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BIOLOGICAL INDICATORS OF WETLAND CONDITION FOR ISOLATED DEPRESSIONAL HERBACEOUS WETLANDS IN FLORIDA

By

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A DISSERTATION PRESENTED TO THE GRADUATE SCHOOL
OF THE UNIVERSITY OF FLORIDA IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

UNIVERSITY OF FLORIDA

2003

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This dissertation is dedicated to my family: my grandmother Helen D. Whelan, my mother Virginia Lieneck and stepfather Herman Lieneck, my aunt June Whelan, my sister Christine Takayama, brother-in-law Chris Takayama, and niece Allison Takayama, and in loving memory of my grandfather, Raymond M. Whelan.

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ACKNOWLEDGMENTS

Many people played a large part in providing support and inspiration for this dissertation. I would first like to acknowledge the love and support of Venessa Rumancik, who has been with me through thick and thin. Dr. Mark T. Brown provided an excellent intellectual environment at the Howard T. Odum Center for Wetlands, and was instrumental in seeing this research to fruition. In addition to Dr. Mark Brown, Drs. Thomas Crisman, Wiley Kitchens, and Clay Montague comprised the doctoral committee and were invariably available for discussions and guidance. Earlier biological indicator research on macrophytes and macroinvertebrates by Mike Murray-Hudson and Benjamin Vivas, respectively, provided the groundwork for the further development of germane metrics.

My family, as always, was there to offer encouragement, love, and material support, for which I am eternally grateful. My friends provided the needed balance in life and were always good for a canoe trip or other diversion. The "research group" of the Howard T. Odum Center for Wetlands helped sculpt this work both by their efforts in the field and by their important participation in research discussions, especially Kelly Reiss, Matt Cohen, Jim Surdick, Benjamin Vivas, and Susan Carstenn. Finally, this research was supported by a grant to Mark T. Brown from the Florida Department of Environmental Protection (FDEP), and I would like to acknowledge the support of Russ Frydenborg, Ashley O'Neal, Ellen McCarron, Liz Miller, Lori Wolfe, Joy Jackson, and Johnny Richardson of the FDEP.

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Abstract of Dissertation Presented to the Graduate School
of the University of Florida in Partial Fulfillment of the
Requirements for the Degree of Doctor of Philosophy

BIOLOGICAL INDICATORS OF WETLAND CONDITION FOR ISOLATED DEPRESSIONAL HERBACEOUS WETLANDS IN FLORIDA

By

Charles Raymond Lane

August 2003

Chair: Mark T. Brown
Major Department: Environmental Engineering Sciences

In order to develop tools to assess the biotic integrity of isolated herbaceous depressional wetlands of peninsular Florida, 75 small (~1ha) wetlands along a gradient of human disturbance were studied, and algae (diatoms), macrophytes, and macroinvertebrates collected and analyzed. Physio-chemical constituents of the water and soil were also collected and analyzed. Three indices of wetland integrity were developed based on measurable attributes (metrics) of the assemblages sampled: the Diatom Index of Wetland Condition (DIWC, 14 metrics), Vegetative Index of Wetland Condition (VIWC, 5 metrics), and the Macroinvertebrate Index of Wetland Condition (MIWC, 5 metrics). Each index was significantly correlated (Spearman's $r \geq |0.75|$, $p < 0.0001$) with an independent geographic information systems-based measure of human landscape modification, the Landscape Development Intensity index.

A secondary goal of this research was to elucidate the various landscape and soil/water physio-chemical constituents responsible for determining the community

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composition of the sampled wetlands, as understanding the driving forces may aid in managing wetland resources. The dimensionality of each dataset was reduced using non-metric multidimensional scaling (NMDS), and correlations of site scores in ordination space made with measured physio-chemical constituents, LDI score, and latitude/longitude. Four variables acting on various spatial scales were correlated (Pearson's r^2>0.30) with all three assemblages: soil pH, water column specific conductivity, water column total phosphorous (TP), and LDI score. In addition, water color (macroinvertebrates) and latitude (macrophytes) were also correlated with the ordination site scores.

The indices of biotic integrity developed from this research may be used to provide quantitative and objective measurement of the aquatic resources of peninsular Florida and may form the scientific basis for an objective and quantifiable rapid assessment procedure. In addition, results from this study suggested that efforts to restore community composition and associated processes and functions should focus on amending inflows and fluxes of water, materials, and energy that affect soil pH, specific conductivity, and water TP, as well as decreasing the abundance of human-developed lands within 100m of wetlands.

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CHAPTER 1
INTRODUCTION AND OVERVIEW

Statement of the Problem

Wetlands are defined as "areas that are inundated or saturated by surface or ground water at a frequency and duration to support, and that under normal circumstances do support, a prevalence of vegetation typically adapted for life in saturated soil conditions" (42 Fed. Reg. 37, 125-126, 37128-29; July 19, 1977). Wetlands on the landscape provide many important functions, including biogeochemical cycling, wildlife habitat, floodwater attenuation, and water quality improvement. In the mid-1970s the United States Environmental Protection Agency (U.S. EPA) mandated that wetlands, as protected under the Clean Water Act (Clean Water Act §101(a)), required biological monitoring and assessment to ensure that the biological integrity (*sensu* Karr 1981) of these systems was not diminished. Wetland biological monitoring and assessment, as differing from synoptic water or soil chemistry sampling, characterizes the wetland flora and fauna to measure the relative state of wetland resources.

The use of biotic characteristics, including taxonomic composition and the abundance of functional guilds (i.e., "scrapers"), has provided robust assessments of the relative state of various aquatic systems (see Barbour et al. 1996, Gerritsen and White 1997), as organisms integrate exogenous perturbations that occur at local (i.e., nutrient loading) and landscape scales (i.e., increases in residential land use) (Karr and Chu 1999). These observations have led to the development of indices of biological integrity (IBIs, Karr 1981), which relate measured attributes (or metrics) of various assemblages

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measured on-site to changes in human disturbance (Karr and Chu 1999, U.S. EPA 2002a).

Often, the human disturbance gradient is interpreted subjectively (i.e., best professional judgment – Adamus et al. 1991, Miller and Gunsalus 1997) or confounded through circular logic (i.e., a metric correlated to the disturbance gradient may also be directly incorporated into scoring the disturbance gradient – Miller and Gunsalus 1997). To decrease these problems associated with on-site assessments, as well as the costs and logistics related to on-site visits, techniques have also been developed to examine biological response to landscape scale measurements through the use of geographic information systems (GIS) analysis (O'Conner et al. 2000, Cuffney et al. 2000). As compared with gradients assessed on-site that examine conditions *within* the system, landscape scale measurements assume a direct relationship between land use *surrounding* an aquatic body and the composition and relative condition of the system (Galatowitsch et al. 1999a). For example, studies have related biological data (e.g., richness) to the abundance of particular land use measurements (e.g., percent impervious surface meters – Doberstein et al. 2000, percent agricultural land use within watershed – Roth et al. 1996). However, GIS based analyses are subject to errors of land use interpretation from aerial photos, as well as delays (often of several years) between flight dates and available geo-rectified and digitized information, which can affect their accuracy. On the other hand, GIS based analyses of relative condition do not require an on-site visit, which decreases sampling effort and thus increases the number of sites quantifiable. In addition, GIS based analyses have been reported as generally precise and correlated with biological conditions within nested systems (Roth et al. 1996, Galatowitsch et al. 1999a, Karr and

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Chu 1999). These attributes increase the allure of GIS based analyses in assessing the relative condition of isolated depressional herbaceous wetlands on the Florida landscape.

However, a disconnection may exist between the wetland system and the GIS-based assessment method due to the assumption of causal mechanisms between land use within a given distance from the wetland and relative wetland condition. For instance, a site may be perched on the landscape and thus not as highly affected by the inputs from the surrounding lands as a site in a low-lying area within the same land use. In this example, the expected variance in the biological data vis-à-vis the two hypothetical sites would not be assessed through GIS analyses as such biological analyses would be at too coarse a scale for spatial analyses. However, incorporating on-site measurements of biota, although more difficult than GIS analyses due to sampling and identification efforts, provides additional information germane to accurately assessing relative wetland condition (Karr and Chu 1999). In addition, combining GIS analyses with on-site biological data can improve the accuracy, precision, and sensitivity of spatial metrics by providing causal pathways to perceived variance in the dataset, and may instigate future improvements in assessment methods.

These observations lead to the following hypotheses. First, it is hypothesized that measurable attributes (or metrics, such as the abundance of exotic taxa) of the flora (diatoms and macrophytes) and fauna (macroinvertebrates) of isolated depressional marshes in Florida are highly correlated with a GIS based measurement of landscape modification, the Landscape Development Intensity index, which is described below. Second, it is hypothesized that interregional compositional differences between the organisms sampled from within three wetland regions of peninsular Florida (after Lane

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2000), if present, will be attributed to latitudinal/physiological gradients and necessitate the development of unique measurable attributes for each wetland region. Third, it is hypothesized that surrounding land use, in addition to measured soil and/or water chemistry parameters within a given wetland, will exhibit a controlling effect on the composition of sampled wetland assemblages.

GIS Measurements and Wetland Metrics

The Landscape Development Intensity index (LDI, Brown and Vivas *submitted*) was used to assess the development intensity of lands within 100m around each sampled wetland, independent of the wetland biota. The LDI was developed by Brown and Vivas (*submitted*) and assesses the intensity of various land uses by measuring the annual non-renewable energy flow, given in terms of emergy, or embodied energy (Odum 1995), for each land use. They calculated their emergy values by identifying the energy pathways for each land use (land use data obtained from local water management districts, based on 1995 aerial photos), obtaining the value in joules/year from published sources, and multiplying the joules/year value by solar-emjoules/joule (sej/J, Odum 1995) to relate the amount of energy to a solar unit per year (see Brown and Vivas *submitted*). The use of an independent energy-based spatial measurement in this study permitted the incorporation of many disparate processes (e.g., pesticide use, water diversion and ditching, plowing, road building) into a single, agglomerative value. The actual LDI calculation was made for a 100m buffer directly surrounding each wetland using the following equation: $LDI = \sum (LDC \cdot \% LU)$; where LDI is the Landscape Development Intensity index, LDC is the Landscape Development Coefficient (Table 1-1), the %LU is the percent of each land use within the 100m buffer directly surrounding each wetland. The relationship of land use intensity with other buffer sizes (18m, 250, 500m, 1000m) is

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Table 1-1. Landscape Development Intensity Disturbance Coefficients.

Land Use	Non-Renewable Empower Density (E14 sej/ha/yr)	Ln Non-Renewable Empower Density	LDI Coefficients
Natural System	0		1.00
Natural Open water	0		1.00
Pine Plantation	5.10	1.63	1.58
Woodland Pasture (with livestock)	8.00	2.08	2.02
Pasture (Bahia) (without livestock)	17.20	2.84	2.77
Recreational / Open Space (Low-intensity)	17.20	2.84	2.77
Low Intensity Pasture (Bahia) (with livestock)	33.31	3.51	3.41
Citrus	44.00	3.78	3.68
High Intensity Pasture (Bahia, with livestock)	46.74	3.84	3.74
Row crops	107.13	4.67	4.54
Single Family Residential (Low-density)	1077.00	6.98	6.79
Recreational / Open Space (High-intensity)	1230.00	7.11	6.92
High Intensity Agriculture (Dairy farm)	1349.20	7.21	7.00

Note: Modified from Brown and Vivas (*submitted*).

currently being investigated (M.B. Vivas, Department of Environmental Engineering Sciences, University of Florida, *personal communication*).

Strong correlations between measured wetland attributes and the LDI would suggest a connection between surrounding land use and nested community composition (Roth et al. 1996, Brown and Vivas *submitted*). The relationship between any given metric and the LDI would be further strengthened through evidence of accuracy,

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precision, and sensitivity to changes in the LDI. High variance within metric responses for any given LDI could suggest causal mechanisms affecting community composition that were not accurately captured by the LDI, such as sources of stress not visible to GIS analyses (i.e., low relief ditch from degraded water source). An examination of metric sensitivity to LDI values would permit the development of land use thresholds for various assemblages, and furthermore may provide predictive ability for future wetland composition in areas undergoing land use change.

Regional Composition

Testing the effects of physiographic and/or climatic regions on wetland community composition can provide germane information on the development of ground-based biometrics. Lane (2000) delineated freshwater wetlands of Florida into four regions based on a climatic and physiographic model. Composition may differ between regions due to physiographic and climatic differences and possibly render biometrics developed in one area of the state impotent in another wetland region.

Environmental Correlates and Assemblage Response

Strong correlations between LDI values and community composition would suggest a causal mechanism and may assist in explaining metric relationships with the LDI. Developing an understanding of water and soil environmental parameters correlated with community composition may also provide the means for environmental managers to address specific ecosystem driving forces affecting wetland systems. In addition, strong correlations between environmental parameters and community composition can advance the knowledge of autecological relationships for diatom, macrophyte, and macroinvertebrate taxa.

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Background

With passage of the Clean Water Act (1972, "Water Pollution and Control Act"), states were charged with the task to "restore and maintain the chemical, physical, and biological integrity of the Nation's waters," including wetlands (Water Pollution and Control Act §101(a), Danielson 1998). Adhering to this mandate for wetland systems required first that a working definition for wetland biological integrity be defined, and second that a method to ascertain the relative ecological state of wetlands be developed.

Integrity, as it has been applied to Clean Water Act mandates and thus may be applied to Florida's wetlands, has been defined as "the ability of an aquatic ecosystem to support and maintain a balanced, integrated, adaptive community of organisms having a species composition, diversity, and functional organization comparable to that of the natural habitats of the region" (Karr and Dudley 1981, pg. 60). Implicit within this definition is maintenance of ecosystem driving forces that operate at different spatial and temporal scales to maintain the resilience and pattern of the ecosystem, such as the proper hydrology, fire regime, and cross-scale community interactions (Figure 1-1, Peterson et al. 1998, Gunderson and Pritchard 2002).

Historically, aquatic monitoring and assessment inferred ecosystem condition from either toxicity samples or ambient water quality monitoring (Karr 1981, 1993, McCarron and Frydenborg 1997). At best, these data were rudimentary in their ability to reflect more than a temporal constituent concentration within the water body. If a toxicity parameter was not specifically targeted for detection, then the water body could be considered "clean" yet replete with undetected toxics, metals, or physically altered such that it no longer resembled a fully functioning water body. Additionally, synergisms within water bodies from multiple impacts or "spike" loading events were

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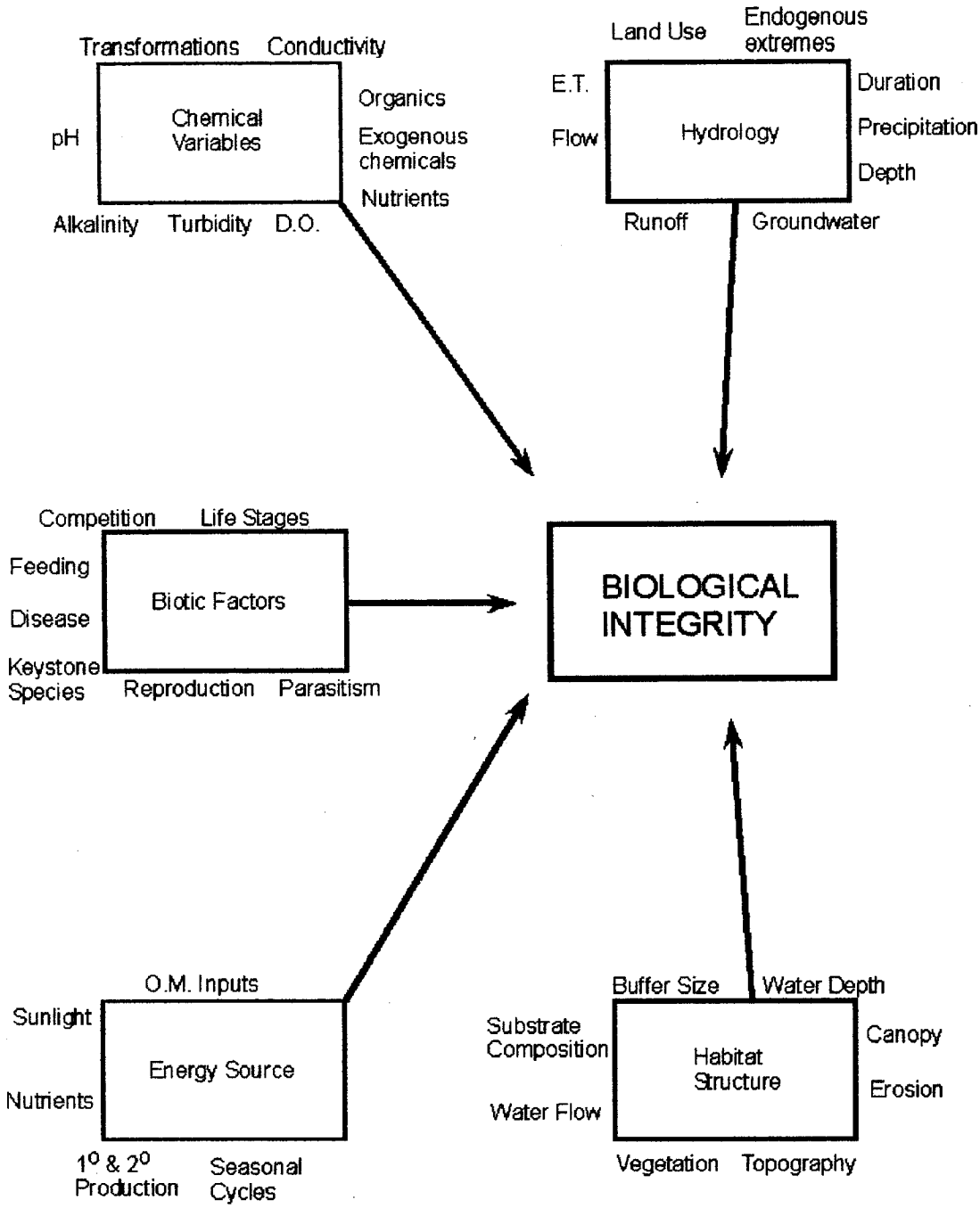

Figure 1-1. Schematic of the relationship between various ecosystem influences and biological integrity. (Modified from U.S. EPA 2002a).

typically not accounted for in toxicity or ambient monitoring programs (Karr 1993, Karr and Chu 1997).

In the late 1980s, the United States Environmental Protection Agency realized the potential of biological criteria to assess the Nation's waters and mandated states to use

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biological indicators to achieve the goals of the Clean Water Act (United States General Accounting Office 1988, U.S. EPA 1990, Karr 1995). A premise behind the use of biological indicators versus synoptic physical or chemical sampling to assess aquatic condition was that due to their intricate relationship with their environment, organisms *in situ* reflect current conditions as well as integrate cumulative impacts (Karr 1981, Hunsaker and Carpenter 1990, Karr and Chu 1999). Biological criteria and monitoring programs embraced by the U.S. EPA have since been utilized to assess lakes and streams throughout the United States (Barbour et al. 1996, Karr and Chu 1999, Barbour et al. 1999, Gerritsen et al. 2000). Only recently have efforts be undertaken to assess wetland condition using biological criteria (U.S. EPA 2002a).

