# EVALUATION OF BENTHIC MACROINVERTEBRATE ASSEMBLAGES AS INDICATORS OF LAKE CONDITION

# **Final Report**

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#### **ABSTRACT**

Responding to the mandates of the Clean Water Act to protect surface water resources, Florida Department of Environmental Protection (FDEP) has collected chemical, physical, and biological measures of its rivers, streams, and lakes in order to assess their abilities to support their designated uses. For this study, samples of benthic macroinvertebrates collected from lakes were evaluated for their association with measures of water quality and human disturbance. Macroinvertebrate metrics included measures of taxa richness and percentages of individuals belonging to taxonomic or functional feeding groups. Human disturbance was quantified using the Landscape Development Intensity (LDI) index measured within the 100 m buffer surrounding each lake. A development (N = 101 lakes) and a validation data set (N = 51 lakes) were used to evaluate metric response to water quality and human disturbance. None of the macroinvertebrate metrics were significantly correlated with LDI; however, several metrics were highly correlated with measures of nutrient concentration and lake clarity. Human disturbance was not correlated with water clarity or nutrient concentration. Metrics were tested again for correlation with LDI while controlling for water clarity by using multiple regression. Many of the metrics were significantly associated with lake clarity, but again failed to consistently correlate with human disturbance. Results suggest that macroinvertebrate assemblages in lakes may be better predicted by natural conditions rather than human disturbance. These results differ from a previous study of macroinvertebrates in Florida lakes which developed a multimetric index for bioassessment based on differences observed between reference and non-reference lakes derived from best professional judgment of lake condition by local biologists. When the land use intensity was compared for these two sets of lakes they were found not to differ. Given the lack of correlation with independent measures of human disturbance and the high correlation with nutrients and water clarity which may be due to either anthropogenic and natural sources, macroinvertebrate indicators may not be reliable for assessing and reporting the biological condition of Florida lakes.

#### **CHAPTER 1: INTRODUCTION**

The federal Clean Water Act (CWA) directs states "to restore and maintain the chemical, physical, and biological integrity of the Nation's waters." Under the CWA, states are required to develop water quality standards for all surface waters (Gerritsen et al., 1998). Water quality standards include designated uses assigned to a water body, water quality criteria to protect the uses, and an antidegradation policy (Ransel, 1995; Karr, 1991; Davies and Jackson, 2006). Within the context of the CWA, bioassessments can be used to define impairment, evaluate best management practices, develop targets for management plans or restoration, or identify exceptional resources for protection (Yoder and Rankin, 1998; Karr and Yoder, 2004).

While most states have biological assessment programs in place for rivers and streams, Florida is one of only nine states with lake or reservoir bioassessment programs in place and one of only three that is developing numeric biocriteria for lakes (as of 2002 which was the last year that EPA collected these statistics; USEPA, 2007). In many states, lakes may represent a smaller proportion of surface waters; however, with more than 7700 lakes greater than 10 acres in size, lakes represent a significant natural resource in Florida.

This report is part of a series of documents developed by the Florida Department of Environmental Protection (FDEP) to support water quality standards for biota by linking independent measures of human disturbance to observed changes in the biological assemblage. FDEP uses multimetric indexes to summarize diverse aspects of each biological assemblage including taxonomic richness and composition, percentage of individuals that belong to different trophic groups, and presence of tolerant or sensitive individuals (Karr and Chu, 1999). This report describes results for testing biological measures derived from benthic macroinvertebrate samples in lakes against an independent measure of human disturbance, the Landscape Development Intensity (LDI) index (Brown and Vivas, 2005; Lane and Brown, 2006b).

The original intention for the current study was to test macroinvertebrate metrics against independent measures of human disturbance and develop a multimetric index that would be used to quantify biological expectations and define biological criteria for lakes. This study did not support development of a multimetric index for macroinvertebrate samples based on the results of metric testing reported below.

## **Background**

FDEP recognizes the importance of biological monitoring of water resources and has developed sampling protocols to assess the condition of streams, lakes, and wetlands based on their biological assemblages and is currently developing numeric biological criteria for all three habitat types.

