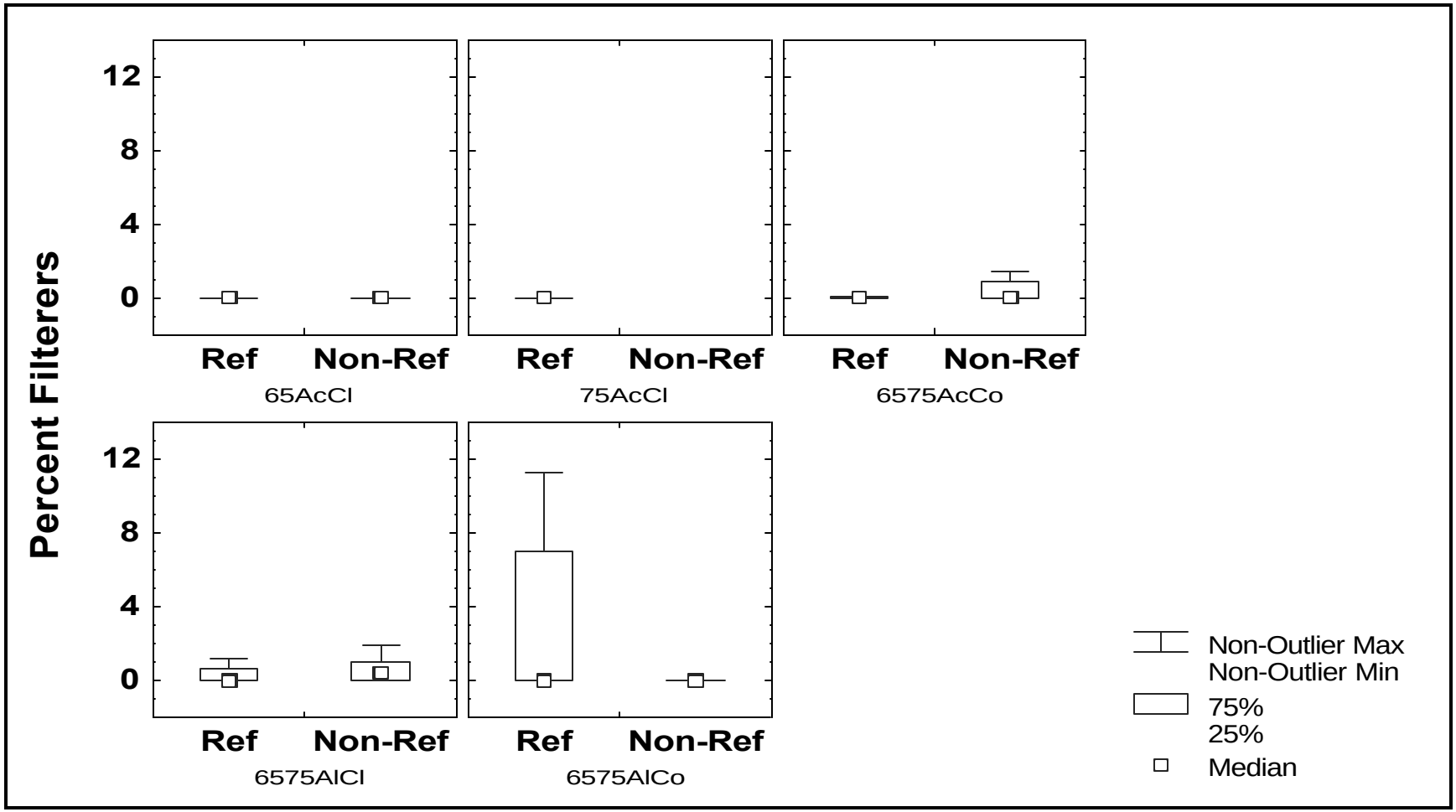


Figure B-1 (continued). Metric responses by ecoregion and lake type. AcCl = Acid-Clear; AcCo = Acid-Colored; AlCl = Alkaline-Clear; AlCo = Alkaline-Colored.