While other assessment methods exist, such as the hydrogeomorphic (HGM) approach (Brinson and Reinhardt 1996), or rapid, "best scientific judgment" approaches (e.g., Wetland Evaluation Technique–WET, Adamus et al. 1991; Wetland Rapid Assessment Procedure–WRAP, Miller and Gunsalus 1997), the U.S. EPA has supported the use of multimetric indices of biotic integrity, or IBIs (*sensu* Karr 1981), to assess the condition of wetland resources (U.S. EPA 2002a). A multimetric index of biotic integrity combines measured biological attributes (or metrics) of trophic, community, and/or functional structure of various species assemblages within a wetland ecosystem into a single value. Ideal metrics are accurate, highly sensitive to change, and precise. The developed index can then be compared to either a reference condition (a suite of previously characterized wetlands located in undisturbed landscapes such as state parks and preserves) or along a gradient defined by changes in land use (Reynoldson et al. 1997, Karr and Chu 1999, Brown and Vivas *submitted*). The condition of the aquatic

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system in question is based on the relative similarity of the metric value obtained from that system vis-à-vis previously measured metrics in similar aquatic systems located in "reference conditions."

Wetland Trends

Wetlands on the Florida landscape provide services to the state including water retention and flood attenuation, water quality improvement and nutrient immobilization, aquifer recharge, biogeochemical processes, wildlife habitat, and climate regulation. Historically, wetlands covered more than half of Florida, approximately 8.2 million hectares (Figure 1-2). Ditching and filling for agricultural operations, water control, and urban development over the past 200 years have substantially decreased the abundance and functional capacity (i.e., habitat, water storage) of wetlands on the Florida landscape (Dahl 1990, Kautz 1993). A historical perspective of the areal extent of marshes in Florida and concurrent increases in agricultural and urban landscapes from 1936 – 1987 is presented in Table 1-2. Approximately 23% of Florida (3.6 million ha) is currently classified as inland freshwater wetlands by the National Wetlands Inventory (Table 1-3), of which greater than 34% are palustrine emergent marshes, the focal wetland class for this study (Doherty et al. 2000).

Wetlands are typically found in topographic lows in the landscape and tend to accumulate nutrients, metals, and toxins from up-slope sources as rainfall percolates through the wetland basin (Kirkman et al. 1998, Mitsch and Gosselink 2000). The constituents of surface and sub-surface flow, if transported into down-slope wetlands, can result in changes in processes and floral and faunal composition of receiving wetlands.

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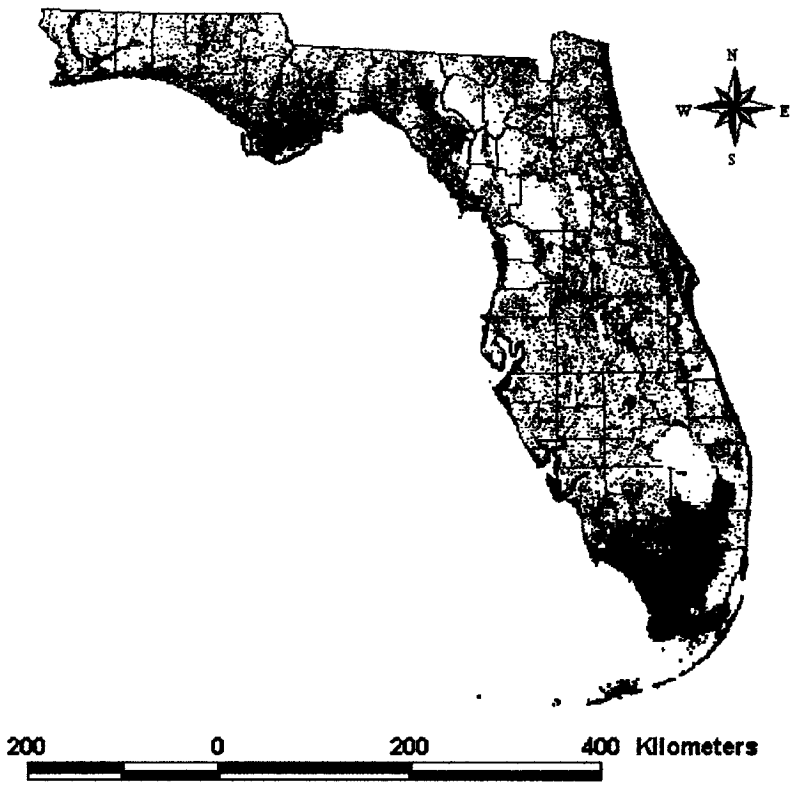

Figure 1-2. Approximate landscape extent of wetlands in Florida. Riverine, palustrine, and estuarine wetlands are indicated in black on the map. (Modified from Lane 2000).

Table 1-2. Historical perspective of marsh extent (in millions of hectares) on the Florida landscape and concurrent increases in agricultural and urban land use.

Land Use	1936	1949	1959	1970	1980	1987
Marsh	2.82	2.15	2.45	1.48	1.23	1.25
Agriculture	2.45	2.73	3.29	4.13	4.38	4.17
Urban	0.29	0.43	0.72	1.16	1.46	1.89

Notes: Values from 1936 are estimates. Modified from Kautz (1993).

Should loading rates be high enough, wetlands can even become sources of exogenous compounds for other aquatic systems connected during high rainfall events (Agami et al. 1990, Flaig and Reddy 1995).

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Table 1-3. National Wetland Inventory categories and landscape abundance of freshwater wetlands in Florida.

NWI Code	Wetland Class Description	% Wetland Area	% State Area
R 2 AB	Riverine, Lower Perennial, Aquatic Bed	<1	<1
R 2 EM	Riverine, Lower Perennial, Emergent, non-persistent	<1	<1
R 3 AB	Riverine, Upper Perennial, Aquatic Bed	<1	<1
R 4 SB	Riverine, Intermittent, Streambed	<1	<1
L 1 AB	Lacustrine, Limnetic, Aquatic Bed	<1	<1
L 2 AB	Lacustrine, Littoral, Aquatic Bed	1	<1
L 2 EM	Lacustrine, Littoral, Emergent, non-persistent	<1	<1
P AB 3	Palustrine, Aquatic Bed, Rooted Vascular	1	<1
P AB 4	Palustrine, Aquatic Bed, Floating Vascular	<1	<1
P EM	Palustrine, Emergent	34	8
P SS	Palustrine, Scrub Shrub	11	3
P FO 1	Palustrine, Forested, Broad-Leaved Deciduous	6	1
P FO 2	Palustrine, Forested, Needle-Leaved Deciduous	9	2
P FO 3	Palustrine, Forested, Broad-Leaved Evergreen	5	1
P FO 4	Palustrine, Forested, Needle-Leaved Evergreen	11	2
P FO 6	Palustrine, Forested, Deciduous (mixed)	<1	<1
P FO 7	Palustrine, Forested, Evergreen (mixed)	22	5
		~100	
	% NWI inland freshwater wetlands		23
	% upland area		63
	% other (estuarine) wetlands and deepwater habitats		14
	State total area		100

Source: Doherty et al. (2000)

Effect of Land Use Change on Wetland Systems

Changes in land use within wetland watersheds (hydrologic units where precipitation falling within will move via percolation and interstitial flow or overland flow to the wetland) can cause significant changes in wetland structure and function. Some of these changes are: 1) increased nutrient (mainly phosphorus) and chemical loading from lands shifted into production (Flaig and Reddy 1995, Bedford 1999, Daoust and Childers 1999, Maul and Cooper 2000), 2) selective herbivory and trampling from

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cattle (Winchester et al. 1985, Long et al. 1986, Blanch and Brock 1994, van Oene et al. 1999, Grace and Jutila 1999, Reader and Craft 1999, Vulink et al. 2000), 3) altered hydroperiod due to ditching or filling (Anderson and Vondracek 1999, Euliss and Mushet 1999, Kirkman et al. 2000, Babbit and Tanner 2000), 4) increased sedimentation due to ditching or removing topsoil (Martin and Hartman 1987, Freeland et al. 1999, Shutler et al. 2000), 5) colonization of invasive exotic organisms (Bedford 1999), 6) destruction of wetland/upland transition zones (Winchester et al. 1985), and 7) alteration of landscape-level processes such as fire frequency (Moss 1992, Kirkman et al. 1998, Bedford 1999, Semlitsch 2000). Of particular relevance to this study are the effects of nutrient and chemical loading, selective herbivory and trampling by cattle, and ancillary effects of changes in wetland watershed land use. As such, a review of those three effects is provided below.

Nutrient and Chemical Loading

Nutrient and chemical loading of wetland systems alters community composition (Davis and Ogden 1994, Freeland et al. 1999, Doherty et al. 2000). Changes in land use development surrounding wetlands can create large stores of exogenous nutrients, which can act as potential sources for ecosystem-altering nutrients and chemicals. Graetz and Nair (1995), studying dairy and beef ranching lands in Okeechobee County, Florida, found mean concentrations of total phosphorus (TP) in the soil of 5300–6500 ug/kg, approximately 40% of which was soluble reactive phosphorus readily available to organisms. Graetz and Nair (1995) also found significant differences in soil phosphorus along a gradient of grazing intensity from 79 kg/ha in natural areas (zero grazing pressure) to 4939 kg/ha in intensely grazed dairy feedlots. Rainfall and the subsequent movement of surface and sub-surface flows through phosphorus (P) laden landscapes can

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mobilize and deliver P into wetlands and other aquatic systems, resulting in alterations in community composition, structure, and function with potential effects on food web dynamics (Winchester et al. 1985, Moss 1992, Flaig and Reddy 1995, Daoust and Childers 1999, Freeland et al. 1999, Maul and Cooper 2000). For instance, Freeland et al. (1999) reported that periphyton assimilating increased exogenous P caused shifts in macrophyte species composition (Freeland et al. 1999). In studies in the Everglades (Davis and Ogden 1994), higher plants, such as *Typha* spp. tolerate nutrient loading and out-compete other less tolerant macrophytes, such as *Cladium jamaicense*. Likewise, floating plants *Lemna* spp., which grow readily in nutrient enriched waters, can become so prolific to create oxygen stress for aquatic communities in the water column (e.g., fish, macroinvertebrates, microbes, macrophytes) through shading of photosynthetic, oxygen-producing algae and other macrophytes (Dierberg and Brezonik 1984).

The direct nutrient loading of wetland systems can also occur as a result of land use development intensity in the surrounding wetland watershed (Graetz and Nair 1995). Tanner et al. (1984) tracked cattle movements on a South Florida rangeland and found that cattle spent the majority of time in herbaceous wetlands (fall months) or upland/herbaceous wetland ecotones (summer months). They also reported no significant difference in cattle defecation/urination location among herbaceous wetlands, upland/herbaceous wetland ecotones, canal banks, or flat woods, suggesting near constant landscape loading of available nutrients. Several authors suggested encouraging cattle to graze in marshes during summer as *Panicum* spp. and *Andropogon* spp. typically have the highest crude protein levels of any available forage during that period (Tanner et al. 1984, Kalmbacher et al. 1984, Long et al. 1986). Direct grazing within the marsh could

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lead to higher concentrations of P and N as there would be no biochemical degradation of the nutrient constituents in the process of overland or interstitial flow before their deposition in the wetland.

Reddy et al. (1996) reported that the uptake of available phosphorous by wetland vegetation was estimated at 28%-70% of the available P transported to the wetland. This P uptake is dependent upon plant growth rate, growth structure (woody or herbaceous material), P concentration in the soil, and the physical and chemical characteristics of the soil (Agami et al. 1990, Flaig and Reddy 1995). However, aquatic plant storage of P is a temporary phenomenon as approximately 80% of the nutrients are released to the water column by decaying vegetation (Agami et al. 1990, Reddy et al. 1996), suggesting that wetland plants are nutrient storages, and not sinks.

Agrochemical application within a wetland basin may also affect wetland processes and functions. Movement of pesticides, herbicides, and agrochemical fertilizers into wetlands is generally related to either direct or drift application or absorption of the chemical onto soil particles, which are subsequently mobilized into the wetlands during rainfall events (Anderson and Vondracek 1999, Freeland et al. 1999). Martin and Hartman (1987) reported influx of sediments to wetlands in cultivated landscapes at twice the rate from uncultivated landscapes. Euliss and Mushet (1999) noted that chemicals and chemical-laden soil particles transported from uplands into wetlands could be toxic to zooplankton and reduce foraging and food assimilation rates of aquatic invertebrates. Semlitsch (2000) and Shutler et al. (2000) reported impacts to non-target wetland organisms from pesticides. Several authors found changes in wetland

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community assemblages due to agrochemical loading (Anderson and Vondracek 1999, Bedford 1999, Kolozsvary and Swihart 1999, Maul and Cooper 1999).

Selective Herbivory and Trampling

While low-density grazing in rangeland (native forage) maintains species richness and mimics natural processes, such as foraging by deer or other herbivores (Blanch and Brock 1994), high-density herbivory by cattle can alter native upland and wetland vegetation communities and have long-term successional consequences (Dobkin et al. 1998). Tanner et al. (1984) and others (Kalmbacher et al. 1984, Long et al. 1986) noted that cattle selectively graze wetland plants on the landscape, thus altering community composition. Van Oene et al. (1999) found that grazing prevented dominance of tall wetland species of grasses, shrubs, and trees, as they were selectively harvested by cattle, which led to structural and functional changes in the wetland, such as decreased wildlife habitat. Vulink et al. (2000) reported that wetland vegetation community changes occurred due to modifications of light availability as livestock consumed the shading vegetation. Additionally, Vulink et al. (2000) found that grazing substantially impacted plants with apical meristems within the reach of livestock, which altered community composition and structure.

Reader and Craft (1999) and Vulink et al. (2000) demonstrated that the trampling and physical destruction of wetland vegetation by livestock directly altered community structure and composition. In addition, several authors have noted increased fluxes of nutrient and sediment flow into wetlands through destruction of vegetative buffers, such as the fringing *Hypericum* spp. or *Serenoa repens* (Winchester et al. 1985, Blanch and Brock 1994, Vulink et al. 2000).

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Ancillary Impacts from Landscape Modification

Changes in landscape level processes as a result of modification of wetland watershed land use have effects on wetland composition and function (Bedford 1999). Wetlands in cultivated and urban landscapes have highly variable water-level fluxes compared to wetlands in non-developed landscapes, which can affect soil anoxia and wildlife habitat (Rushton and Carr 1993, Tilley and Brown 1998, Euliss and Mushnet 1999). In order to facilitate movement of water off the landscape, wetlands in agricultural land use matrices are often inter-connected by ditches that can decrease the hydroperiod of all wetlands if attached to an outflow (such as a canal or stream), which can provide conditions for colonization by upland taxa. If not connected to an outflow, an increased hydroperiod may result from ditching as water is rapidly shunted off the landscape and into the wetland, instead of moving slowly via overland and sub-surface flows. In some cases, canals and streams can back-flood connected marshes, altering hydrology and possibly allowing marsh access to stream/canal species (such as predatory fish), with potential impacts to wetland fauna that require fish-free habitat for breeding (Babbit and Tanner 2000).

Florida ecosystems have evolved with fire as a driving force to the extent that some organisms require fire for reproductive success (Ewel 1990). In wetland watersheds used for agricultural purposes, fire is typically excluded (Kirkman et al. 1998). Whereas the fire season in Florida is related to lightning strikes, which are highest in summer, prescribed burns currently occur in spring (Kirkman et al. 1998). This alteration to the timing and extent of natural processes may affect reproduction and foraging success of wetland organisms, which have evolved with seasonal fire.

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Wetland diversity across the landscape (i.e., the number of wetlands in a given area) is also affected through alterations of wetland watershed land use that include ditching, draining, and filling (Kirkman et al. 1996, Kirkman et al. 1998, Kolozsvary and Swihart 1999). Whereas depressional marshes may be scattered on the landscape and provide metapopulation dynamics (i.e., increased gene flow) of organisms between wetlands, loss of wetlands on the landscape decreases landscape diversity and can cause increased crowding and subsequent decreases in population as organisms compete for decreasing resources and space (Pearlstine et al. 1997).

Wetland Assemblages and Biological Assessment

Various assemblages (i.e., macrophytes, macroinvertebrates, microbes, birds, etc.) within an ecosystem have been used as biological indicators for aquatic systems, including wetlands (U.S. EPA 2002b-e). Appropriate indicator assemblages are those that measurably respond to the gradient defined along the abscissa (i.e., land use change, total phosphorus, etc.), and ideal indicator assemblages are also composed of organisms relatively easy to identify, abundant, and available for sampling or mensuration for an extended period (Karr and Chu 1999). Appropriate metrics derived from indicator assemblages are accurate, exhibit high precision, and are likewise sensitive to increases in change along the abscissa (Karr and Chu 1999).

As most aquatic assemblages respond to alterations occurring at both large (i.e., abundance of croplands in a wetland basin) and small scales (i.e., pH alterations; Allan and Johnson 1997), determining the particular stressor or component of anthropogenic disturbance that an aquatic assemblage responds to is often difficult. Various scales of temporal response further compound the identification of stressors. Incorporating multiple assemblages (i.e., macrophytes and macroinvertebrates) can assist in

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determining sources of perturbations, especially if the autecological requirements of the selected assemblages are well established (e.g., Reed 1986, Lenat 1993, Van Dam et al. 1994). Characterizing various edaphic, hydrologic or landscape variables and identifying trends can also assist in elucidating sources of wetland perturbation.

By including multiple assemblages in the initial development of biological indicators, the likelihood increases that a suite of potential metrics can be identified that are strongly correlated with the abscissa. In addition, sampling multiple assemblages permits an examination of the temporal responses of wetland organisms to perturbations, which, depending on the speed of community change, has the potential to alter conclusions. For instance, diatoms respond extremely rapidly to environmental conditions, on the order of a few hours to a few days, macroinvertebrates respond slower (with turnover times ranging from hours to years), and macrophytes generally react the slowest to alterations (responses occur over days to decades). Substantial information also exists on the natural history and autecological requirements of diatoms (Bahls 1993, Van Dam et al. 1994), macroinvertebrates (Hilsenhoff 1988, Lenat 1993, Barbour et al. 1999), and macrophytes (Reed 1997, Taft et al. 1997).