For streams, multimetric indexes for macroinvertebrate samples were developed in the early 1990's and were recently updated and recalibrated using a continuous measure of human disturbance rather than a categorical comparison of reference vs. non-reference sites to test metrics (Barbour et al., 1996; Fore et al., 2007). The stream condition index (SCI) for macroinvertebrate samples has been calibrated using a method recommended by EPA called the Tiered Aquatic Life Uses framework and is currently in the process of being implemented as numeric biocriteria into Florida water quality standards (Davies and Jackson, 2006; Fore et al., 2007). Development and testing of indicators for diatoms and algae in streams will be initiated in 2008.

For lakes, a multimetric index for macroinvertebrate samples (the Lake Condition Index – LCI), was developed in the late 1990's based on best professional judgment (BPJ) to designate reference and non-reference lakes (Gerritsen et al., 2000). The study described by the current report used some of the same data from the earlier study, but used a continuous measure of human disturbance (LDI) to quantify and test macroinvertebrate metrics rather than a categorical comparison of reference and non-reference

lakes (Brown and Vivas, 2005). LDI was also used to test metrics and develop a multimetric index for macrophytes sampled around the lake perimeter (Cohen et al., 2004; Fore, 2005). Development and testing of indicators for diatoms and algae in lakes will be initiated in 2008.

For depressional and isolated wetlands in Florida, multimetric indexes have been developed for diatoms, macrophytes, and macroinvertebrates based on correlation with LDI (Reiss and Brown, 2005, 2007; Lane and Brown, 2006a, b; Lane, 2007).

## **CHAPTER 2: METHODS**

#### Field collection of macroinvertebrates

Macroinvertebrates were collected from the lake bottom using a grab sampler. Samples were taken at a depth of 2–4 m to avoid the littoral zone which is typically covered with macrophytes. From each lake, 12 grab samples were collected from segments equally spaced around the lake. The 12 samples were composited and a 100-organism count subsample was drawn and identified to the lowest practical taxonomic level, typically species. Some large lakes were divided into more than one section and treated as independent lakes with separate macroinvertebrate samples. FDEP divides these lakes because the type and intensity of human development can differ by section. In addition, large lakes can be composed of large bays which can restrict water movement between sections.

#### Site selection and datasets

The full data set for lake macroinvertebrates had 394 samples from 310 lakes. Of these, 52 samples collected during 1994 were eliminated because some samples were collected from the profundal zone of the lake and these could not be easily identified from the data. Of the remaining 352 visits to 283 lakes, the most recent lake visit was selected in order to eliminate replicate samples for testing. Of the 283 (most recent) samples, only 152 had a Secchi score and habitat information and these were retained for metric testing. In order to test that patterns observed in the data set were consistent, the data set was divided into two parts with two-thirds used for metric testing and evaluation (development data sets: N = 101 lakes) and one-third retained to confirm that observed patterns were consistent and reliable (validation data set: N = 51 lakes). To divide the data set, lakes were sorted by LDI index values and every third sample selected for the validation data set.

A different data set of "reference" sites was also carved out of the 283 most recent lake visits, again excluding lake-visits from 1994. The 70 reference sites had an LDI index value < 2. Macroinvertebrate data from these sites were used to evaluate patterns related to geographic differences using non-metric multidimensional scaling (NMS).

# **Quantifying human disturbance**

At the center of each lake, FDEP biologists measure temperature, dissolved oxygen, conductivity, and nutrient concentrations. Total Kjeldahl nitrogen (TKN), chlorophyll A, total phosphate (TP), color, algal growth potential, ammonia (NH<sub>3</sub>), nitrites/nitrates (NO<sub>x</sub>), and orthophosphate were measured to summarize nutrients. In addition, sediment composition was evaluated in some lakes and percent fine

sediment was calculated as particle size < 0.063 mm and percent organic sediment measured as the percent by weight that could be ignited.

A lake habitat assessment was also completed for each lake (FDEP, 2004). Secchi depth, vegetation quality, stormwater inputs, bottom substrate quality, lakeside adverse human alterations, upland buffer zone and adverse watershed land use were scored and the scores summed for an overall measure of habitat quality, the habitat index. Because the number of elements in the habitat index changed during the time of lake sampling, the habitat index was standardized by dividing by the total possible points so that the index ranged from 0–100% for all years.