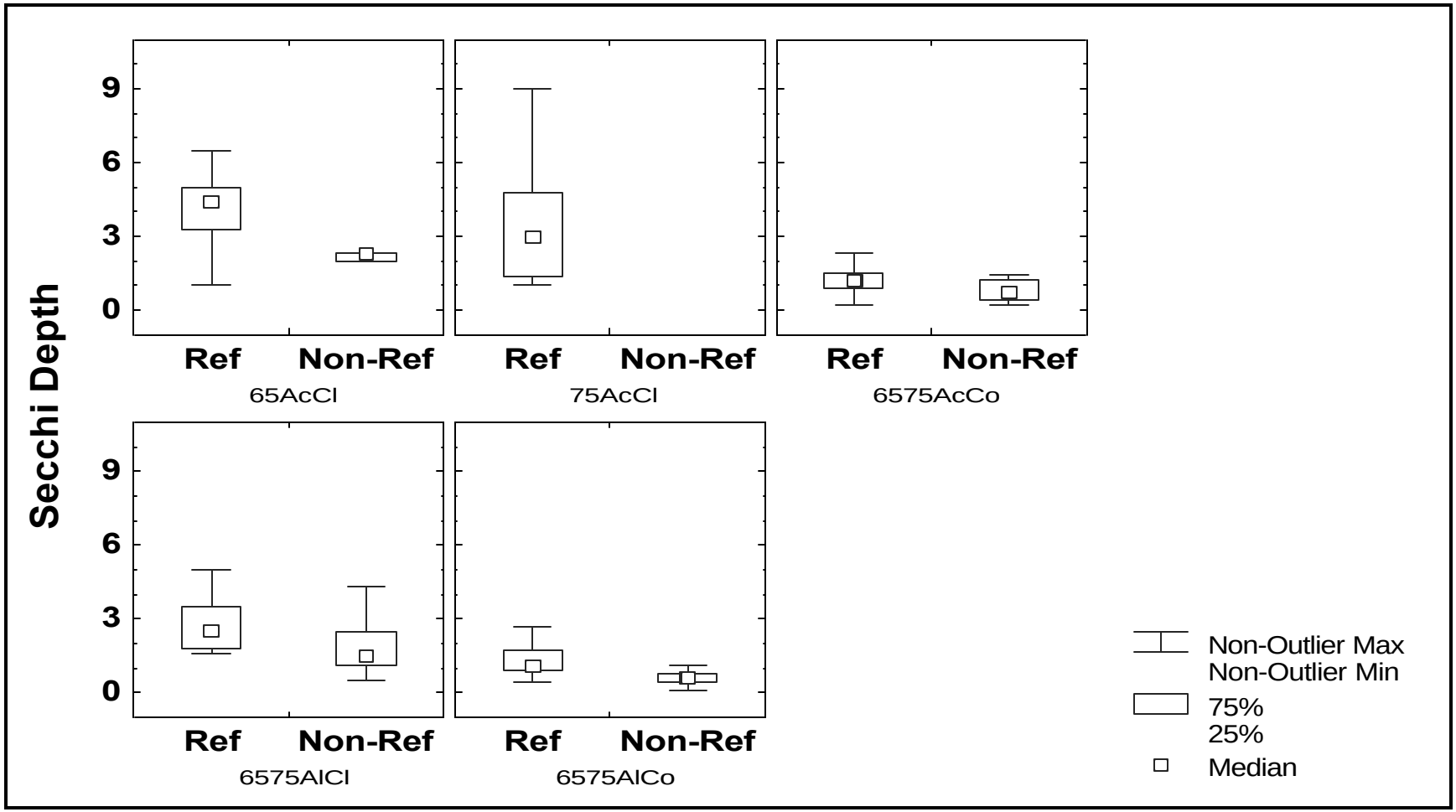


Figure B-2. Water column measure responses by ecoregion and lake type. AcCl = Acid-Clear; AcCo = Acid-Colored; AlCl = Alkaline-Clear; AlCo = Alkaline-Colored.