Plan of Study

In this study, diatoms, macrophytes, and macroinvertebrates were sampled from isolated depressional herbaceous wetlands located within human-modified land use matrices throughout peninsular Florida. Compositional differences among sampled assemblages driven by latitudinal or physiographical regions were examined to ascertain the importance in developing regionally specific biological assessment metrics. Analysis of water and soil physical-chemical parameters and land use identified variables affecting the composition and structure of sampled biotic assemblages in the study wetlands. GIS

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analyses assigned empirically derived values to each wetland based on the abundance (% area) and land use intensity of developed lands within a 100m boundary around each wetland site. Compositional and functional attributes, or metrics, that correlated with the empirically derived GIS land use measure were identified from each assemblage and combined into three unique assemblage-specific field based indices of biological integrity. In addition, the ability of the GIS land use measure to accurately and precisely reflect the environmental conditions on-site was assessed through examination of the variance within metric responses. Conclusions on the use of the wetland organisms and GIS based measures of relative wetland condition and suggestions for improvements were made.

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CHAPTER 2
DIATOMS AS BIOINDICATORS

Introduction

Diatoms (Class Bacillariophyceae) are important components of wetlands and other aquatic systems and provide several functions including: 1) primary production, 2) nutrient and biogeochemical cycling, 3) oxygen cycling, 4) water chemistry regulation, and 5) physical barriers to erosion (Rader and Richardson 1992, Stevenson et al. 1996, Goldsborough and Robinson 1996, McCormick et al. 1997, Doherty et al. 2000, U.S. EPA 2002e). Stream and lake researchers, and more recently wetland researchers, noted the response of diatoms to land use changes, increases in nutrient loading, alterations in pH, and changes in specific conductivity (Lange-Bertalot 1979, Whitmore 1989, Bahls 1993, Van Dam et al. 1994, Pan and Stevenson 1996, McCormick and Cairns 1997, Danielson 1998, Barbour et al. 1999, Stevenson 2001, U.S. EPA 2002e). The U.S. EPA (2002e, p.2) reports diatoms as "amongst the most widely used indicators of biological integrity and physico-chemical conditions in aquatic ecosystems." Changes in diatom taxonomic composition correlated with changes in physical-chemical parameters have been noted in the Florida Everglades (Raschke 1993), prairie potholes (Adamus 1996), riverine bottomland wetlands, marshes, swamps, and bogs (Pan and Stevenson 1996, Stevenson et al. 1999), streams (Bahls 1993, McCormick and Cairns 1997, Barbour et al. 1999, Winter and Duthie 2000, Munn et al. 2002), large rivers (Fore and Grafe 2002), lakes in Florida (Whitmore 1989), and other systems (see McCormick and Cairns 1997). The diatom community response has also been examined for heavy metals (Charles et al.

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1996), pH (Pan and Stevenson 1996), saprobity levels (Lange-Bertalot 1979), nutrient and physical-chemical parameters (Van Dam et al. 1994), and other perturbations (see reviews in Stoermer and Smol 1999).

In this chapter, diatoms community attributes were correlated with the Landscape Development Intensity index (LDI, Brown and Vivas *submitted*). Strongly correlated attributes (or metrics) identified through regression with the LDI were scaled so disparate metrics could be combined. The scaled metrics were summed to create the Diatom Index of Wetland Condition (DIWC). Analysis of measured abiotic and biotic environmental data identified parameters strongly correlated with diatom composition to advance synecological understanding on driving forces affecting the distribution and composition of wetland diatoms. Variance within diatom metrics was examined to explore relationships between landscape scale measurements and local, wetland specific responses.

Methods

Site Selection and Agricultural Development Gradient

Seventy isolated depressional marshes throughout peninsular Florida were sampled in landscape matrices with varying amounts of human-modified land uses during summer 1999 and 2000. Using best scientific judgment, sites were initially stratified into reference (no obvious human modified landscapes within >100m), and impaired (obvious human landscape modification within 100m) categories. The Landscape Development Intensity index (LDI, Brown and Vivas *submitted*) was later independently calculated through GIS analyses for each site by identifying varying land use abundance within each wetland basin (100m around wetland edge). LDI scores of <2.0 were characteristic of minimally disturbed ("reference") wetlands and allowed the reference condition to

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incorporate small disturbances such as low density park paths. Slightly less than half the sites (33) were defined as reference sites, which were generally located in state and federal parks, preserves, and state forests. The remaining 37 wetlands were located within agricultural landscape matrices and were considered "impaired" sites (LDI > 2.0). Twenty-seven impaired sites were located in cattle ranches of varying stocking densities, 7 were located in truck crops (tomatoes, peppers), 2 were in citrus groves, and 1 was sampled in a silvicultural forest. All sites selected were hydrated when sampled. Site coordinates and LDI score for each sampled wetland may be found in Appendix A.

As isolated depressional marshes are not uniformly distributed throughout Florida, the sites were likewise generally located in counties with the proper wetland hydrology and geomorphic setting (Lane 2000, Figure 2-1). The sites were initially stratified into three peninsular wetland regions, or areas of similar climatic and physiographic conditions (after Lane 2000). A compositional test, the multiple response permutation procedures (MRPP) available in PCOrd (MjM Software, Gleneden Beach, Oregon, version 4.10) was used to identify the ecological significance of the modeled wetland regions for diatom distribution. MRPP (Mielke 1984, Mielke and Berry 2000) provides a rank-based non-parametric multivariate test of the difference between *n* samples based on a distance matrix calculated between sites in ordinate space. The Sorensen distance measure was used for this test, as it is an effective distance measure for use in community data (McCune and Grace 2002). MRPP returns a statistic, *A*, which is the "chance-corrected within group agreement" (McCune et al. 2002). Zero values for *A* indicate that heterogeneity within groups equals that expected by chance, and values of 1.0 indicate perfect agreement between groups (McCune and Grace 2002, McCune et al. 2002).

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MRPP also returns a T value test statistic. The more negative the test statistic T the greater the separation between test groups (McCune and Grace 2002). The probability value, p, is based on the calculated possibility of finding differences as or more extreme

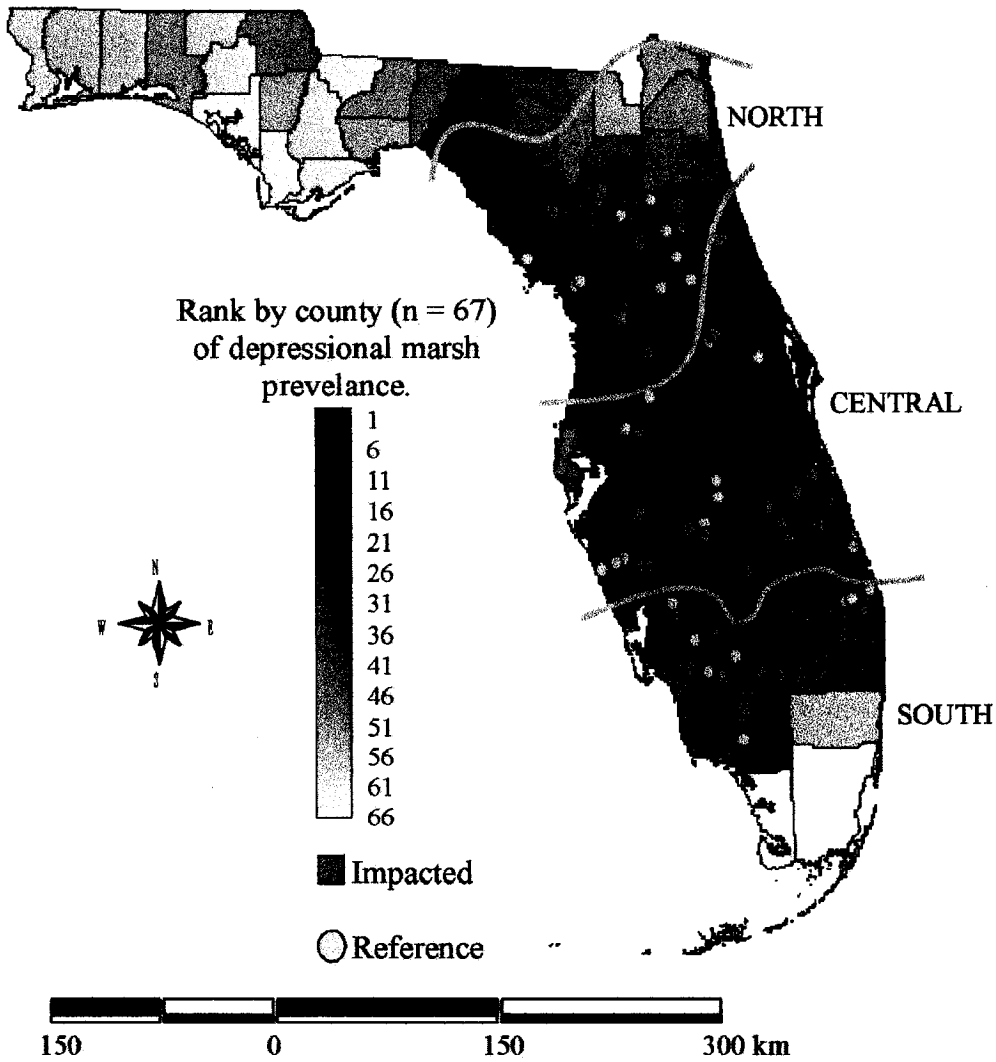

Figure 2-1. Algal study site locations and encounter probability for isolated depressional wetlands. Counties shaded darker have a higher density of isolated depressional wetlands <1ha (from U.S. Fish and Wildlife Service 2000).

than the observed group differences (Lesica et al. 1991). MRPP was calculated to compare composition across all regions (e.g., South versus Central versus North), and was also iteratively calculated to compare between individual wetland regions (e.g., South region composition versus North region composition). Significance

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for this test, as for all tests unless otherwise noted, was set at the conventional significance of 0.05.

Field Data Collection, Sample Preparation and Laboratory Procedures

Diatoms

Diatom, soil, and water physical-chemical samples were taken at each wetland, and care was exercised to ensure minimal cross-sample contamination of diatom communities. Three different types of diatoms were sampled: benthic, epiphytic, and phytoplanktonic. Benthic diatoms were collected from wetland sediments. Epiphyton was collected from submerged portions of macrophytes. Phytoplankton was sampled from the water column.

Benthic diatoms were sampled throughout hydrated areas of each wetland. Ten samples were proportionally distributed in a randomized fashion among the vegetation zones and a composite sample fixed. For each sample, a hollow cylinder with a diameter of approximately 10 cm was placed on the substrate, and a bulb pipette was used to mobilize the material at the water/sediment interface. A 10mL sample was taken with the bulb pipette and placed in a composite sampling jar. The total sampled volume in the composite sample was approximately 100mL. Epiphytic diatoms were sampled at ten locations in the wetland by snipping representative submersed and floating vegetation and placing the samples into a 3.8L Ziploc bag. Approximately 0.5L of wetland water was added and the bag gently kneaded to mobilize the epiphyton. A 10mL sample was obtained using a bulb pipette rinsed with wetland water and placed into a composite sampling jar. Total volume of the composite sample was approximately 100mL. Phytoplanktonic diatoms were sampled at ten different locations through the wetland in areas of at least 10cm of water. A sealed 100mL cylinder was placed under the surface of

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the water, the cap was removed and the cylinder filled. The cap was replaced underwater, the cylinder removed, and a composite sample taken. The total volume of the composite phytoplankton sample was approximately 1L. Once all ten samples were taken of each diatom type, the composite samples were preserved using M3 (APHA 1995): 5mL for benthic and epiphytic diatoms, and 20mL for phytoplanktonic diatoms.

All samples were homogenized prior to subsampling for identification. To clean organic matter from the diatom frustules for identification the subsamples were digested following Hasle and Fryxell (1970). The samples were rinsed using distilled water and mounted on microscope slides using Naphrax (Northern Biological Supplies Limited, Ipswich, England). Following FDEP procedures (SOP #AB-03.1, available at www.dep.state.fl.us/labs/sop/), 250 diatom valves were then counted along microscope transects and identified to the lowest taxonomic level possible (usually species). For samples that were sparse (i.e., phytoplankton), sampling effort was capped at 1 hour. Diatom data were combined and entered into the FDEP database for further analysis.

Soil and water physical-chemical sampling and analysis

To relate the distribution of wetland diatoms to environmental parameters, soil and water physical and chemical parameters were obtained at each site and determined in the laboratory as described in Appendix B. The following soil chemistry parameters were determined in the lab: total phosphorous (soil TP, mg/kg), percent total nitrogen (%TN), percent total carbon (%TC), percent organic matter (%OM), and pH. The following water chemistry parameters were obtained from samples collected from each site: color (PCU), specific conductivity (umhos/cm), turbidity (NTU), pH, ammonia (mg/L), nitrate/nitrite (mg/L), total Kjeldahl Nitrogen (TKN, mg/L), and total Phosphorus (water TP, mg/L). In the event that the constituent measured was below detection levels,

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the value was entered for analysis was half of the lowest detectable level. See Appendix B for additional information.

Data Analyses

Summary statistics

Summary measures of sample richness, evenness, Shannon diversity, and Simpson diversity were calculated for each site and diatom type and provided information on the distribution of species vis-à-vis land use. Richness is a measure of the total number of taxa found in a sample (Whittaker 1972). Evenness is a measure of distribution among taxa at a site. Shannon diversity and Simpson diversity indices measure the diversity of a site based on the abundance and total number of taxa (Brower et al. 1990). Two additional diversity indices were also calculated from the diatom data and compared between LDI classes of <2.0 or ≥ 2.0: beta and gamma diversity (Ricklefs 1990, McCune and Grace 2002). Gamma diversity, or species richness over a range of habitat (i.e., reference conditions, Whittaker 1972), was calculated as the total richness of a class of samples (LDI classes of <2.0 or ≥ 2.0). Beta diversity incorporates gamma diversity and indicates compositional variation in a sample. Beta diversity was calculated as gamma diversity divided by the average richness at a site (McCune and Grace 2002).

Diatoms-compositional tests

The Percent Similarity Index (PSI, Wolda 1981) and MRPP were used to assess the fidelity of habitat types for each of the three types of diatoms sampled. PSI (Wolda 1981) provides an index of overlap between diatom types and was calculated as

$$PSI = [\sum (\text{minimum } p_{ij}, p_{ik})] \cdot 100$$

where PSI is the percentage similarity between diatom types j and k (for instance benthic and epiphytic diatoms), p_{ij} is the percentage of species i in diatom type j, and p_{ik} is the

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percentage of species *i* in diatom type *k*. PSI was calculated to compare local, within-site diatom community composition (i.e., benthic, epiphytic, and phytoplanktonic diatom types from a single site) and for a global comparison (i.e., the abundance of all benthic, epiphytic, and phytoplanktonic diatoms identified were compared across all sites). While no significance was calculated, PSI values greater than 0.70 are generally considered highly similar (Matthews et al. 1988).

MRPP, using the Sorensen distance measure, was also used to identify global group differences and provided a measure of the significance of the difference. Differences in summary statistics, such as evenness and diversity indices, were examined between landscape development classes (i.e., LDI <2.0 and LDI ≥ 2.0) with the Mann-Whitney U-test, and among diatom types using Tukey's Honest Significant Difference (HSD) test (Zar 1999). The Mann-Whitney U-test and Tukey's HSD test are non-parametric comparison procedures that test for equal medians between groups. The Mann-Whitney U-test was performed using two classes (i.e., LDI <2.0 and LDI ≥ 2.0) and Tukey's HSD was calculated for tests of more than two classes (i.e., South, Central, and North regions).

Diatoms community composition and correlation with soil and water parameters

The following soil variables were arcsine square root transformed to decrease measured skewness and kurtosis in the dataset: %TN, %OM, and %TC. Log_{10} transformations were completed on soil TP, color, specific conductivity, turbidity, ammonia, nitrate/nitrite, TKN, and water TP for similar reasons. Multivariate colinearity among environmental variables, described as strong correlations between independent variables (Zar 1999), was tested using two multicolinearity diagnostic statistics available through linear regression analysis in SAS, the variance inflation factor and tolerance (SAS 1990). While no formal cutoff value exists, a conservative measure was used, and

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variables were considered collinear if they had a variance inflation factor (VIF) of ≥5.0 and a tolerance of <0.20 (ter Braak 1987, SAS 1990). The Mann-Whitney U-test was used to test the null hypothesis of equal medians between reference and impaired classes for each environmental variable (Zar 1999).

The relationship between measured environmental values (including LDI score) and species composition was examined with the Mantel test (Mantel 1967) and with non-metric multidimensional scaling (NMDS; Kruskal 1964, Mather 1976), both available in PCOrd. A Mantel test, which evaluates the correlation between distance measures of two matrices (McCune and Grace 2002), was computed to test the null hypothesis of no relationship between the arcsine square root transformed diatom species abundance and measured environmental parameters. The Sorensen distance measure was used for the abundance matrix, and the Euclidean distance measure was utilized for the second matrix due to the negative values of the log-transformed environmental parameters. The significance of the hypothesis of no relationship between the matrices was tested with a Monte Carlo simulation of 9999 randomizations. The standardized Mantel statistic (Mantel's r), a t statistic, and the significance of the relationship were calculated for each test.

NMDS was used to ordinate the samples based on arcsine square root transformed species abundances and to correlate environmental parameters to sites in ordination space. NMDS is an ordination method well suited for data that are non-normal, as it uses ranked distances to avoid the assumption of linear relationships among variables (Waichler et al. 2001, McCune and Grace 2002). To decrease chances of finding local minima, twenty-five runs with random seeds were made using six-dimensional starting

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space and the Sorensen distance measure. The instability criterion was set at 0.00001, the maximum number of iterations to find a stable solution was set to 400, 50 runs were conducted with real data, and 50 randomized runs were completed to calculate a *p*-value of the solution. The number of dimensions that accurately described the data set was obtained as a measure of the "stress" (or fit) of the ordination. A final run was made with the starting seed that provided the best final fit. A two-dimension solution was selected for benthic and epiphytic diatoms, and a three-dimension solution was selected for phytoplanktonic diatoms, as increased dimensionality only marginally improved the fit. The final NMDS solution was repeated several times to ensure stability of the solution. Pearson's correlation coefficients between site scores and environmental parameters, including LDI score, were calculated. A bi-plot of environmental parameters with Pearson correlations to site scores >0.30 was constructed.

Metric Development

Diatom metrics were developed using literature sources and iterative tests (i.e., Bahls 1993, Van Dam et al. 1994, McCormick and Cairns 1997, Stevenson 2001, U.S. EPA 2002e, Fore and Grafe 2002, Fore 2003) and correlated using Spearman's *r* with the Landscape Development Intensity index (Brown and Vivas *submitted*). Final metrics were those with significance values of <0.05 and Spearman correlations of $\geq|0.50|$ with the LDI. Final metrics were also tested for bivariate correlation using Spearman's *r*. Metrics with bivariate correlations ($r \geq |0.80|$) reduced to one metric based on the strength of the correlations with the LDI. All metric evaluation were conducted with SAS (SAS Institute, Cary N.C., version 8.02).