To summarize human disturbance, Brown and colleagues have developed an index to estimate the intensity of human land use based on nonrenewable energy flow (Brown and Vivas, 2005, Lane and Brown, 2006b). The Landscape Development Intensity (LDI) Index is calculated as the percentage area within a catchment of particular types of land use multiplied by the coefficient of energy use associated with that land use, summed over all land use types in a buffer or catchment area:

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

Where:

 $LDI_i$  = the nonrenewable energy land use for land use i, and  $%LU_i$  = the percentage of land area in the catchment with land use i.

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

Land use was derived from aerial photos manipulated as layers in a geographic information system (GIS) computer program. LDI was calculated for the area within a 100 m buffer area surrounding each lake. LDI was not calculated for a lake's watershed because watersheds are difficult to define for Florida lakes due to low topographical relief and groundwater movement between watersheds.

# **Data analysis**

Non-metric multidimensional scaling (NMS) is a nonparametric ordination procedure used to identify structure and pattern associated with taxonomic distributions. For these lake samples, taxa abundances were summarized at the genus level. Genera that occurred at less than 5% of the sites, that is, genera with < 4 occurrences (70 sites x .05 = 3.5 sites) were eliminated leaving 60 unique genera for the analysis. Data were summarized using the Sorenson similarity index (Bray-Curtis) to calculate taxonomic similarity between all pairs of reference site samples.

Candidate metrics for macroinvertebrates were evaluated for their range of values in lakes and those with a very narrow range were eliminated from further analysis. Remaining metrics were tested for correlation with measures of site condition and nutrient concentration. Because statistical significance of a correlation coefficient (r) is a function of the sample size (a smaller r-value will be significant for larger sample sizes), and because sample size varied for the different disturbance measures, a correlation coefficient was defined as significant if it was > 0.4 (or < -0.4). In this way, results from metric testing were made more comparable. An r-value > |0.4| was statistically significant in all cases.

Multiple regression analysis tested for metric association with LDI when the influence of water clarity on macroinvertebrate metrics was included in the model. For each multiple regression model, a single metric served as the independent variable with LDI and Secchi score as the two predictor variables.

# **CHAPTER 3: RESULTS**

Lake samples were dominated by insects (68% of individuals, N = 152 lakes) with oligochaetes (15%), crustaceans (6%) and bivalves (6%) representing the next largest percentages. Of the insects, Diptera dominated (90%) with Ephemeroptera (6%) and Trichoptera (3%) comprising most of the remaining taxa. Of the Diptera, 75% belonged to the family Chironomidae and 20% to Chaoboridae (genus *Chaoborus*); an additional 6% belonged to Ceratopogonidae. Of the oligochaetes, tubificids dominated at 76% of organisms; another 20% were naidids. Of the crustaceans, the majority were hyallelid amphipods. Bivalves included organisms from the family Sphaeriidae (33%), Pisidiidae (28%), Corbiculidae (17%), and Veneridae (16%).

#### **Human disturbance**

Measures of human land use (LDI), lake clarity (Secchi score, Secchi depth, and color), nutrient concentration (TKN, NOx, TP, algal growth potential, NH $_3$ , chlorophyll A, orthophosphate), conductivity, percent fine sediment (particle size < 0.063 mm), and percent organic sediment were evaluated for their pair-wise correlation (Spearman's |r| > 0.4). Data were incomplete for many samples, e.g., 131 (out of 152 lakes) had data for color and 67 had Secchi depth. Data for Secchi score were available for all 152 lakes and was used to measure water clarity. Secchi score was derived from information recorded as part of the habitat assessment. Four categories were defined (> 3 m; 1–3 m; 0.5–1 m; < 0.5 m) and five possible values could be assigned within each category such that the final score could range from 1 to 20.

Most notable was the lack of correlation between measures of land use around the lake (LDI and the habitat index) and measures of nutrient concentration (Table 1). The high correlation between LDI and the habitat index (r = -0.64) suggested that land cover calculations from photographs matched observations of site disturbance made from the lake.

Some nutrient measures were more consistently associated with each other while several others showed little or no association with other measures of human influence. TKN, TP, AGP, chlorophyll A, and measures of water clarity tended to correlate with each other while NO<sub>x</sub>, NH<sub>3</sub>, and orthophosphate failed to correlate with many of the other measures.

Secchi score was highly correlated with Secchi depth and color (r = 0.94 for n = 67 and -0.74 for n = 131). Because Secchi score was also highly correlated with several nutrient measures and summarizes the effects associated with both natural and anthropogenic nutrient sources, it was included in the multiple regression to test for effects of human land use while controlling for water clarity.