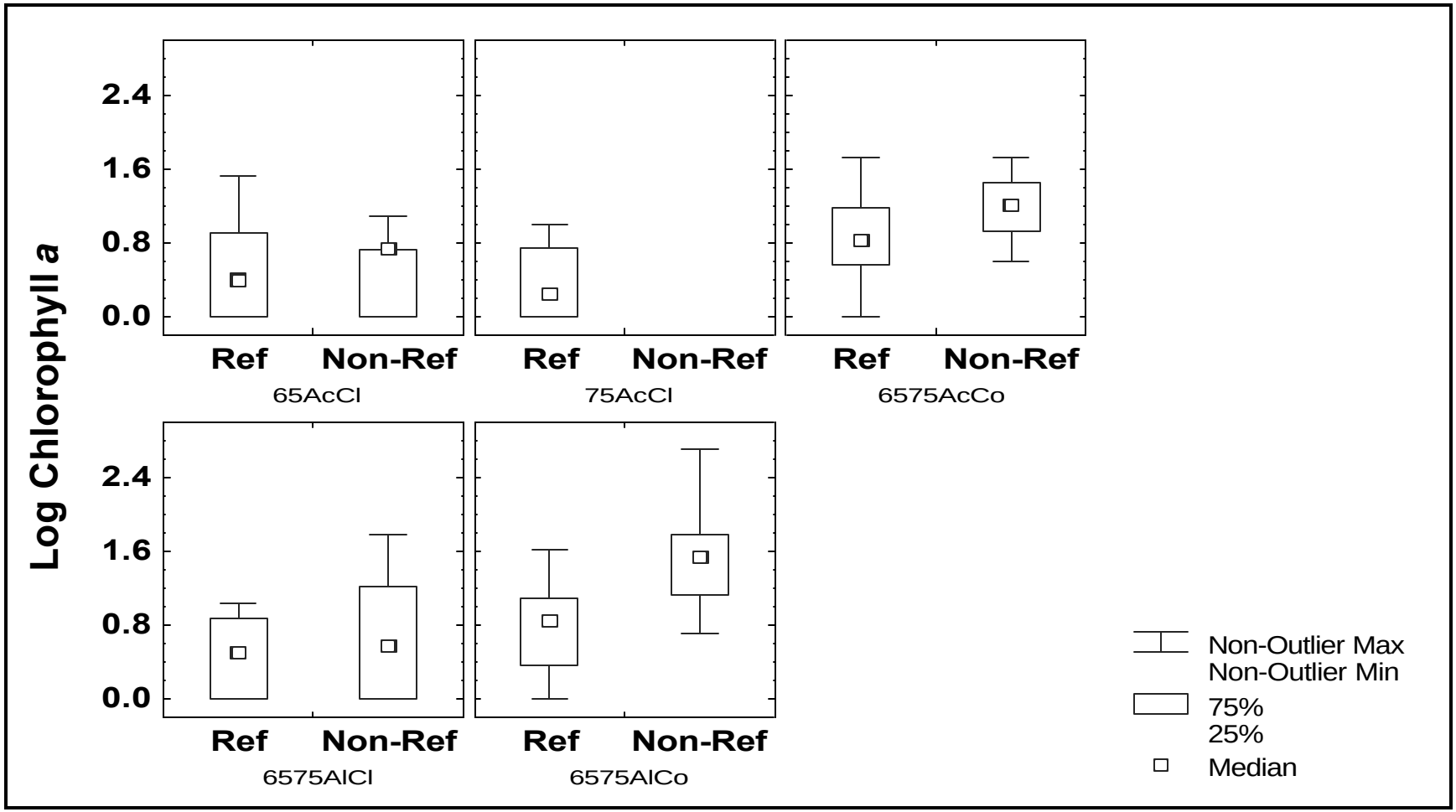


Figure B-2 (continued). Water column measure responses by ecoregion and lake type. AcCl = Acid-Clear; AcCo = Acid-Colored; AlCl = Alkaline-Clear; AlCo = Alkaline-Colored.