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Sensitive and tolerant taxa

The use of Indicator Species Analysis (ISA) as a method of identifying species with specificity (i.e., exclusive to a given group) and fidelity (i.e., always present within a given group) to ecological conditions is growing in ecological literature (Chytry et al. 2002). The randomization test and the corresponding *p*-value provide a statistically rigorous and defensible presentation of identified species (McCune and Grace 2002). Indicator Species Analysis (Dufrene and Legendre 1997) with non-transformed abundance data identified species with significant association to LDI classification of impaired or reference conditions.

ISA, available in PCOrd, contrasts the fidelity of species between *a priori* defined groups (in this case, sites with LDI<2.0 or LDI≥2.0) using the algorithms of Dufrene and Legendre (1997). First, the mean abundance (x_{kj}) of species *j* in group *k* was calculated by examining the proportional abundance of species *j* in group *k* relative to species *j* in all groups. Second, the relative abundance (RA_{kj}) of species *j* in group *k* was calculated by dividing the mean abundance (x_{kj}) by abundance in each group. The proportional frequency (RF_{kj}) of species *j* in group *k* was then calculated by summing the total occurrences (presence) of species *j* in group *k* and dividing by the number of sample units in group *k*. The two proportions (RA_{kj} and RF_{kj}) combined in the following equation that solves for $IndVal_{kj}$, the indicator value of species *j* for group *k*:

$$IndVal_{kj} = 100 \cdot (RA_{kj} \cdot RF_{kj})$$

As RA and RF are multiplied, both must be high for IndVal to be high. Additional descriptions of ISA may be found in McCune and Grace (2002). A Monte Carlo test with 10,000 permutations and *p*-value of 0.10 was used to test significance of the indicator species for a particular group *k* (i.e., sites with LDI<2.0 or LDI≥2.0). The abundance of

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species with significant specificity and fidelity to reference or impaired conditions (i.e., sensitive and tolerant taxa) was assessed for each site and correlated with the LDI.

Autecological metrics

An ecological indicator value based on published tolerances to particular physical/chemical condition for each diatom species identified was determined using a coded checklist of autecological guilds (Appendix C; Bahls 1993, Van Dam et al. 1994). Bahls (1993) categorized diatoms in Montana streams as very tolerant, tolerant, or sensitive to pollution based on initial classifications of Lange-Bertalot (1979) and Lowe (1974). Van Dam et al. (1994) provided a list of attributes describing the tolerance of European diatoms to varying pH, salinity, dissolved oxygen content, nutrient enrichment, and saprobic conditions, which Fore (2003) and Fore and Grafe (2002) used to develop indicators for streams in Mid-Atlantic states and large rivers in Idaho, respectively.

As agricultural operations can increase soil alkalinity through fertilizer application, and evaporative salts from irrigation can be transported into wetlands through precipitation (Leland and Porter 2000, Fore and Grafe 2002), it was expected that alkaliphilous diatoms and diatoms that tolerate higher salinity would increase with increasing agricultural operations (and hence LDI scores). Agrochemical fertilizers, as well as direct application of cattle wastes on the landscape, increase nutrient loading into aquatic systems (Flaig and Reddy 1995, Reddy et al. 1996, Reddy et al. 1998). It was therefore anticipated that diatoms tolerant of higher nutrient levels and saprobity (or biodegradable organic matter and low dissolved oxygen levels), as well as higher trophic state (driven by high levels of inorganic nutrients), would increase with increasing LDI scores. In contrast, acidophilous diatoms and those indicative of low salinity would be expected to dominate wetlands in reference conditions. Similarly, diatoms sensitive to

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nutrient enrichment, low dissolved oxygen and saprobic conditions would be expected to decrease with increasing LDI scores.

For each of the autecological guilds identified by Van Dam et al. (1994) and the pollution tolerance value of Bahls (1993), diatoms were coded (guilds with between 3 and 7 categories) to denote tolerance to environmental variables. The ordinal trophic classes of Van Dam et al. (1994), "oligotraphentic" and "oligo-mesotraphentic," were combined into a single class as these diatoms indicative of low-trophic status were expected to respond in a similar fashion to increasing LDI scores. The abundance of diatoms for each class was determined for each autecological index. The abundance of diatoms for each class was then correlated (Spearman's r) with the LDI score for each site. Bivariate correlations between taxa guild values were assessed using Spearman's r, and a tolerance of $r \geq |0.80|$ was arbitrarily established to delimit highly correlated guild values. Guilds having lower correlations with the LDI were parsed. The median abundances for potential metrics based on autecological indices between LDI classes were tested with the Mann-Whitney U-test.

Constructing the Multimetric Index

To complete development of the multimetric index of wetland condition based on diatom metrics, it was necessary to scale the metrics to a uniform ranking so they could be added together and a single value representative of wetland condition be obtained (Karr and Chu 1999). In this study, metrics were scored into four categories: 0,3,7, and 10, based on quartile scores for the 95th percentile of each metric (Mack 2001). A hypothetical example of this scoring is presented in Figure 2-2. Using the 95th percentile decreases the influence of outliers on metric scoring (Mack 2001). For measures that decreased in abundance with increasing LDI scores, the lowest quartile was given a 0, the

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next quartile a 3, the third a 7, and the highest and last quartile a 10. This scale was inverted for measures that increased in abundance with increasing LDI scores.

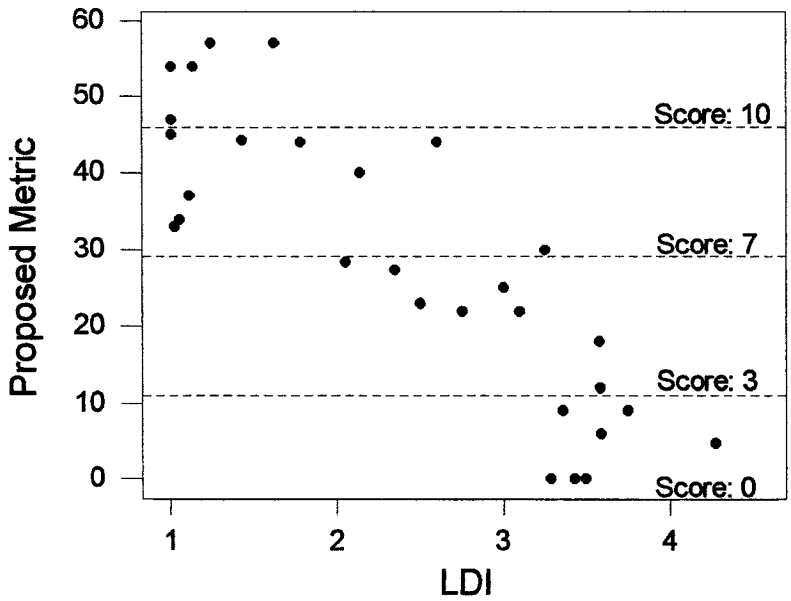

Figure 2-2. Hypothesized example of metric scoring using the 95th percentile of the dataset. The scoring would switch for metrics that increase with increasing LDI scores.

Results

Wetland Diatom Composition

Seventy herbaceous depressional wetlands throughout peninsular Florida with standing water were sampled in 1999 and 2000. Twenty-three were sampled in the South region, thirty in the Central region, and seventeen in the North region. Based on the dominant land use around the wetland, twelve sites sampled in the South region were *a priori* considered impaired, sixteen in the Central region, and eight in the North region.

Two hundred ninety-nine diatom taxa from the combined three diatom types were identified. Seventy benthic and sixty-nine epiphytic and phytoplanktonic samples were

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enumerated and identified, as data were incomplete for two sites: (IFASII - epiphyton and CMWRef – phytoplankton). The average count of frustules for benthic diatoms was 261.6 (+/- 19.7) and for epiphytic diatoms, 265.5 (+/- 31.9). The sparse nature of the phytoplanktonic diatoms resulted in an average of 67.9 diatoms identified in each sample and a high standard deviation of 57.7. One hundred ninety-five diatom taxa were identified in the benthic samples, one hundred seventy-four in the epiphytic samples, and two hundred forty-four in the phytoplankton samples.

Summary measures of sample richness, evenness, Shannon diversity, and Simpson diversity were calculated for each site (Brower et al. 1990). Summary statistics are presented in Tables 2-1 to 2-3. Tukey's HSD results (Table 2-4) indicated that richness was significantly higher in phytoplankton and benthic diatoms than in epiphyton. No significant differences were found for measures of evenness and Simpson's diversity measure, but phytoplankton and benthic diatoms, and benthic diatoms and epiphytic diatoms, did not significantly differ for Shannon diversity values (see Table 2-4). No significant differences were found for measures of richness, evenness, Shannon diversity, or Simpson's diversity between impaired and reference sites for all diatom types (Mann-Whitney U-test, Table 2-5).

Alpha (richness), beta, and gamma diversity values for each diatom type and wetland condition are given in Table 2-6. Beta and gamma diversity were higher in phytoplankton samples than at the other samples despite the low numbers of diatoms identified at each site. Beta diversity was higher in impaired sites for epiphytic and

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Table 2-1. Summary statistics of richness (S), evenness (E), Simpson's diversity index (H) and Shannon diversity index (D') for benthic diatoms.

Name	S	E	H	D`
Audubon	14	0.824	2.176	0.8380
BearScat	32	0.884	3.064	0.9336
BigCow	33	0.866	3.028	0.9296
BRSebastian	14	0.791	2.088	0.8246
CabPatch	10	0.705	1.623	0.6885
Caravelle	22	0.884	2.731	0.9194
Chuluota	23	0.817	2.561	0.8862
CLBayard	13	0.752	1.928	0.8053
CLCove	19	0.805	2.370	0.8694
CMWPast	24	0.820	2.606	0.8885
CMWRef	20	0.760	2.277	0.8359
COBurgle	21	0.827	2.518	0.8887
COHole	22	0.848	2.622	0.8872
Crew	24	0.799	2.540	0.8654
Deerfly	21	0.899	2.737	0.9171
DEMelon	18	0.661	1.912	0.7495
Garber	18	0.734	2.122	0.8230
GbarE	18	0.751	2.171	0.7914
GLDonut	28	0.797	2.654	0.8967
GLPont	26	0.797	2.597	0.8624
Goethe	14	0.820	2.165	0.8536
GreenSwamp	23	0.893	2.800	0.9212
HagueI	18	0.812	2.346	0.8759
HagueII	33	0.894	3.126	0.9424
HalfMoon	16	0.827	2.294	0.8711
HARare	19	0.766	2.255	0.8575
HEBad	9	0.796	1.748	0.7885
HEL2	16	0.768	2.129	0.8178
HEOkay	20	0.869	2.605	0.8998
HighPast	23	0.828	2.597	0.8884
HighRef	22	0.882	2.726	0.9160
HillsRef	23	0.914	2.865	0.9322
HuntCamp	30	0.879	2.989	0.9298
IFASI	15	0.802	2.173	0.8431
IFASII	12	0.791	1.966	0.8210
Immokalee	23	0.879	2.756	0.9206
IRBlueCypress	21	0.627	1.908	0.6833
IRCanal	12	0.675	1.676	0.6772
IROJ	28	0.857	2.857	0.9191
JD6	14	0.818	2.159	0.8373
KellyPark	11	0.847	2.031	0.8318
LCork	26	0.847	2.759	0.8962
LEGo	17	0.864	2.449	0.8913
LESuwan	20	0.861	2.579	0.9081
LLeeCounty	21	0.828	2.522	0.8769
MALudy	16	0.712	1.973	0.7781
MASpray	15	0.643	1.741	0.7564
McArthur	23	0.666	2.089	0.7236
MNElmer	16	0.725	2.009	0.7628
MNOcala	14	0.862	2.275	0.8769
MRPepper	26	0.813	2.649	0.8819
Myakka	14	0.827	2.182	0.8504
OKCara	17	0.861	2.439	0.8854
OKKISS	14	0.842	2.221	0.8476
OKPast	22	0.807	2.493	0.8791
PacificTom	17	0.825	2.338	0.8697
PallMar	14	0.717	1.892	0.7778
PBCorbett	11	0.745	1.787	0.7686
PBEnjay	10	0.738	1.698	0.7783
Penner	13	0.483	1.240	0.4755
POWales	10	0.757	1.744	0.7956
POWeowak	19	0.816	2.404	0.8797
RiceCreek	20	0.895	2.680	0.9178
SandhillCrane	11	0.661	1.585	0.6512
SANorthMya	11	0.815	1.954	0.8178
SAOscer	12	0.733	1.820	0.7762
Savannas	16	0.811	2.250	0.8412
STCow	14	0.672	1.773	0.7398
UNHealthy	19	0.846	2.491	0.8915
Weikiva	9	0.481	1.057	0.4270

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Table 2-2. Summary statistics of richness (S), evenness (E), Simpson's diversity index (H) and Shannon diversity index (D') for epiphytic diatoms.

Name	S	E	H	D`
Audubon	14	0.736	1.943	0.7716
BearScat	19	0.858	2.526	0.8888
BigCow	27	0.862	2.841	0.9220
BRSebastian	10	0.784	1.806	0.7785
CabPatch	33	0.916	3.201	0.9493
Caravelle	16	0.833	2.309	0.8732
Chuluota	12	0.756	1.878	0.7772
CLBayard	7	0.713	1.388	0.7151
CLCove	9	0.760	1.669	0.7463
CMWPast	12	0.756	1.879	0.7944
CMWRef	18	0.846	2.445	0.8841
COBurgle	15	0.706	1.912	0.7493
COHole	12	0.759	1.885	0.8026
CREW	21	0.888	2.705	0.9168
Deerfly	16	0.792	2.197	0.8366
DEMelon	23	0.802	2.516	0.8770
Garber	16	0.642	1.779	0.6725
GbarE	12	0.437	1.086	0.4106
GLdonut	15	0.774	2.097	0.8129
GLpont	14	0.866	2.287	0.8775
Goethe	14	0.798	2.106	0.8367
GreenSwamp	18	0.895	2.588	0.9078
HagueI	15	0.750	2.031	0.8071
HagueII	28	0.902	3.006	0.9359
HalfMoon	13	0.826	2.118	0.8475
HARare	14	0.853	2.250	0.8660
HEBad	7	0.793	1.544	0.7242
HEL2	18	0.678	1.960	0.7955
HEOkay	19	0.818	2.409	0.8620
HighPast	17	0.806	2.285	0.8490
HighRef	19	0.767	2.258	0.8243
HillsRef	23	0.788	2.471	0.8733
HuntCamp	12	0.747	1.856	0.7757
IFASI	15	0.752	2.036	0.8096
Immokalee	12	0.880	2.188	0.8668
IRBlueCypress	16	0.673	1.865	0.7372
IRCanal	20	0.753	2.255	0.8312
IROJ	18	0.813	2.350	0.8597
JD6	14	0.754	1.989	0.8054
KellyPark	11	0.733	1.758	0.7610
LCork	13	0.716	1.836	0.7423
LEgo	11	0.920	2.206	0.8808
LESuwan	20	0.883	2.645	0.9126
LLeeCounty	20	0.870	2.607	0.9034
MALudy	16	0.705	1.954	0.7649
MASpray	8	0.662	1.376	0.7101
McArthur	14	0.602	1.589	0.6392
MNElmer	13	0.769	1.973	0.8199
MNOcala	14	0.817	2.156	0.8464
MRPepper	28	0.834	2.778	0.8810
Myakka	16	0.802	2.224	0.8477
OKCara	18	0.828	2.394	0.8739
OKKiss	13	0.736	1.889	0.7722
OKPast	21	0.709	2.159	0.7994
PacificTom	19	0.837	2.465	0.8755
PallMar	10	0.818	1.884	0.8183
PBCorbet	20	0.786	2.355	0.8742
PBenjay	11	0.815	1.955	0.8175
Penner	9	0.507	1.114	0.4502
POWales	7	0.889	1.730	0.8078
POWeowak	16	0.847	2.349	0.8795
RiceCreek	16	0.766	2.123	0.8107
SandhillCrane	25	0.821	2.642	0.8872
SANorthmya	17	0.796	2.257	0.8646
SAOscer	13	0.583	1.494	0.6955
Savannas	12	0.633	1.574	0.7035
STCow	18	0.758	2.191	0.8224
UNHealthy	12	0.794	1.973	0.8097
Weikiva	11	0.573	1.373	0.5789

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Table 2-3. Summary statistics of richness (S), evenness (E), Simpson's diversity index (H) and Shannon diversity index (D') for phytoplanktonic diatoms.

Name	S	E	H	D`
Audubon	18	0.739	2.137	0.8197
BearScat	27	0.874	2.881	0.9152
BigCow	36	0.864	3.096	0.9344
BRSebastian	14	0.712	1.879	0.7611
CabPatch	35	0.925	3.290	0.9541
Caravelle	26	0.960	3.129	0.9486
Chuluota	40	0.998	3.680	0.9744
CLBayard	13	0.709	1.819	0.7172
CLCove	14	0.651	1.718	0.6958
CMWPast	27	1.000	3.296	0.9630
COBurgle	13	0.748	1.920	0.7610
COHole	19	0.813	2.394	0.8667
CREW	25	0.968	3.116	0.9506
Deerfly	22	0.922	2.850	0.9304
DEMelon	19	0.781	2.299	0.8441
Garber	19	1.000	2.944	0.9474
GbarE	16	0.651	1.804	0.6838
GLDonut	15	0.719	1.948	0.7634
GLPont	22	0.816	2.521	0.8902
Goethe	15	0.986	2.670	0.9273
GreenSwamp	23	0.940	2.949	0.9275
HagueI	20	0.974	2.918	0.9408
HagueII	24	1.000	3.178	0.9583
HalfMoon	14	0.871	2.300	0.8773
HARare	15	0.710	1.923	0.7543
HABad	8	0.690	1.434	0.6892
HEL2	21	0.774	2.356	0.8325
HEOkay	29	0.883	2.973	0.9313
HighPast	8	0.660	1.372	0.6048
HighRef	12	0.887	2.203	0.8715
HillsRef	21	0.781	2.378	0.8048
HuntCamp	9	0.702	1.542	0.6503
IFASI	11	0.663	1.590	0.6957
IFASII	26	0.992	3.233	0.9592
Immokalee	24	0.939	2.985	0.9348
IRBlueCypress	22	0.864	2.671	0.9004
IRCanal	12	0.749	1.862	0.7750
IROJ	24	0.868	2.760	0.8998
JD6	14	0.728	1.921	0.7687
KellyPark	13	0.446	1.143	0.4485
LCork	20	0.704	2.109	0.7544
LEGo	13	0.858	2.201	0.8565
LESuwan	17	0.907	2.569	0.9078
LLeeCounty	16	0.824	2.285	0.8569
MALudy	21	0.732	2.230	0.7943
MASpray	10	0.625	1.439	0.6719
McArthur	15	0.439	1.189	0.4819
MNElmer	13	0.748	1.919	0.7845
MNOcala	14	0.838	2.211	0.8381
MRPepper	25	1.000	3.219	0.9600
Myakka	20	0.878	2.631	0.9061
OKCara	18	0.852	2.462	0.8854
OKKiss	32	0.975	3.377	0.9612
OKPast	22	0.917	2.835	0.9211
PacificTom	36	0.952	3.410	0.9595
PallMar	16	0.812	2.250	0.8086
PBCorbett	16	0.793	2.199	0.8473
PBEnjay	12	0.838	2.081	0.8379
Penner	14	0.750	1.979	0.7628
POWales	10	0.835	1.922	0.7937
POWeowak	19	0.801	2.358	0.8736
RiceCreek	19	0.833	2.453	0.8782
SandhillCrane	29	0.893	3.005	0.9326
SANorthMya	12	0.827	2.056	0.8262
SAOscer	9	0.402	0.883	0.3623
Savannas	18	0.736	2.128	0.8048
STCow	17	0.771	2.183	0.8442
UNHealthy	15	0.818	2.215	0.8473
Weikiva	12	0.924	2.296	0.8852

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Table 2-4. Summary statistics and diatom similarities using Tukey's HSD.