**Table 1. Correlation between lake condition measures.** Sample size (number of lakes) and Spearman's correlation coefficients (for |r| > 0.4) for measures of land use, nutrient concentration, and sediment condition.

	LDI	Secchi score	Habitat score (%)	TKN	NO <sub>x</sub>	TP	AGP	NH <sub>3</sub>	Chl. A	ОР	Cond.	Color	% Org. sed.	%Fines
N =	152	152	99	138	123	110	116	110	127	107	147	131	93	88
LDI	_		-0.64											
Secchi Score		_		-0.75		-0.65	-0.47		-0.56			-0.74		
Habitat score (%)	-0.64		_											
TKN		-0.75		_		0.72	0.59	0.41	0.59			0.62		
NO <sub>x</sub>												0.48		
TP		-0.65		0.72		_	0.60	0.49	0.52			0.67		0.40
AGP		-0.47		0.59		0.60			0.45			0.48		
NH <sub>3</sub>				0.41		0.49		_				0.41		
Chlorophyll A		-0.56		0.59		0.52	0.45		_			0.50		
ОР										_		0.56		
Conductivity											_			
Color		-0.74		0.62	0.48	0.67	0.48	0.41	0.50	0.56		_		
% Organic sediment													_	0.55
% Fines (<0.063)						0.40							0.55	_

# Multivariate analysis of reference sites

Results from the non-metric multidimensional scaling analysis suggested a tendency for lakes located in the panhandle and peninsula to group separately according to the macroinvertebrates collected (Figure 1). The analysis illustrated in Figure 1 was derived from counts of individual organisms. A similar analysis (not shown) based on simple presence or absence of each genus provided very similar results.

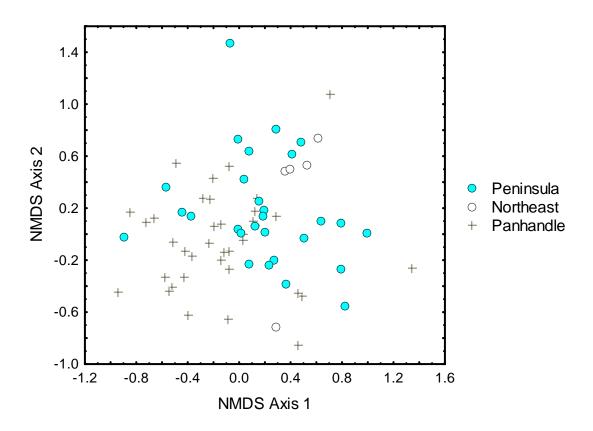


Figure 1. NMDS for macroinvertebrate genus counts. Shown are 70 'reference' sites (LDI < 2) illustrating a tendency for peninsula and panhandle lakes to separate.

# **Metric testing**

#### **Correlation testing**

From an initial list of about 40 candidate macroinvertebrate metrics about half were eliminated from additional testing due their narrow range of values, e.g., < 3 unique taxa for taxa richness measures or a predominance of 0 values and a narrow range of values for percentage metrics (Blocksom, 2003). The 19 metrics retained for testing were the Florida and Hulbert indexes, which multiply a taxon's tolerance value by the number of individuals in that taxon and divide by the total number of individuals in the sample to yield a single weighted value for each sample; total taxa richness with and without chironomid taxa included, chironomid taxa, Oligochaete taxa; and the percentage of individuals belonging to amphipods, chironomids, dipterans, oligochaetes, Tanytarsini chironomids, dominant taxon, ETO (Ephemeroptera, Trichoptera, and Odonata), epilithic collector and depositional feeders, predators, shredders, substrate collector and depositional feeders, filterers, and very tolerant taxa.

None of the 19 candidate metrics were significantly associated with LDI or the habitat index for either the development or validation data sets (with significance defined as |r| > 0.4; Table 2). Of the 19, 8 metrics were highly correlated with Secchi score for both the development and the validation data sets. Most of these 8 metrics were also correlated with TKN and chlorophyll A and some were also correlated with TP, color, and AGP. In contrast none of the metrics were correlated with NO<sub>x</sub>, NH<sub>3</sub>, or orthophosphate. Although more metrics were correlated with disturbance measures for the validation than the development data set, these differences were most likely attributable to noise in the data.

#### **Multiple regression**

Multiple regression was used to test for a relationship between macroinvertebrate metrics and LDI while including the influence of water clarity measured as Secchi score. Results for multiple regression were similar to the correlation analysis and many of the metrics were significantly associated with Secchi score but not with LDI (see Table 2). A few macroinvertebrate metrics were significantly associated with both Secchi score and LDI, but the relationships with LDI were quite weak and none of the metrics were significantly associated with LDI for both the development and validation data sets.

**Table 2. Correlation between macroinvertebrate metrics and measures of lake condition.** Shown are sample sizes for the development (and validation) lakes, Spearman's *r*-values for which the absolute value was >= 0.4, and the number of macroinvertebrate metrics that were highly correlated with each measure of lake condition.

	Secchi Score	N	Chlorophyll A	£	Color	Sed. < 0.063	AGP	ΓĐ	Habitat index (%)	Nox	NH3	Orthophosphate
Valid N	101(51)	92(46)	84(43)	74(36)	89(42)	55(33)	75(41)	101(51)	67(32)	84(39)	73(37)	75(32)
Florida index	0.61 (0.75)	-0.62 (-0.63)	(-0.77)	(-0.59)	(-0.73)		(-0.51)					
Hulbert Index	0.50 (0.75)	-0.56 (-0.73)	-0.5 (-0.72)	-0.44 (-0.6)	(-0.74)		(-0.43)					
Total taxa (no Chi)	0.47 (0.45)	-0.5										
Total taxa	0.67 (0.67)	-0.63 (-0.58)	-0.5 (-0.68)	-0.45 (-0.55)	-0.52 (-0.56)		(-0.5)					
Chironomid taxa	0.61 (0.69)	-0.53 (-0.67)	-0.42 (-0.65)	-0.42 (-0.65)	-0.52 (-0.71)		(-0.58)					
Oligochaete taxa												
% Amphipod	(0.53)	(-0.59)	(-0.53)	(-0.69)	(-0.45)		(-0.53)					
% Chironomid	(0.45)					(-0.49)						
% Diptera												
% Oligochaete												
% Tanytarsini	0.51 (0.60)	(-0.57)	(-0.64)	-0.46 (-0.67)	-0.49 (-0.65)	(-0.52)	(-0.63)					
% Dominant	-0.42 (-0.5)		0.41 (0.54)		(0.47)							
% ETO	(0.63)	(-0.57)	-0.43 (-0.6)	(-0.55)	(-0.52)							
% Epi-Coll-Dep	0.44 (0.53)	-0.49 (-0.59)		(-0.54)	-0.45 (-0.57)	-0.43 (-0.7)						(-0.45)
% Predator												
% Shredder						-0.45						
% Sub-Coll-Dep												
% Filterer	(0.51)	-0.42 (-0.47)	-0.4 (-0.5)	(-0.5)	(-0.46)	(-0.41)	(-0.46)					
% Very tolerant							(0.51)					
Number Significant	8 (12)	7 (9)	6 (9)	4 (9)	4 (10)	2 (4)	0 (8)	0 (0)	0 (0)	0 (0)	0 (0)	0 (1)

#### Connection to previous work

Gerritsen et al. (2000) selected six metrics for lake assessment based on a comparison of metric performance in reference and non-reference sites that were defined according to the best professional judgment of FDEP biologists. The six metrics were combined into an index, the LCI, and included total taxa richness, EOT taxa, %EOT individuals, Hulbert Index, Shannon-Wiener diversity index, and % Diptera. At the time of the previous report, information to calculate LDI was not available. That study used lake data from 122 lakes designated as reference and 84 lakes designated as non-reference. Within the current data set were 81 of their reference lakes and 43 of their non-reference lakes. Lakes missing from the comparison may have been sampled before 1995 (recall that these earlier samples were excluded from the present analysis due to differences in data collection methods). Comparing the LDI for these two data sets revealed very slightly more disturbed sites for non-reference (higher median), but the range and overlap of LDI values were nearly identical for lakes designated as reference and non-reference (Figure 2). Furthermore, the LCI was not correlated with LDI when tested across all lakes, or when stratified according to color (Figure 3).