Variable	Algal Type	N	Mean	Median	Standard Deviation
S	Benthic[a]	70	18.4	18.0	5.9
	Epiphytic[b]	69	15.7	15.0	5.2
	Phyto.[a]	69	18.8	18.0	7.1
E	Benthic[a]	70	0.792	0.814	0.087
	Epiphytic[a]	69	0.773	0.788	0.094
	Phyto.[a]	69	0.815	0.827	0.132
H	Benthic[ab]	70	2.280	2.276	0.431
	Epiphytic[b]	69	2.101	2.118	0.418
	Phyto.[a]	69	2.236	2.296	0.609
D'	Benthic[a]	70	0.834	0.860	0.095
	Epiphytic[a]	69	0.808	0.820	0.097
	Phyto.[a]	69	0.831	0.857	0.124

Note: Diatom types (Benthic, Epiphytic, Phytoplanktonic) with a similar letter superscript were not significantly different at p=0.05.

Table 2-5. Mann-Whitney U-test results of summary statistics between reference and impaired LDI classes within benthic, epiphytic, and phytoplanktonic diatom groups.

	Benthic Diatoms	Epiphytic Diatoms	Phytoplanktonic Diatoms
S (Richness)	-1.515 (0.130)	-1.780 (0.075)	-1.175 (0.240)
E (Evenness)	1.306 (0.192)	0.560 (0.576)	0.595 (0.551)
H (Shannon Diversity)	-0.077 (0.939)	-0.746 (0.456)	-0.169 (0.866)
D' (Simpson's Diversity)	0.435 (0.663)	0.048 (0.962)	-0.078 (0.938)

phytoplanktonic diatoms, while gamma diversity in impaired sites was higher than in reference sites for all three diatom types sampled.

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Table 2-6. Alpha, Beta, and Gamma diversity between diatom types and wetland condition.

	Alpha Diversity	Beta Diversity	Gamma Diversity
All Benthic (n=70)	18.4	10.60	195
Impaired	19.4	7.94	154
Reference	17.4	8.05	140
All Epiphyton (n=69)	15.7	11.08	174
Impaired	16.9	8.70	147
Reference	14.4	6.81	98
All Phytoplankton (n=69)	18.8	12.98	244
Impaired	19.5	10.00	195
Reference	17.9	9.11	163

Comparisons of the fidelity of species to particular habitats (benthic, epiphytic, and phytoplanktonic) were made using PSI and MRPP. For benthic versus epiphytic diatoms, within site PSI ranged from 6% to 89%, and averaged 55% (+/- 17.2%).

Within site PSI ranged from 2% to 78%, and averaged 42% (+/- 19.0%) for benthic versus phytoplanktonic diatoms. The range between epiphytic and phytoplanktonic diatoms was 1% to 77%, and averaged 46% (+/- 19.4%). Global comparisons using pooled data (i.e., all benthic diatoms from all sites pooled into a "benthic" heading versus all epiphytic diatoms from all sites pooled into an "epiphytic" heading) indicated a remarkable lack of fidelity to specific habitats among the diatom community as the benthic and epiphytic communities were 84% similar, the benthic and phytoplanktonic communities were 75% similar, and the epiphytic and phytoplanktonic communities were 76.3% similar. MRPP results supported the lack of uniqueness between benthic, epiphytic, and phytoplanktonic communities. MRPP analyses (Table 2-7), were completed using both abundance data and simple presence data, and were calculated for

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Table 2-7. MRPP analyses of the compositional similarity between benthic, epiphytic, and phytoplanktonic diatoms.

	Test	*A*	*T*	*p*-value
Abundance Global Test	B vs. E vs. P	-0.0005	0.321	0.558
Presence Global Test	B vs. E vs. P	-0.0008	-0.492	0.262
Abundance Iterative Tests	B vs. E	-0.0020	1.186	0.994
	B vs. P	0.0013	-0.841	0.166
	E vs. P	-0.0004	0.252	0.503
Presence Iterative Tests	B vs. E	-0.0019	1.012	0.916
	B vs. P	0.0021	-1.262	0.108
	E vs. P	0.0016	-0.951	0.151

Note: Abbreviations are B (benthic), E (epiphyton), P (phytoplankton). Global tests refer to simultaneous multivariate tests of the pooled diatoms from all benthic, epiphytic, and phytoplanktonic samples. Iterative tests exclude one group (of three) during the MRPP procedure.

both a simultaneous comparison of all three diatom types and an iterative comparison of one type versus another. In all cases, MRPP results indicated no significant differences between the benthic, epiphytic, and phytoplanktonic communities at the conventional *p*-value of 0.05.

Diatom Composition and Environmental Correlates

As a lack of habitat fidelity was demonstrated by the benthic, epiphytic, and phytoplanktonic communities, it was necessary to select one community to pursue the development of biological indicators of wetland condition. Selecting multiple communities would "double-count" the effect of landscape influences on the wetland environment. The criterion for further metric development was to identify the diatom community with the strongest relationship to the environmental parameters, including the

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LDI score. This was accomplished by examining matrix correlations between abundance values and environmental parameters using the Mantel test, and by examining the NMDS ordination of the abundance values for each diatom community and the correlation of site scores with environmental parameters.

Including the LDI, fourteen environmental variables were measured at each site. The measured water and soil parameters are given in Appendix B. Significant differences between impaired and reference conditions, as measured by the Mann-Whitney U-test, were found for the following parameters: soil pH, soil TP, specific conductivity, water pH, ammonia, TKN, and water TP (Table 2-8). Water color was significantly lower in reference conditions at $p<0.10$. Colinearity among the variables was recognized for %TC, TKN, and water pH following regression analyses of the transformed data (SAS 1990). These variables were removed.

The Mantel test was used to test the hypothesis of no relationship between the abundance data for benthic, epiphytic, and phytoplanktonic diatoms and the measured environmental parameters and LDI score. The relationship between the matrices was strongest for epiphytic diatoms (Mantel's $r=0.49$, $p<0.001$). Benthic diatoms (Mantel's $r=0.39$, $p<0.001$) and phytoplanktonic diatoms (Mantel's $r=0.30$,$p<0.001$) were also significantly correlated to the environmental parameters, albeit not as strongly as the epiphyton.

NMDS ordinations of the diatom community abundance data were able to capture approximately equal total variance in the dataset for each of the three diatom types (total variance for Benthic: 81.1%; Epiphyton: 77.4%; Phytoplankton 80.1%). The stress, or

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Table 2-8. Comparison of the medians of water and soil variables between LDI classes with the Mann-Whitney U-test.

Water Values	Statistic (Z)	*p*-value
Color	-1.868	0.062
Specific Conductivity	-3.919	<0.001
Turbidity	-1.524	0.127
Water pH	-4.677	<0.001
Ammonia	-2.690	0.007
Nitrates/Nitrites	0.771	0.441
TKN	-2.932	0.003
TP	-4.836	<0.001

Soil Values	Statistic (Z)	*p*-value
Soil pH	-3.036	0.002
%TC	-0.818	0.414
%OM	-1.253	0.210
%TN	-1.147	0.251
%TP	-2.771	0.006

"goodness of fit" of the ordination was also approximately equal, and indicated an adequate and representative decreased dimensionality of the dataset (Kruskal 1964, Clarke 1993). The calculated stress for each diatom was: benthic diatoms: 16.68; epiphytic diatoms: 18.45; phytoplanktonic diatoms: 15.66. Site scores for each diatom community type, overlaid by a vector diagram of environmental variables with strong ($r^2 \geq 0.30$) correlations to the first two axes, are presented in Figures 2-3 to 2-5. A two-dimensional solution was identified for benthic and epiphytic diatoms, and a three dimensional solution was identified for phytoplankton. Total variance accounted for by the ordination of the diatoms was approximately equal between types. The benthic diatom ordination accounted for 81.1% of the variation in the dataset (first axis: 26.2%, second axis 54.9%); 77.4% of the variation in the epiphytic diatom dataset was accounted for by the ordination (first axis: 49.2%, second axis: 28.2%); and 80.1% of the variation

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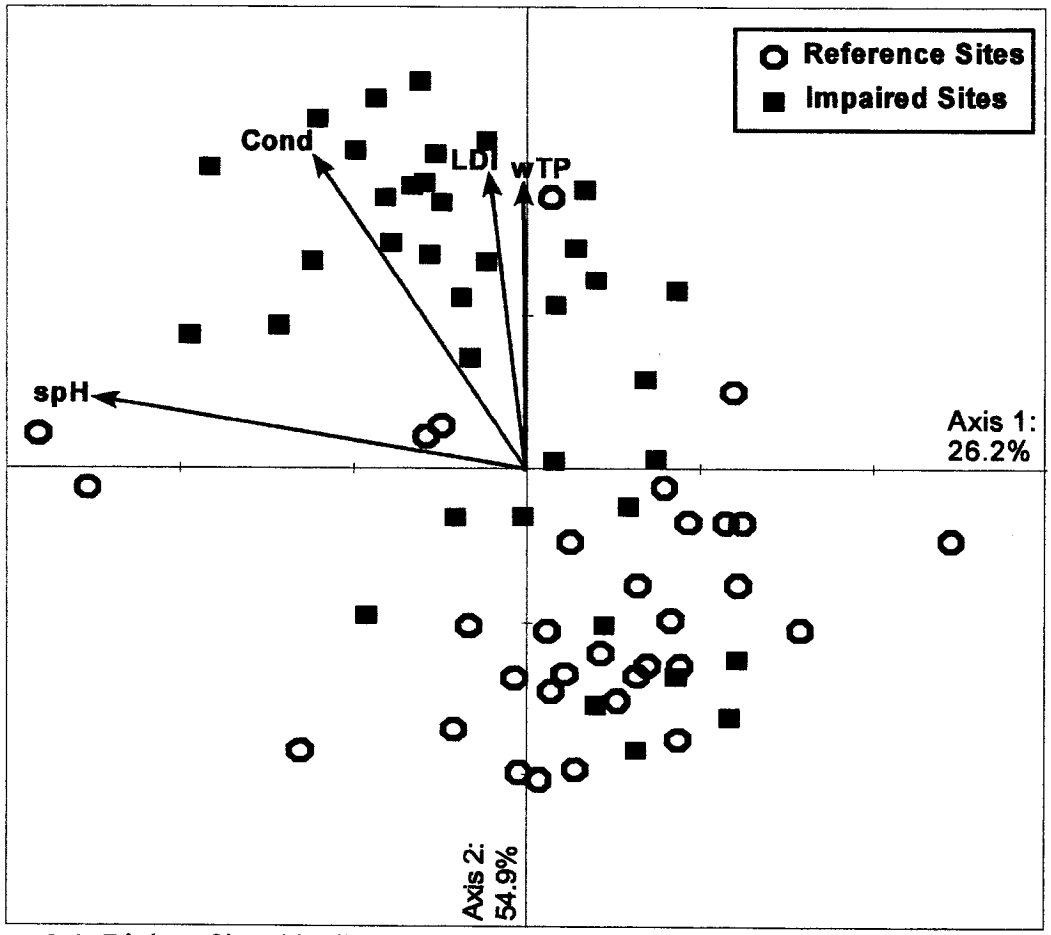

Figure 2-3. Biplot of benthic diatom NMDS ordination scores for each site and strongly correlated environmental variables. The vectors are shown at 150% of original length for clarity. The percent variance explained by each axis is noted on the axis. The length of the vectors represents the strength of the correlation (all >|0.30|) and the angle represents the direction of maximum change. Four variables are shown: pH (soil pH), Cond (specific conductivity), TP (water total phosphorus), and LDI (Landscape Development Intensity index).

was reflected in the ordination of the phytoplankton (first axis: 28.7%, second axis: 36.5%, third axis: 14.8%). The relationship between the first two axes for phytoplankton are shown in Figure 2-5 as the third axis, despite accounting for approximately 15% of the variation in the dataset, had no strongly correlated environmental variables.

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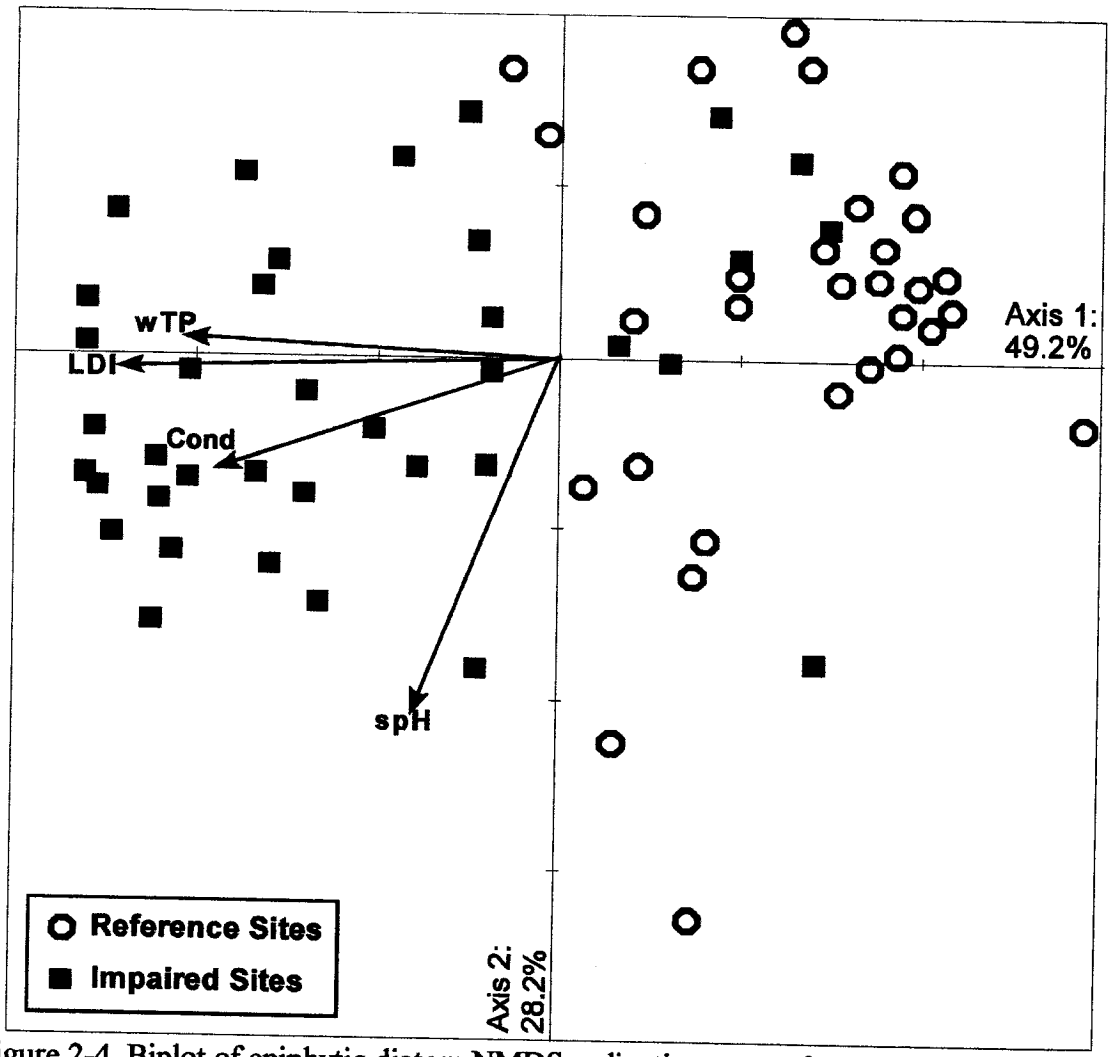

Figure 2-4. Biplot of epiphytic diatom NMDS ordination scores for each site and strongly correlated environmental variables. The vectors are shown at 125% of the original length for clarity. The percent of variance explained by each axis is given on each axis. Four environmental variables were strongly ($r>|0.30|$) correlated with NMDS ordination scores: pH (soil pH), Cond (specific conductivity), TP (water TP), and LDI.

Correlations of environmental variables with the ordination score for benthic, epiphytic, and phytoplanktonic diatoms are presented in Tables 2-9 to 2-11. Four environmental variables were correlated ≥ 0.30 with the ordination scores of benthic and epiphytic diatoms (LDI, soil pH, specific conductivity, and water TP). Three variables were correlated with phytoplanktonic diatoms (LDI, specific conductivity, and soil pH).