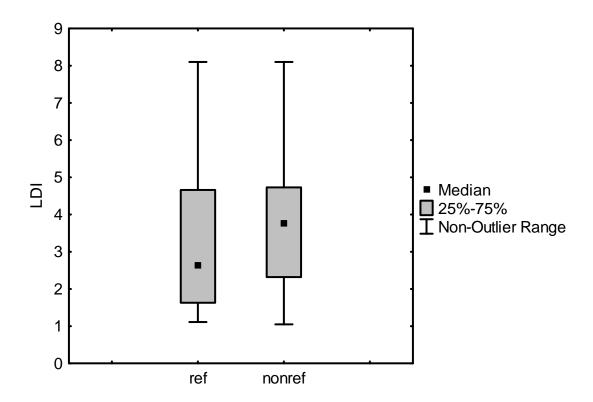


Figure 2. Comparison of LDI values for lakes in the current data set that were designated as reference or non-reference by FDEP in Gerritsen et al. (2000).

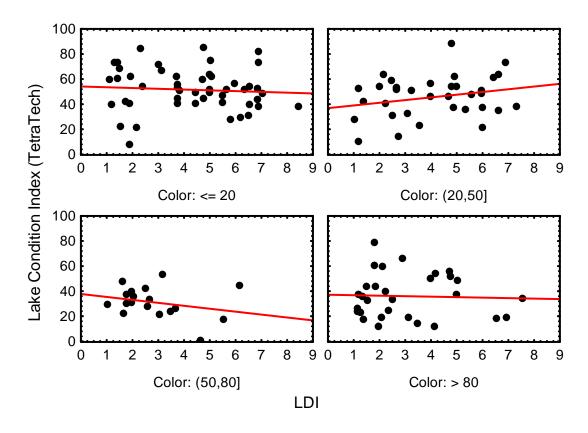


Figure 3. Lake condition index values (LCI; Gerritsen et al. [2000]) based on macroinvertebrate samples were not significantly correlated with LDI values (Pearson's r < 0.3, p > 0.05 for tests stratified by color or for all lakes considered together; N = 131 lakes).

#### **CHAPTER 4: DISCUSSION**

Macroinvertebrate metrics were highly correlated with measures of water clarity and some measures of nutrient concentration, but were not associated with measures of human disturbance or site condition in the surrounding watershed. Because neither nutrient concentration nor water clarity was associated with human disturbance or site condition, the question remains to be answered: are high nutrient concentrations in Florida lakes due to anthropogenic or natural sources? When measures of human disturbance fail to agree, additional questions remain regarding how to combine the measures for a single assessment of site condition in order to test biological response to human disturbance (Fore, 2003).

This section reviews the results from the current analysis of macroinvertebrate samples collected from lakes, compares the results to previous studies in lakes using macroinvertebrates and macrophytes, and discusses alternative explanations for the results.

# Macroinvertebrate assemblages in Florida lakes

Non-metric multidimensional scaling was used to test for natural patterns associated with geography or other physical variables in structuring the macroinvertebrate assemblages observed in lakes. The NMS used only reference lakes (LDI < 2) in order to look for patterns in the absence of human disturbance. There was a tendency for different taxa to be found in panhandle and peninsula areas of the state. The ability to test for other gradients that might be structuring the macroinvertebrate assemblage was limited by the many missing values for water chemistry and physical parameters in the data set. Given the strong correlation between macroinvertebrate metrics and measures of water clarity and nutrient concentrations in subsequent analyses, additional multivariate tests were not pursued with the taxa count data.

For metric testing, the data set was divided into two parts: a development data set for initial metric testing and a validation data set to ensure that patterns observed in one data set would be confirmed by the second. Results from this analysis were clear and consistent, several macroinvertebrate metrics were highly correlated with water clarity (measured as Secchi score, Secchi depth, or color) and several measures of nutrient concentration (TP, TKN, chlorophyll A, and AGP). Metric values associated with good biological condition (e.g., high taxa richness) declined as nutrient concentrations increased and water clarity declined. In contrast, metrics were not correlated with LDI or the habitat index.

Macroinvertebrate metrics also failed to correlate with three other measures of nutrient concentration (NO<sub>x</sub>, NH<sub>3</sub>, and OP) which tend to be associated more directly with human sources such as sewage and wastewater; however, none of the three measures were associated with the LDI or the habitat index. Lack of correlation between nutrient concentrations and measures of human land use intensity or site condition suggests a natural source for nutrients or a path unrelated to current measures of human disturbance.

Many Florida lakes are naturally eutrophic due to nutrients from the soil and leaf litter decaying in the lakes. High correlations with color for all nutrient measures suggest that leaf litter is a primary source for nutrients. Nutrients make the water opaque and provide food for algae which further limit light to the substrate. Even though macroinvertebrates were collected from shallow water (2–4 m), most lakes are dark at this depth with 90% of lakes having a Secchi depth < 3 m. Thus, for the depths at which most of the macroinvertebrate samples were collected, the substrate was dark.

## Results of previous studies in Florida lakes

A related study by FDEP tested biological metrics developed for macrophytes found in the littoral zone of lakes (Fore, 2005). For that study, macrophyte metrics that were correlated with human disturbance tended to be correlated with nutrient concentrations, the habitat index, and the LDI. The consistent correlation between macrophyte metrics and both watershed disturbance and nutrient concentration was worth noting given the contrasting results observed in the current study for macroinvertebrates. Also worth noting from that study was the lack of correlation between LDI and measures of nutrient concentration, similar to results observed for the macroinvertebrate lake data set. To summarize, lake macroinvertebrate metrics were only correlated with measures of water clarity and nutrients, but lake macrophyte metrics were correlated with both types of measures even though nutrient concentrations were not strongly associated with LDI for either data set.

A previous study tested macroinvertebrate metrics in Florida lakes for differences between lakes designated as reference and non-reference. Metrics that showed differences were combined in a multimetric index (LCI; Gerritsen et al., 2000). The authors first grouped lakes according to whether they were acid or alkaline, clear or colored, and according to ecoregion; metrics were tested within these groups. The authors concluded that the LCI was a more reliable indicator of lake condition for clear lakes and recommended the LCI not be used for lakes with color > 20 PCU. Unfortunately, > 60% of the lakes in the current data set had color > 20 PCU which limits the broad applicability of LCI.

Gerritsen et al. (2000) also tested for correlation between the LCI and urban land use and found little or no association. These results repeat what was found in the current study, that is, lack of correlation between macroinvertebrate metrics and watershed disturbance. The LCI also failed to correlate with human disturbance as measured by LDI for the current data set. Looking at the lakes identified as reference and non-reference from that study, there was almost no difference in the range of their LDI values suggesting broad overlap in the watershed condition and human disturbance surrounding these lakes designated as reference and non-reference for metric testing. If lakes were designated as reference based on water clarity, and if water clarity was associated with natural condition as well as anthropogenic disturbance, the LCI may not be a reliable indicator of resource condition. If reference lakes were selected according to the biological samples, e.g., presence of mayflies, metric selection and development of the LCI may be based on circular reasoning.

#### Macroinvertebrate indicators of lake condition

Three competing conclusions could be drawn from the results of this study: 1) macroinvertebrate assemblages respond to the trophic condition of lakes that is associated with natural sources of nutrients; 2) macroinvertebrate assemblages are correlated with water quality measures because water chemistry is a better indicator of lake condition than LDI or the habitat index; 3) anthropogenic and natural sources of nutrients are confounded and cannot be distinguished for metric testing with the current data set.

Macroinvertebrate metrics were correlated with water clarity and nutrient concentrations rather than measures of site condition or watershed land use. Results suggested that natural sources of nutrients may be more important to macroinvertebrate assemblages than anthropogenic sources given higher metric correlations with nutrient measures typically associated with natural sources such as leaf litter breakdown, e.g., TKN, TP, and color (indicating tannins from leaves), than with anthropogenic sources, e.g., nitrates-nitrite ( $NO_x$ ), ammonia ( $NH_3$ ), and orthophosphate. An alternative interpretation could be that TKN and TP were by far the larger sources of nutrients and eclipsed any association with other nutrient sources ( $TKN \sim 10x > NO_x$  and  $NH_3$ ) and that TKN and TP include both natural and anthropogenic sources of nutrients.

Whatever the source of the nutrients, they contribute to algal growth and loss of light to the lake benthos. Most macroinvertebrate samples were collected from below the photic zone. Many freshwater insects have highly developed eyes and are very specific in the types of food they eat, presumably they use their eyes to identify and select appropriate food. Their eyes need light to function and samples for

this study were collected from substrates that were dim or dark. Many freshwater insects also graze on attached diatoms and algae which need light to grow on the substrate. Worth noting is the fact that macroinvertebrate metrics derived from sweep net samples in Florida wetlands were highly correlated with LDI, suggesting that macroinvertebrates collected from the photic zone might be more indicative of lake condition and human disturbance (Lane et al., 2004).