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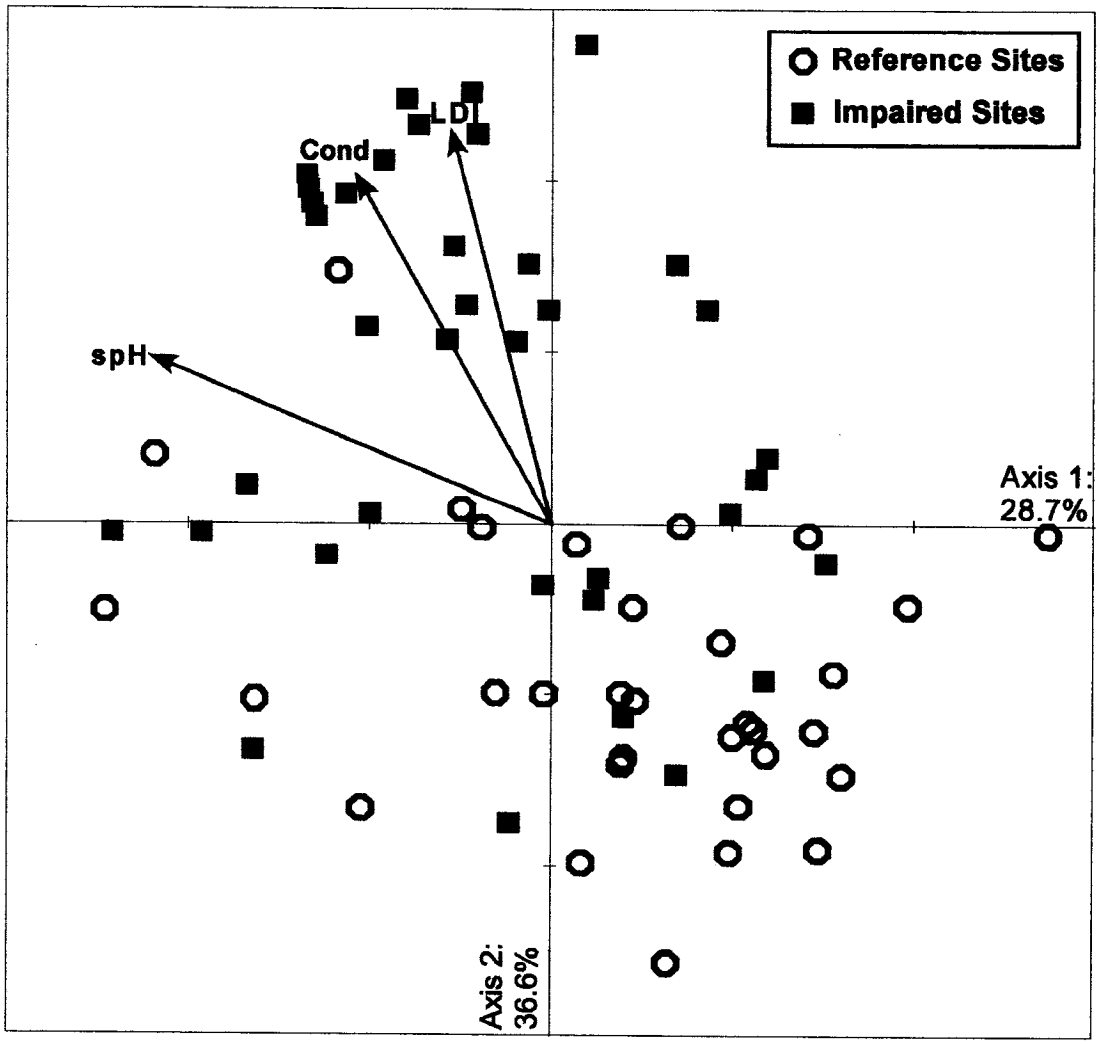

Figure 2-5. Biplot of phytoplanktonic diatom NMDS ordination scores for each site and strongly correlated environmental variables. The vectors are shown at 200% of the original length for clarity. The percent of variance explained by each axis is given on each axis. Three environmental variables were strongly ($r>|0.30|$) correlated with NMDS ordination scores: Cond (specific conductivity), TP (water TP), and LDI.

None of the environmental variables were correlated ≥ 0.50 for phytoplanktonic diatoms, while one of four were ≥ 0.50 for benthic diatoms and three of four were ≥ 0.50 for epiphytic diatoms.

Based on results of the Mantel tests and the NMDS ordination scores and correlations, as well as the lack of significant differences between the benthic, epiphytic,

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Table 2-9. Correlations of environmental variables and ordination scores for benthic diatoms.

Parameter	Axis 1 (26.2%) r^2	Axis 1 (26.2%) tau	Axis 2 (54.9%) r^2	Axis 2 (54.9%) tau
Soil pH	0.62	-0.55	0.20	0.25
% TN	0.03	0.08	0.02	0.17
TP (mg/kg)	0.01	0.07	0.09	0.23
% OM	0.07	0.14	0.01	0.14
Color (PCU)	0.18	0.25	0.05	0.18
Spec. Cond. (umhos/cm)	0.28	-0.39	0.48	0.51
Turbidity (NTU)	<0.01	-0.02	0.06	0.17
Ammonia (mg/L)	<0.01	-0.09	0.05	0.19
Nitrate/Nitrite (mg/L)	0.02	0.10	0.02	-0.08
Water TP (mg/L)	<0.01	-0.04	0.42	0.49
LDI Score	0.06	-0.20	0.42	0.36

Note: Underlined correlations were ≥ 0.30 and were considered strongly correlated with NMDS ordination scores. Kendal's tau, a non-parametric measure of association, is also given.

Table 2-10. Correlations of environmental variables and ordination scores for epiphytic diatoms.

Parameter	Axis 1 (49.2%) r^2	Axis 1 (49.2%) tau	Axis 2 (28.2%) r^2	Axis 2 (28.2%) tau
Soil pH	0.23	-0.34	0.59	-0.58
% TN	<0.01	-0.07	0.03	0.08
TP (mg/kg)	0.11	-0.24	<0.01	0.06
% OM	<0.01	-0.07	0.08	0.14
Color (PCU)	0.07	-0.17	0.22	0.26
Spec. Cond. (umhos/cm)	0.46	-0.50	0.19	-0.32
Turbidity (NTU)	0.06	-0.15	0.02	0.06
Ammonia (mg/L)	0.03	-0.21	0.02	<-0.01
Nitrate/Nitrite (mg/L)	<0.01	<-0.01	0.03	0.17
Water TP (mg/L)	0.53	-0.55	<0.01	0.05
LDI Score	0.63	-0.50	0.02	-0.11

Note: Correlations marked with an asterisk were $r^2 \geq 0.30$ and were considered strongly correlated with NMDS ordination scores for each site. Kendal's tau, a non-parametric measure of association, is also given.

and phytoplanktonic communities as measured by MRPP and indicated by high PSI values, a decision to base bioassessment metric development on the epiphytic community was made. The results of the Mantel test indicated that the epiphytic diatom community

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Table 2-11. Correlations of environmental variables and ordination scores for phytoplanktonic diatoms.

Parameter	Axis 1 (28.7%) r^2	Axis 1 (28.7%) tau	Axis 2 (36.5%) r^2	Axis 2 (36.5%) tau	Axis 3 (14.8%) r^2	Axis 3 (14.8%) tau
Soil pH	0.40	-0.49	0.16	0.27	0.06	0.15
% TN	<0.01	0.02	<0.01	0.09	0.040	-0.11
TP (mg/kg)	<0.01	<-0.01	0.02	0.11	0.05	-0.15
% OM	<0.01	0.03	0.02	0.01	0.05	-0.17
Color (PCU)	0.13	0.20	0.03	0.15	0.19	-0.27
Spec. Cond. (umhos/cm)	0.19	-0.34	0.35	0.45	0.02	0.11
Turbidity (NTU)	<0.01	-0.02	<0.01	0.03	0.07	-0.24
Ammonia (mg/L)	<0.01	-0.06	<0.01	0.18	0.01	-0.03
Nitrate/Nitrite (mg/L)	0.03	0.12	0.04	-0.15	0.05	-0.18
Water TP (mg/L)	<0.01	-0.08	0.28	-0.22	0.11	-0.27
LDI Score	0.11	-0.25	0.38	0.38	0.05	-0.15

Note: Correlations marked with an asterisk were ≥ 0.30 and were considered strongly correlated with NMDS ordination scores for each site. Kendal's tau, a non-parametric measure of association, is also given.

had a stronger relationship with the measured environmental parameters than either the benthic diatoms or phytoplankton. NMDS ordinations also suggested stronger correlations between epiphytic diatoms and environmental parameters, notably LDI score and water TP, than were found with benthic diatoms, although goodness of fit and percent of variance explained were slightly lower. Phytoplanktonic diatoms were removed from consideration due to the low Mantel statistic values and weaker NMDS correlations with environmental parameters, most notably the LDI score, as compared to epiphyton or benthic diatoms.

Metric Development

Regional composition

MRPP analyses of the arcsine square root transformed abundance data were completed for epiphytic diatoms to determine if regional factors contributed to epiphyton community composition. When simultaneously compared (i.e., South vs. Central vs.

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North), diatom distribution did not differ significantly (at $p<0.05$). However, when iteratively tested, diatom distribution differed significantly ($p<0.05$) between the South and North regions, but the differences were insignificant between the South and Central, and the Central and North regions. This was examined further by testing the regional distribution of reference sites and impaired sites. When simultaneously tested, the reference sites differed across all regions (Table 2-12). Reference sites also differed between South and Central regions, and South and North regions, but not between Central and North regions. In contrast, the impaired sites did not differ when simultaneously compared, nor when regions were contrasted, indicating similar species composition across peninsular Florida in disturbed landscapes.

Indicator species analyses

Indicator Species Analysis was used to identify species that exhibited specificity and fidelity to LDI scores of <2.0 (i.e., "reference indicators") and ≥2.0 (i.e., "impaired indicators") for each region. The significant species for reference indicators and impaired indicators are presented in Tables 2-13 and 2-14, respectively. Ten indicators

Table 2-12. MRPP analyses of the compositional similarity between epiphytic diatoms of South, Central, and North wetland regions.

	Test	*A*	*T*	*p*-value
Simultaneous Tests	%: S vs. C vs. N	0.0056	-1.254	0.111
	P/A: S vs. C vs. N	0.0096	-1.794	0.057
Abundance Iterative Tests	S vs. C	0.0045	-1.080	0.132
	S vs. N	0.0113	-2.038	0.045
	C vs. N	-0.0015	0.320	0.533
Presence Iterative Tests	S vs. C	0.0086	-1.843	0.057
	S vs. N	0.0195	-2.818	0.019
	C vs. N	-0.0040	0.662	0.711

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Table 2-13. Reference site diatom indicator taxa identified using Indicator Species Analysis for each wetland region.

Region	Indicator Species (LDI <2.0)	Observed Ind. Value	*p*-value
South Reference	*Anomoeneis serians acuta*	45.5	0.013
	Anomoeneis serians brachysira	43.8	0.046
	Anomoeneis serians	27.3	0.092
	Anomoeneis vitrea	59.5	0.015
	Eunotia bilunaris mucophila	45.8	0.054
	Eunotia flexuosa eurycephala	27.3	0.091
	Eunotia naegelii	63.0	0.014
	Frustulia rhomboides crassinerva	32.0	0.084
	Frustulia rhomboides saxonica	70.2	0.006
	Navicula mediocris	36.4	0.035
Central Reference	*Anomoeneis serians acuta*	38.5	0.009
	Anomoeneis serians brachysira	45.9	0.016
	Desmogonium rabenhorstianum	44.0	0.016
	Eunotia bilunaris mucophila	43.1	0.033
	Eunotia denticulata	23.1	0.069
	Eunotia paludosa	53.8	0.001
	Eunotia paludosa trinacria	46.2	0.002
	Eunotia pirla	38.5	0.009
	Eunotia soleirolii	30.8	0.027
	Eunotia zygodon	38.5	0.010
	Frustulia rhomboides	50.3	0.021
	Frustulia rhomboides saxonica	81.2	<0.001
North Reference	*Desmogonium rabenhorstianum*	50.0	0.081
	Frustulia rhomboides	58.7	0.024
	Frustulia rhomboides saxonica	72.0	0.050
	Pinnularia subcapitata	68.3	0.098

of reference conditions were identified in the South region, 12 in the Central region and 4 in the North region. Five impaired indicators were identified in the South region, 12 in the Central region, and 6 in the North region. There was substantial overlap of indicator species between regions.

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Table 2-14. Impaired site diatom indicator taxa identified using Indicator Species Analysis for each wetland region.

Region	Indicator Species (LDI ≥2.0)	Observed Ind. Value	*p*-value
South Impaired	*Gomphonema parvulum*	58.3	0.005
	Navicula confervacea	58.3	0.005
	Nitzschia palea debilis	85.2	<0.001
	Nitzschia sociabilis	41.7	0.038
	Pinnularia acrosphaeria	33.3	0.094
Central Impaired	*Gomphonema angustatum*	29.4	0.054
	Gomphonema gracile	52.9	0.002
	Gomphonema parvulum	60.1	0.004
	Navicula confervacea	70.3	0.001
	Navicula minima	33.9	0.060
	Navicula seminulum	45.8	0.018
	Nitzschia frustulum	35.3	0.038
	Nitzschia gracilis	29.4	0.072
	Nitzschia palea	52.2	0.026
	Nitzschia palea debilis	77.0	<0.001
	Nitzschia sociabilis	30.8	0.089
	Pinnularia gibba	37.3	0.067
North Impaired	*Gomphonema parvulum*	62.5	0.026
	Navicula difficillima	62.5	0.027
	Navicula minima	49.2	0.080
	Navicula seminulum	50.0	0.081
	Nitzschia palea	58.3	0.033
	Nitzschia palea debilis	63.3	0.066

ISA was also used to identify significant indicator species for impaired and reference sites for the combined peninsular regions (South, Central, and North regions; n=69). A total of 43 indicator species were identified for the combined analysis, 22 reference condition indicators and 21 impaired condition indicators (Tables 2-15 and 2-16, respectively). With the exception of two indicators of reference conditions (*Eunotia soleirolii* and *Pinnularia subcapita*) and two indicators of impaired conditions (*Nitzschia frustulum* and *N. gracilis*) found in regional analyses, there was complete overlap of

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Table 2-15. Statewide indicators of reference conditions as determined from Indicator Species Analysis.

Indicator Species (LDI <2.0)	Observed Ind. Value	*p*-value
Anomoeneis serians acuta	34.4	<0.001
Anomoeneis serians brachysira	39.1	<0.001
Anomoeneis serians	9.4	0.099
Anomoeneis vitrea	22.0	0.052
Desmogonium rabenhorstianum	26.6	0.027
Eunotia bilunaris mucophila	41.4	0.003
Eunotia denticulata	17.5	0.021
Eunotia flexuosa	20.2	0.099
Eunotia flexuosa eurycephala	9.4	0.093
Eunotia monodon	9.4	0.095
Eunotia naegelii	59.5	0.002
Eunotia paludosa	29.4	0.007
Eunotia paludosa trinacria	29.7	0.017
Eunotia pirla	27.7	0.005
Eunotia rhomboidea	37.0	0.029
Eunotia zygodon	23.1	0.015
Frustulia rhomboides	46.3	<0.001
Frustulia rhomboides crassinerva	17.8	0.046
Frustulia rhomboides saxonica	75.1	<0.001
Navicula mediocris	25.0	0.001
Navicula subtilissima	17.3	0.030
Stauroneis kriegeri	16.6	0.084

regional and peninsular indicator species. This was not unexpected considering the lack of significance when composition between regions was simultaneously tested with MRPP. The larger *n* allowed for greater statistical power in the analysis of indicator species, thus 5 reference indicator species and 9 impaired indicator species were identified using the peninsular (n=69) dataset that were not in any regional indicator species list. Little additional information was garnered from identifying regionally specific indicator species, and 14 additional indicator species were identified using the peninsular dataset. Furthermore, no significant difference was found in the simultaneous

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Table 2-16. Statewide indicators of impaired conditions as determined from Indicator Species Analysis.

Indicator Species (LDI ≥2.0)	Observed Ind. Value	*p*-value
Caloneis bacillum	16.2	0.030
Gomphonema angustatum	22.8	0.020
Gomphonema gracile	46.4	0.002
Gomphonema lanceolatum	21.6	0.005
Gomphonema parvulum	60.9	<0.001
Melosira italica	18.9	0.014
Navicula confervacea	59.3	<0.001
Navicula difficillima	35.4	0.001
Navicula minima	33.8	0.002
Navicula molestiformis	13.5	0.053
Navicula pupula rectangularis	15.9	0.057
Navicula seminulum	42.2	<0.001
Nitzschia amphibia	21.2	0.041
Nitzschia microcephala	13.5	0.058
Nitzschia palea	48.4	0.005
Nitzschia palea debilis	76.3	<0.001
Nitzschia palea tenuirostris	15.5	0.065
Nitzschia perminuta	12.3	0.010
Nitzschia sociabilis	32.8	0.002
Pinnularia acrosphaeria	21.6	0.006
Pinnularia gibba	27.5	0.036

multivariate MRPP test of regional significance of community composition. Thus, it was concluded that the remaining analyses would utilize the peninsular dataset. Therefore metrics developed are not regionally specific but apply throughout peninsular Florida.

Using the peninsular dataset of indicator species (n=43), the abundance of the indicator species for both impaired and reference conditions were calculated for each site and correlated with the LDI using Spearman's r (Figure 2-6). Correlations for the abundance of reference indicators and impaired indicators are: % Reference Indicators r = -0.66, p<0.001; % Impaired Indicators r = 0.60, p<0.001. The abundance of reference indicators demonstrated a highly variable distribution, especially for LDI values of

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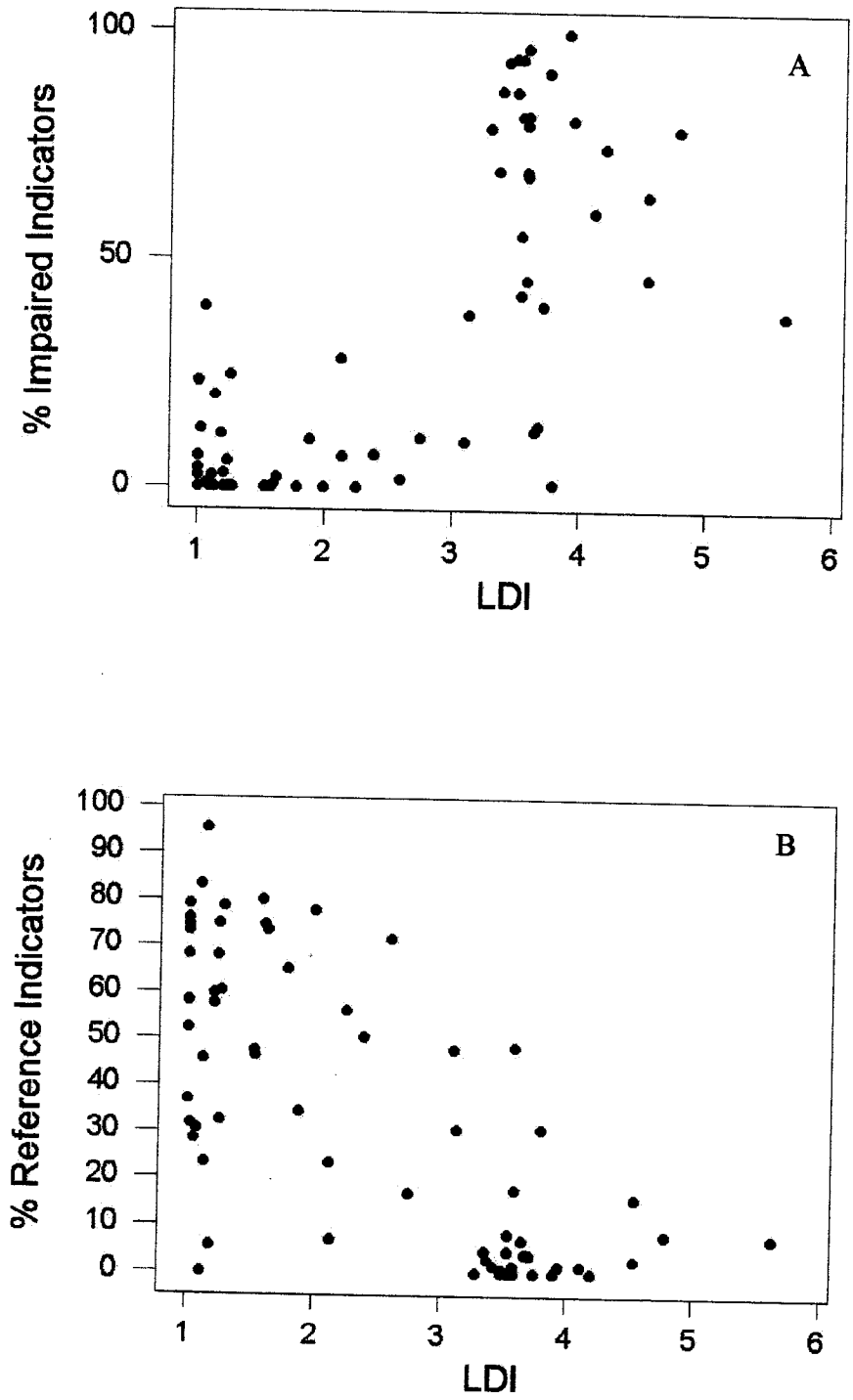

Figure 2-6. Indicator species abundance data plotted against the LDI. A) Abundance of Reference Indicators vs. the LDI. B) Abundance of Impaired Indicators vs. the LDI.