Most of the assemblage was composed of chironomids and there may be other metrics more specific to chironomids that could be related to sediment condition or watershed land use. However, if other metrics derived from chironomid data were to be good indicators, we would expect to see some correlation between human disturbance and either total chironomid taxa richness or % of Tanytarsini individuals, which are typically recognized as more sensitive chironomids.

A second interpretation would be based on the idea that water chemistry samples within the lake are a better indicator of lake condition than the LDI or habitat index. Certainly the cultural eutrophication of lakes has been broadly documented and connected to human sources such as fertilizer, sedimentation, and wastewater (Murtaugh and Pooler, 2006). Many of the studies relating eutrophication to human land use have been done for northern and western areas of the US and results from these areas may not be relevant for Florida where lakes are shallow, warm temperatures promote plant and algal growth, and high nutrient levels occur naturally in soils. For example, a lake index based on macroinvertebrate samples collected from lakes in New Jersey used the response of metrics primarily to Secchi depth as a criterion for metric selection. Nonetheless, the final multimetric index derived from these metrics was highly correlated with urban development in the watershed, suggesting that eutrophication in New Jersey lakes is primarily due to human influence even though the authors did not test this association directly (Blocksom et al., 2002).

To conclude that nutrients are largely related to human activities may be less defensible for Florida lakes without a better understanding of the specific mechanism or pathway describing how human activities influence water clarity and nutrient concentrations. Because LDI was not correlated with nutrients and water clarity, and because nutrients can be naturally occurring, an alternative measure of human disturbance may need to be developed to summarize anthropogenic sources for nutrients. Furthermore, the high correlation between LDI and macroinvertebrate metrics in Florida streams (Fore et al., 2007), macroinvertebrate, macrophyte, and diatom metrics in wetlands (Cohen et al., 2004; Lane et al., 2004; Lane and Brown, 2006a; Lane, 2007), and macrophyte metrics in lakes (Cohen et al., 2004; Fore, 2005) suggests that LDI is a reliable indicator of anthropogenic disturbance and change within a watershed. LDI's consistent correlation with the habitat index further suggests that the LDI assessment derived from photographs of land coverage matches the site assessment made by a biologist from the ground. For these reasons, it seems unlikely that LDI is an unreliable or meaningless measure of site condition. Furthermore, LDI is only a measure of human disturbance; in contrast, nutrient concentrations are influenced by both human and natural sources. If water quality is proposed as the better indicator of lake condition, additional explanations may be needed. An example of an alternative explanation might be that ground water degraded by human activities outside the watershed is influencing lake condition.

A third interpretation would be that both human activities and natural sources contribute nutrients and tannins to lakes, but their relative contribution cannot be determined from the data on hand. Additional data sources for background levels of nutrients based on soil sampling or water samples from lakes with minimal human influence could be used to set expectations for trophic condition or water clarity of lakes.

## **CONCLUSIONS**

- Ordination of macroinvertebrate taxa counts identified some differences in genera associated with peninsula and panhandle locations.
- Nutrient concentrations and water clarity were not correlated with human disturbance in the near shore area around the lake.
- Integrative disturbance measures around the lake agreed (habitat index and LDI).
- Macroinvertebrate metrics were correlated with water clarity (Secchi depth and color) and nutrient concentrations (TKN and TP).
- Macroinvertebrate metrics were not correlated with intensity of human disturbance (LDI) or habitat condition in the near shore area around the lake.
- Macroinvertebrate assemblages responded to eutrophication, but whether the source of nutrients and tannins were anthropogenic or natural could not be determined from the data set.
- Lack of correlation with an independent measure of human disturbance (LDI)
  given that other assemblages in lakes, streams and wetlands were highly
  correlated with LDI, suggests that benthic macroinvertebrates may not be a
  reliable indicator assemblage for the biological condition of lakes.
- If lake bioassessments will be derived from macroinvertebrate sampling, an
  alternative sampling collection protocol should be considered (e.g., from the
  photic zone) and a method should be developed to distinguish between
  anthropogenic and natural sources of eutrophication.

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