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approximately <3.2. In general, sites with LDI scores of >3.2 were composed of <10% reference indicators. The abundance of impaired indicators suggested a low correlation with increasing LDI scores until a threshold at approximately 3.7 was reached. At that point, the abundance of impaired indicators increased markedly.

Autecological indices

One hundred thirty-four of the 174 epiphyton diatom taxa (approximately 77%) had one or more autecological, or ecological indicator, values obtained from Van Dam et al. (1994) and Bahls (1993). On a site-by-site basis, approximately 82% of diatoms found at a given site had ecological indicator values. This ranged from approximately 56% to 100% of the diatoms found in the 69 sampled wetlands.

The abundance of each class for the autecological indicator guilds of Van Dam et al. (1994) and Bahls (1993) was calculated for each site and correlated (Spearman's *r*) with the LDI. Responsive metrics were those with correlations of $>|0.50|$ and significant ($p<0.05$) correlation coefficients. Bivariate correlations using Spearman's *r* were calculated for the autecological indices having negative (n=6 metrics) and positive (n=7 metrics) responses with increasing LDI scores. The abundance of diatoms sensitive to nitrogen levels, or "tolerating very small concentrations of organically bound nitrogen," was strongly ($r = 0.86$) correlated with oligosaprobous diatoms (Van Dam et al. 1994, p. 120). The percent of diatoms sensitive to elevated nitrogen levels index was maintained, as the abundance of nitrogen-sensitive diatoms was more strongly correlated with the LDI than the abundance of oligosaprobous diatoms (Spearman's $r = -0.67$ for percent nitrogen sensitive diatoms, $r = -0.58$ for oligosaprobous taxa). The remaining autecological indices of Van Dam et al. (1994) and Bahls (1993) were significantly ($p<0.001$) correlated (Spearman's r) $\geq |0.50|$ with the LDI (Table 2-17). A plot of the

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Table 2-17. Correlations of autecological abundance data and LDI score.

Metric	Spearman's *r*	*p*-value	Notes
% Tolerate High pH	0.65	<0.001	VD 3
% Require Low pH	-0.68	<0.001	VD Class 1
% Require Low Salinity	-0.51	<0.001	VD Class 1
% Tolerate High Salinity	0.62	<0.001	VD Class 3
% Sensitive High Nitrogen	-0.66	<0.001	VD Class 1
% Tolerate High Nitrogen	0.68	<0.001	VD Class 3
% Require Elevated D.O.	-0.63	<0.001	VD Class 1
% Tolerant Low D.O.	0.64	<0.001	VD Class 4
% Meso- & Polysaprobous	0.69	<0.001	VD Class 4
% Oligotrophic	-0.59	<0.001	VD Classes 1 & 2
% Eutrophic	0.67	<0.001	VD Class 5
% Pollution Tolerant	0.67	<0.001	Bahls Class 1

Note: VD Class and Bahls class represent the autecological index value from Van Dam et al. (1994) and Bahls (1993).

Table 2-18. Mann-Whitney U-test of autecological abundance values between impaired and reference conditions.

Metric	Statistic (Z)	*p*-value
% Tolerate High pH	-5.288	<0.001
% Require Low pH	5.433	<0.001
% Require Low Salinity	4.067	<0.001
% Tolerate High Salinity	-5.171	<0.001
% Sensitive High Nitrogen	5.475	<0.001
% Tolerate High Nitrogen	-5.595	<0.001
% Require Elevated D.O.	5.379	<0.001
% Tolerant Low D.O.	-5.287	<0.001
% Meso- & Polysaprobous	-5.381	<0.001
% Oligotrophic	5.048	<0.001
% Eutrophic	-5.580	<0.001
% Pollution Tolerant	-5.474	<0.001

abundance of diatoms for each uncorrelated metric along the disturbance gradient is presented in Figure 2-7. The Mann-Whitney U-test (Table 2-18) indicated that the

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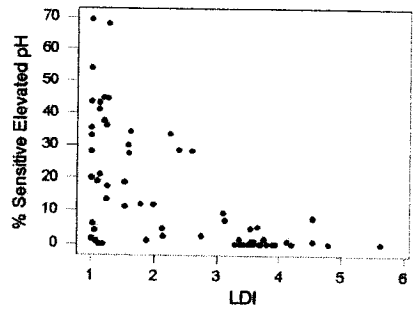
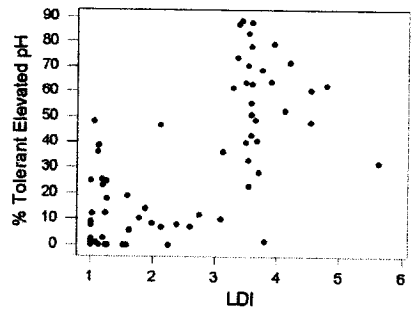
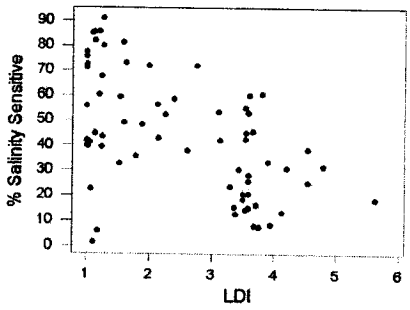


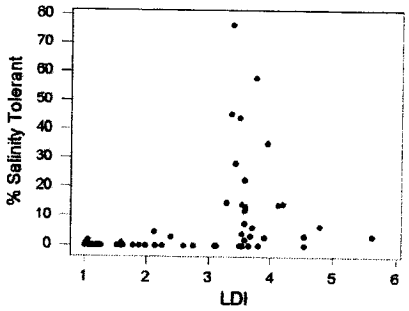
A) % Sensitive Elevated pH



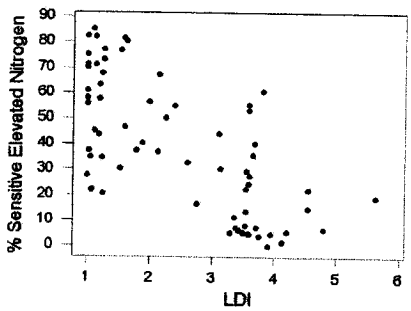
B) % Tolerate Elevated pH



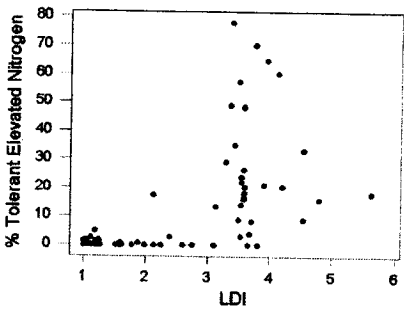
C) % Salinity Sensitive



D) % Salinity Tolerant



E) % Sensitive Elevated Nitrogen



F) % Tolerant Elevated Nitrogen

Figure 2-7. Diatom abundance for autecological metrics plotted against the LDI gradient. A) % Sensitive Elevated pH, B) % Tolerate Elevated pH, C) % Salinity Sensitive, D) % Salinity Tolerant, E) % Sensitive Elevated Nitrogen, F) % Tolerant Elevated Nitrogen, G) % Sensitive Low D.O., H) % Tolerant Low D.O., I) % Oligotrophic, J) % Eutrophic, K) % Meso-Polysaprobous, L) % Pollution Tolerant.

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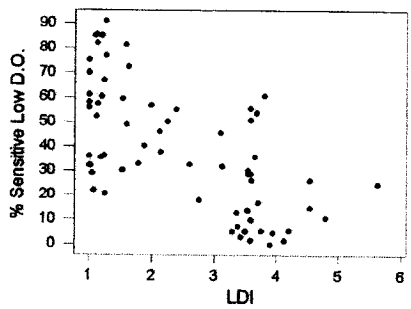
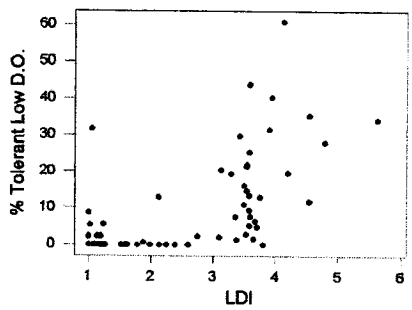
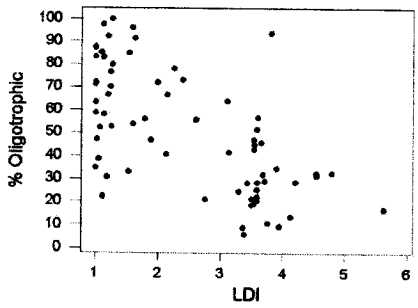


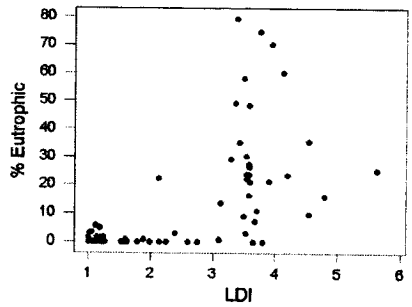
G) % Sensitive Low D.O.



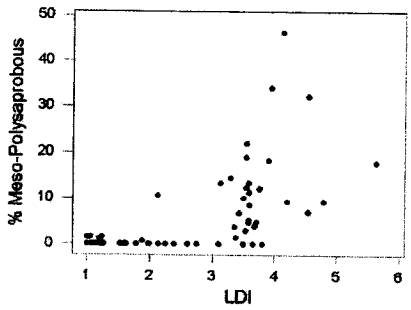
H) % Tolerant Low D.O.



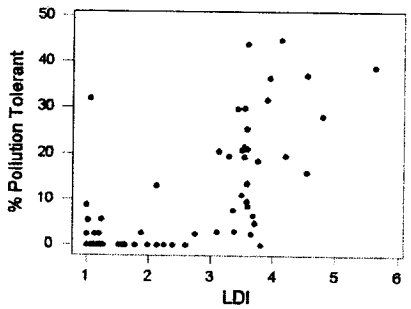
I) % Oligotrophic



J) % Eutrophic



K) % Meso-Polysaprobous



L) % Pollution Tolerant

Figure 2-7 (Continued).

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medians were significantly different ($p \leq 0.001$) between reference and impaired sites for all autecological indices.

In general, autecological metrics that decreased with increasing LDI values appeared to have a greater goodness of fit and to be less sensitive to changes in the LDI. The results suggest metrics that increase with increasing LDI score approach a threshold near approximately 3.8. Thereafter, the abundance of the various metrics increases markedly, but with a high variance.

The Multimetric Index

Two metrics derived from indicator species analysis (Dufrene and Legendre 1997), one metric based on pollution tolerance (Bahls 1993) and eleven metrics identified from autecological indicator values of Van Dam et al. (1994), were selected as final metrics based on the significance and strength of the correlations with the LDI. The quartile values of 95th percentile and the corresponding values of 0,3,7,10 for each metric are given in Table 2-19. The fourteen metrics were summed to create the Diatom Index of Wetland Condition (DIWC), with a possible range of value from 0 to 140. One site had a summed DIWC value of zero, and one site scored a perfect 140 (Table 2-20). Reference sites averaged 114.5 points, with a standard deviation of 26.3 points and a median value of 122.5. Impaired sites averaged 42.1 points (+/- 38.3) and had a median value of 33.0. DIWC scores were correlated with the LDI Score for each site: Spearman's $r = -0.79$, $p < 0.001$ (Figure 2-8). However, similar to the metrics that comprised the index, the results appear to be highly variable for any given LDI score. Metric sensitivity appears to increase near LDI scores of 3.2. Mann-Whitney U-test results indicate a significant difference between DIWC scores for impaired and reference sites ($Z=5.991$, $p<0.001$).

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Table 2-19. Quadrisect values and the 95th percentile data for each proposed biological indicator.

Metric	95th Percentile	0 Scores	3 Scores	7 Scores	10 Scores
% Reference Indicators	0.789	<0.0279	0.0279 – 0.2328	>0.2328 – 0.5620	>0.5620
% Impaired Indicators	0.934	>0.5512	>0.1094 – 0.5512	0.0001 – 0.1094	0
% Sensitive Elevated pH	0.445	0	0.0001 – 0.0234	>0.0234 – 0.2000	>0.2000
% Tolerant Elevated pH	0.818	>0.4820	>0.2300 – 0.4820	0.0571 – 0.2300	<0.0571
% Sensitive Elevated Salinity	0.840	<0.2361	0.2361 – 0.4221	>0.4221 – 0.5889	>0.5889
% Tolerate Elevated Salinity	0.405	>0.1000	>0.035 – 0.1000	0.0001 – 0.035	0
% Sensitive Elevated Nitrogen	0.811	<0.1349	0.1349 – 0.3506	>0.3506 – 0.5590	>0.5590
% Tolerate Elevated Nitrogen	0.589	>0.1622	>0.0118 – 0.1622	0.0001 – 0.0118	0
% Sensitive Low D.O.	0.840	<0.1471	0.1471 – 0.3274	>0.3274 – 0.5551	>0.5551
% Tolerate Low D.O.	0.350	>0.1195	>0.0228 – 0.1195	0.0001 – 0.0228	0
% Meso- & Polysaprobous	0.209	>0.1400	>0.0250 – 0.1400	0.0001 – 0.0250	0
% Oligotrophic	0.932	<0.2830	0.2830 – 0.4582	>0.4582 – 0.6667	>0.6667
% Eutrophic	0.592	>0.2101	>0.0152 – 0.2101	0.0001 – 0.0152	0
% Pollution Tolerant	0.368	>0.1594	>0.0239 – 0.1594	0.0001 – 0.0239	0

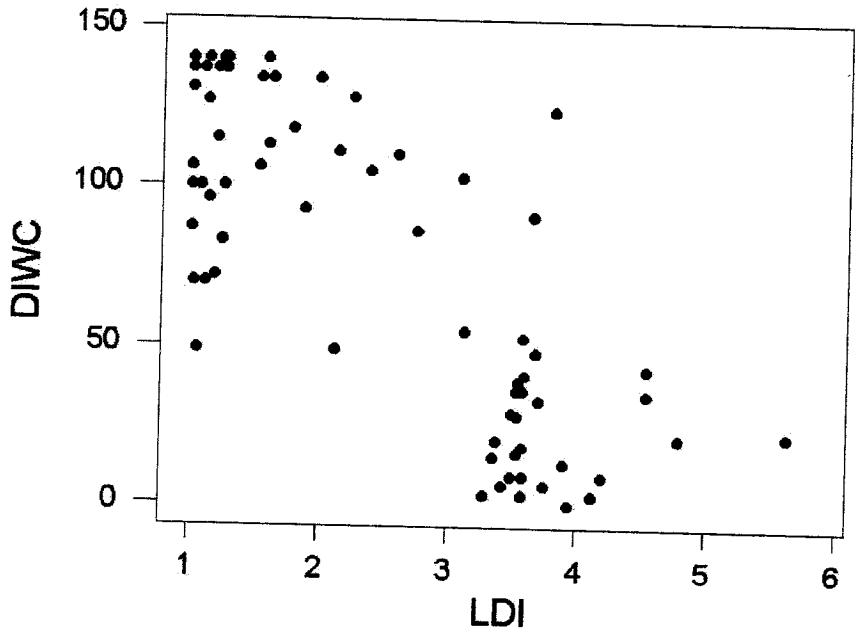

Figure 2-8. Diatom Index of Wetland Condition scores for each site plotted against the disturbance gradient.

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Table 2-20. Diatom Index of Wetland Condition scores for sampled sites.

Sites	DIWC	Sites	DIWC
Audubon	36	**IRBlueCypress**	53
BearScat	72	**IRCanal**	41
BigCow	18	**IROJ**	48
BRSebastian	137	**JD6**	140
CabPatch	28	**KellyPark**	91
Caravelle	39	**LCork**	100
Chuluota	131	**LLeeCounty**	70
CLBayard	100	**LEGo**	106
CLCove	16	**LESuwan**	49
CMWPast	111	**MALudy**	9
CMWRef	134	**MASpray**	124
COBurgle	15	**McArthur**	6
COHole	70	**MNElmer**	55
Crew	118	**MNOcala**	140
DEMelon	9	**MRPepper**	35
Deerfly	105	**Myakka**	100
Garber	86	**OKCara**	21
Gbare	20	**OKKiss**	137
GLDonut	9	**OKPast**	36
GLPont	3	**PacificTom**	43
Goethe	106	**PallMar**	140
GreenSwamp	113	**PBCorbett**	96
HARare	3	**PBEnjay**	137
HagueI	3	**Penner**	140
HagueII	22	**POWales**	137
HalfMoon	134	**POWeowak**	134
HEBad	29	**RiceCreek**	93
HEL2	6	**SANorthMya**	115
HEOkay	49	**SAOscer**	127
Highpast	33	**SandhillCrane**	103
HighRef	87	**Savannas**	140
HillsRef	83	**STCow**	0
HuntCamp	137	**UNHealthy**	13
IFASI	128	**Weikiva**	140
Immokalee	110		

Discussion

Diatom Composition and Environmental Correlates

It was anticipated that environmental variables would drive the wetland community composition such that low overlap (as measured by PSI and MRPP) would occur between

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benthic, epiphytic, and phytoplanktonic diatoms, as reported by Pan and Stevenson (1996) and U.S. EPA (2002e). Benthic diatoms were expected to respond strongly to the nutrient and chemical condition of the wetland sediments (Stevenson 2001). Epiphytic diatoms were expected to respond to nutrients from both the soils and water column, as "nutrients taken up by plants from sediments are released and readily absorbed by epiphytic biofilms" (Stevenson 2001, p. 121). Phytoplanktonic diatoms were expected to respond most strongly to the nutrients and physical/chemical characteristics of the enveloping waters. However, as found by Van Meter-Kasanof (1973), Moss (1981), de Jonge and van Beusekom (1992), there was substantial overlap for each of the wetland diatom communities, indicating a similar response to forcing functions (i.e., pH, specific conductivity, nutrients).

The similarities may also be a result of the spatial location of the sampled diatoms. The benthic community, due to the spatial position at the bottom of the water column, likely integrated not only benthic, but also epiphytic and phytoplanktonic diatoms. This purported ability of benthic diatoms to integrate a longer temporal signature of wetland conditions vis-à-vis the epiphytic or phytoplanktonic diatoms is a reflection of their position within the wetland and could be considered an advantage to using diatoms for biological assessment (Stevenson 2001, U.S. EPA 2002e). Benthic diatoms were thus likely composed not only diatoms of the benthos, but also of the rain of dead and sloughed-off diatoms from the phytoplankton and epiphyton communities. Epiphytic diatoms were composed not only of the diatoms equipped to thrive on macrophytes, but also of phytoplanktonic and mobilized benthic diatoms that became entangled in the often thick web of tendrils of soft algae growing from the macrophytes. U.S. EPA (2002e, p.

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6) considers wetland epiphytic diatoms a reflection of the "last couple of months" – generally meaning the hydrated period for the wetland, although many of the diatoms may persist during periods of drought. The phytoplankton community was composed of diatoms evolved to move within the water column, as well as organisms mobilized from the sediments and plant matter by wind action or disturbances from vertebrates. The phytoplanktonic diatoms generally reflect the shortest period of wetland conditions, as once they fall from suspension, they become assimilated into the benthos (U.S. EPA 2002e).

While high similarities (≥75%) existed on a global level between diatoms found in benthic, epiphytic, and phytoplanktonic communities, a moderate ~50% within-site similarity was identified (using PSI) for the sampled diatom assemblages. These results may be indicative of temporal variations of within-site species abundances, perhaps a reflection of slower or faster responses of diatoms within a particular community to hydrologic or physical/chemical parameters.

Strong correlations were found for soil pH, specific conductivity, and LDI score for the sampled assemblages, and benthic and epiphytic diatoms were also strongly correlated with water TP (see Tables 2-9 to 2-11). It is well established that pH and specific conductivity have a controlling effect on diatom species composition (Lowe 1974, Pan and Stevenson 1996, U.S. EPA 2002e), and that these variables are altered by anthropogenic disturbances such as agro-fertilizer application and hydrologic modifications (Fore and Grafe 2002, U.S. EPA 2002e). Likewise, the link between phosphorus and diatom composition is well established (Lowe 1974, Pan and Stevenson 1996, Stevenson et al. 1999 Barbour et al 1999), and a link would be expected between a

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general impairment indicator (LDI score), which reflects the physical, chemical, and nutrient alterations to the landscape, and wetland diatom species composition (Carpenter and Waite 2000, O'Conner et al. 2000, Cuffney et al. 2000, Munn et al. 2002, Fore and Grafe 2002, Fore 2003).

Despite the high similarity identified with PSI and MRPP, and similar driving forces identified with NMDS, a much stronger relationship with environmental variables (as measured by Mantel tests) was identified with epiphyton than with benthic or phytoplanktonic diatoms. These results (Mantel's r values for each assemblage) do not change by more than 0.01 when species that occur in <2 sites are removed (C. Lane, *unpublished data*). This suggests that the higher richness found in phytoplanktonic and benthic assemblages do not affect the relationship between a particular assemblage and the measured environmental variables through the addition of ecological "noise" from rare species (Marchant 2002). These results further support the use of epiphytic diatoms as the selected diatom assemblage for analyses.

Diatom Composition and Wetland Regions

The lack of significant differences in the composition of epiphytic diatoms between regions, when simultaneously tested with MRPP, was driven by the lack of significant differences in the composition of impaired sites (see Table 2-12). It is theorized that once a wetland crossed a threshold of impairment (e.g., McCormick and Stevenson 1998), whatever effect physiographic (i.e., mineral or organic soil) or climatic variables may have on the community is muted by the driving energies of the impairment (i.e., alterations to specific conductivity, pH, or phosphorus levels). Thus, there were no significant differences among epiphytic diatoms in impaired sites throughout peninsular Florida, but a significant difference between diatoms found in reference conditions in

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each region was identified. The environmental driving forces strongly were specific conductivity, soil pH, water color, and TP (C. Lane, *unpublished data*). Adding latitude and longitude of each site to the second (environmental variables) matrix resulted in the addition of latitude as a strongly correlated variable (C. Lane, *unpublished data*). These results again distinguish the importance of specific conductivity, pH, and TP as driving variables of diatom community composition, and also suggest that physiographic or climatic variables associated with the North to South axis of peninsular Florida affect the distribution of diatoms located in reference conditions. The addition of water color as a driving variable in diatom communities follows from the negative effect of darker waters on photosynthesis rates, and the necessity of adaptations to such environments.

Metric Development

Indicator species analysis

Indicator species analysis identified 43 taxa with specificity and fidelity to LDI scores of <2.0 and ≥ 2.0. Using the abundance of these taxa provided a metric to measure the relationship between the land use around the wetland and the identified sensitive or tolerant taxa within the wetland. Increased sampling would likely affect the composition of the sensitive and tolerant taxa lists as additional taxa would be found that were significantly related to reference or impaired conditions, while some taxa currently listed as sensitive or tolerant would likely be not significantly related to either classification due to increased sampling size clarifying the relationships in the data.

The highly variable abundance of reference indicators, when plotted along the LDI, suggests that reference indicators are not particularly accurate assessors of wetland condition, at least until a threshold of LDI scores of approximately 3.2. After that threshold of measured disturbance is reached, the abundance of reference indicators

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plummets. Impaired indicator abundance appears to react similarly to increasing LDI values, with a similar threshold of approximately 3.4. However, the accuracy and precision of the impaired indicator abundance at LDI values greater than 3.4, appears to be improved vis-à-vis the abundance of reference indicators at low development intensity values.

Autecological indices

The indices of Van Dam et al. (1994) and Bahls (1993) combined autecological values for diatoms of European and the Mountain-west environments, respectively. That the indices developed elsewhere appear to be valid for diatoms of herbaceous depressional wetlands in peninsular Florida is not surprising, considering their use in identifying various disturbance gradients in large rivers of Idaho (Fore and Grafe 2002), streams of the mid-Atlantic states (Fore 2003), Wyoming (Peterson and Porter 2002), and Maine wetlands (U.S. EPA 2002e–see case studies). Not only are many of the diatoms cosmopolitan in their worldwide distribution, but they also exhibit fidelity of response to disturbance gradients throughout the world.

As in the abundance of indicator taxa, a threshold near 3.2-3.4 appears to dramatically affect the distribution of site scores. For instance, the abundance of diatoms that tolerate elevated pH, salinity, nitrogen, low D.O., and pollution, as well as the eutrophic and meso-polysaprobous all increase markedly in that range, although there are highly variable distributions for any given LDI above a 3.0. This suggests a threshold of tolerance exists up to approximate LDI values of 3.2.

Most metrics that decrease with increasing development intensity do so with accuracy and precision (albeit low), especially when compared with metrics that increase with increasing development intensity. A highly variable response is nevertheless

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evident for those six metrics, although it appears that the highest variability in the data exists near values of 1.0, as well as around LDI values of 3.2.

The Diatom Multimetric Index–Conclusion and Recommendations

The DIWC, which combines species composition and autecological response for diatoms of herbaceous depressional marshes in Florida, was correlated $|>0.75|$ ($p<0.001$) with increasing land use modification scores. The high number of metrics (14) provided for a wide-range of responses to various possible perturbations from hydrologic alterations to nutrient loading. However, the metric responses, including that of the DIWC, highlighted discrepancies within the distribution of values along the LDI. The highly variable distribution of metrics for any given LDI value was exhibited by all identified metrics. Thresholds appeared to exists at LDI values near 1.0 and 3.3, although these threshold were muted in the DIWC. These results suggest that LDI values of less than 3.2 could most likely be grouped together into a suite of highly variable reference sites – little structure in the data (i.e., correlation with the LDI) appeared to be manifested for values of <3.2. Similarly, the rapid increase in most metric values after 3.2 was also highly variable by site.

The sites analyzed in the development of this multimetric index were sampled during the late summer. Research on seasonal and annual variation in the distribution of diatoms, and of the variation in the DIWC for diatoms sampled in different years and seasons is currently underway. In addition, the current DIWC requires that the wetland be hydrated for sampling. However, in this study it was unknown how long a given wetland had been hydrated prior to sampling. Thus, although diatoms may respond very quickly to hydration (Stoermer and Smol 1999), sampling response and subsequent analyses may be confounded by temporal variation in hydration. Current research

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underway (K. Reiss, University of Florida Department of Environmental Engineering Sciences, *personal communication*) will determine the utility of benthic diatoms sampled during dry periods to adequately and accurately reflect the condition of the wetland in question. Should the benthic diatoms prove to be year-round indicators of community condition, this multimetric index constructed on epiphytic diatoms should be reassessed.

Finally, epiphytic diatoms are generally expected to change in community composition depending on the macrophytes species to which they are attached (Stevenson 2001). It would be well worth examining, in laboratory settings, community variations among epiphytic diatoms when several different wetland macrophytes species common in both impaired and reference conditions are available for colonization.

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CHAPTER 3
MACROPHYTES AS BIOINDICATORS

Introduction

Wetland macrophytes are well suited for use as reliable biological indicators of the relative condition of Florida freshwater depressional marshes. Defined as plants "growing in water or on a substrate that is at least periodically deficient in oxygen as a result of excess water"(Cowardin et al. 1979, pg. 40), wetland macrophytes are ubiquitous and identifying features of marshes throughout Florida. Since wetland plants are sessile (save for a few free-floating species), they are unable to actively avoid perturbations through flight mechanisms. Thus, the community composition of marsh macrophytes, "...reflect[s] the temporal, spatial, chemical, physical, and biological dynamics of a [wetland] system"(Fennessy et al. 2001, pg. 3), and changes in wetland macrophyte community composition may be used to indicate both past perturbations and current conditions (Doherty et al. 2000, Adamus et al. 2001, Fennessy et al. 2001).

Reliable biological indicators provide information on the condition of a resource relative to "reference," or least disturbed conditions, within an ecologically meaningful region (Barbour et al. 1996, Karr and Chu 1999). Wetland macrophytes possess many advantages for use as reliable biological indicators, most notably their prevalence in wetland systems and response to land use changes (Table 3-1). Wetland macrophytes respond to hydrologic, nutrient, and chemical perturbations associated with increased development to wetland landscape matrices (Adamus and Brant 1990, Adamus 1996,

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Table 3-1. Advantages and disadvantages of using macrophytes as biological indicators of wetlands condition.

Advantages of Macrophyte Biological Indicators • Ubiquitous Features of Wetlands • Sessile Organisms • Established Taxonomy • Great Diversity of Species • Ecological Tolerances Well-Known • Well-developed Sampling Techniques
Disadvantages of Macrophyte Indicators • Possible lag time between disturbance and response • Field Identification Difficult in Certain Seasons • Sampling Window May Only Be the Growing Season • Some Species Non-responsive to Perturbations • Few Published Documentations of Response to Certain Stressors

Note: Modified from Cronk and Fennessy (2001).

Doherty et al. 2000, Fennessy et al. 2001, Adamus et al. 2001). Changes in wetland macrophyte species abundance and composition along nutrient gradients have been noted Bedford et al. 1999). Compositional changes due to chemical loading (i.e., herbicides; McIntyre et al. 1988), hydrologic alterations (Wood and Tanner 1990, Rochow 1994, David 1994, Busch et al. 1998, MaGee et al. 1999) and to destruction of habitat (Blanch and Brock 1994, Winchester et al. 1995, Dobkin et al. 1998, van Oene et al. 1999, Grace and Jutila 1999, Reader and Craft 1999, Vulink et al. 2000) have also been described for wetland macrophytes. Additionally, well-documented collection methods exist for wetland plants (e.g., Brown 1991, Peet et al. 1996, Fennessy et al. 2001), and definitive identification texts abound that permit identification to the species level in most cases (e.g., Godfrey and Wooten 1981, Wunderlin 1998). Moreover, unlike typical macroinvertebrate and algal indicators, standing water need not be present for the collection of wetland macrophytes, thus increasing the sampling opportunities for wetland assessment.

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The use of macrophytes as biological indicators is not without disadvantages. There are seasonal differences in species composition and potential lag times between perturbations and responses (Fennessy et al. 2001). While most wetland plants are easily identified with basic field training, some species may lack diagnostic parts during the sampling window or require expert skills to identify. While detractions from using macrophytes exist (see Table 3-1), wetland macrophytes nevertheless "...provide clear and robust signals of human disturbance" (Cronk and Fennessy 2001, p. 373).

Recognizing the ability and sensitivity of macrophyte bioindicators to differentiate wetland condition along a gradient of landscape modification, several states have included or are exploring the use of macrophytes in their wetland biological assessment programs (e.g., Ohio: Mack 2001, Montana: Apfelbeck 2000, Minnesota: Gernes and Helgen 1999, Galatowitsch et al. 1999a, North Dakota: Mushet et al. 2002). Additionally, the U.S. Environmental Protection Agency's Biological Assessment of Wetlands Working Group (BAWWG) has supported development of biological assessment criteria, including macrophytes, through funding for state initiatives, workshops, and technical publications (e.g., Doherty et al. 2000, Fennessy et al. 2001, U.S. EPA 2002a).

In this chapter, details regarding methods of data analyses and final choices for macrophyte metrics for use in developing biological indicators of wetland condition were given. Analyses of environmental parameters measured (both abiotic and biotic) were also conducted to assess the environmental variables that affected the distribution of macrophyte taxa within wetlands. In addition, metric variation for a given LDI score for

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the macrophyte metrics was also examined to assess the accuracy and precision of the LDI.

Methods

Site Selection and Disturbance Gradient

Seventy-five isolated depressional marshes were sampled for macrophyte community composition throughout peninsular Florida in the summers of 1999 and 2000 (Figure 3-1). Using best scientific judgment, sites were initially stratified into reference (no obvious human modified landscapes within >100m), and impaired (obvious human landscape modification within 100m) categories. Site condition was independently assessed later with the LDI (Brown and Vivas *submitted*), as noted in the diatom methods section (Chapter 2: Site Selection and Agricultural Development Gradient). Slightly less than half the sites (35) were considered reference sites, generally located in state and federal parks, preserves, and state forests. Wetlands that comprised the remaining 40 sites were located within agricultural landscape matrices and were considered impaired sites (LDI > 2.0). Thirty impaired sites were located in cattle ranches of varying stocking densities, 7 in truck crops (tomatoes, peppers), 2 in citrus groves, and 1 was sampled in a silvicultural forest. Site coordinates and LDI score for each sampled wetland are found in Appendix A. All but five sites were hydrated when sampled.

Field Data Collection

Wetland macrophytes were identified and counted in 1m x 5m quadrats along four belt transects. Transects were laid out along each cardinal direction (North, South, East, West) beginning at the wetland edge and extending to the center of the wetland (Figure 3-2). The wetland/upland boundary was determined in the field through plant status (i.e., facultative, obligate, upland) from Tobe et al. (1998) and hydric soil parameters (see U.S.

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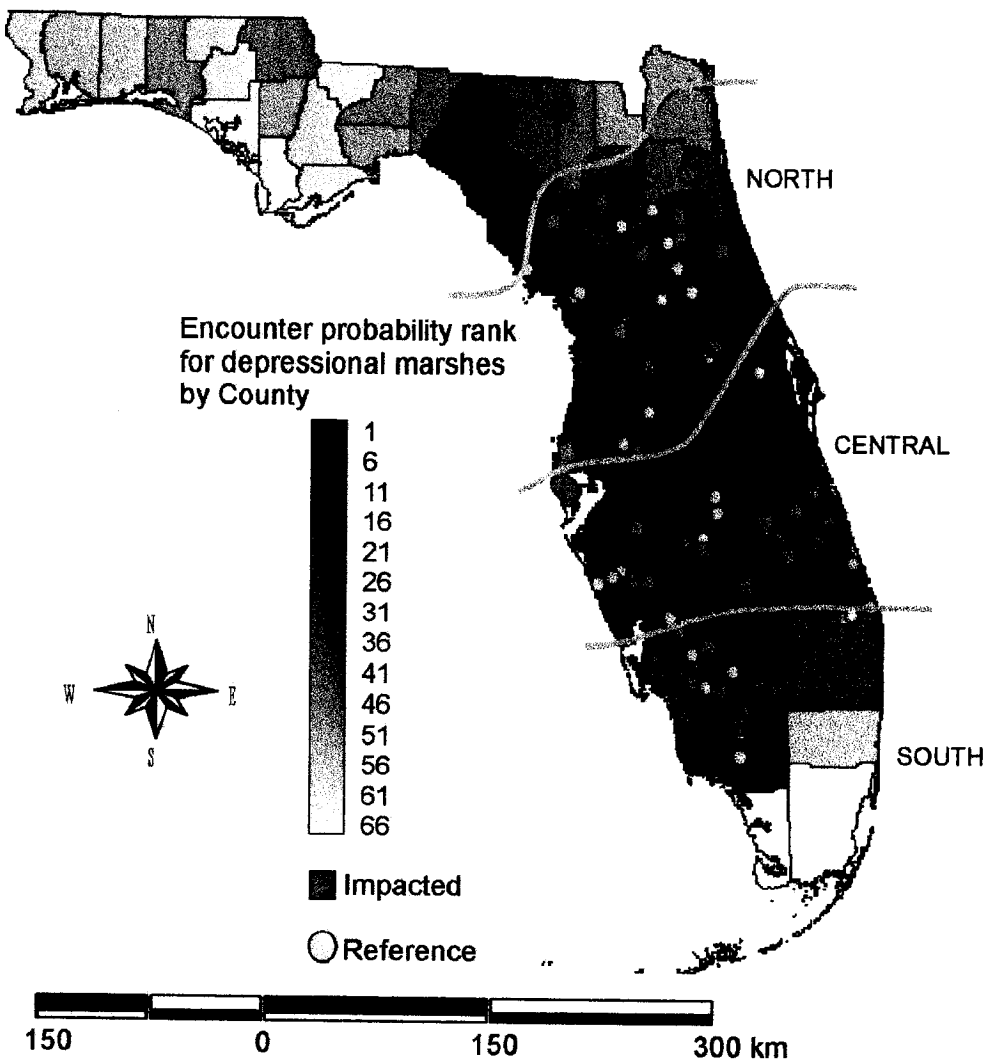

Figure 3-1. Study site locations and encounter probability for isolated depressional wetlands. Counties shaded darker have a higher density of isolated depressional wetlands <1ha (from U.S. Fish and Wildlife Service 2000).

Department of Agriculture 1998). In 1999, the transects continued from the wetland edge until the center was reached or until 15m^2 (3 consecutive quadrats) did not change in species composition. (Approximately 2% of the sites had transects not reaching the measured center of the wetland in 1999.) This was changed in 2000 to ensure full

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characterization of the wetland macrophyte community by collecting data along the entire length of each transect. The presence of living plants was noted within 0.5m along each

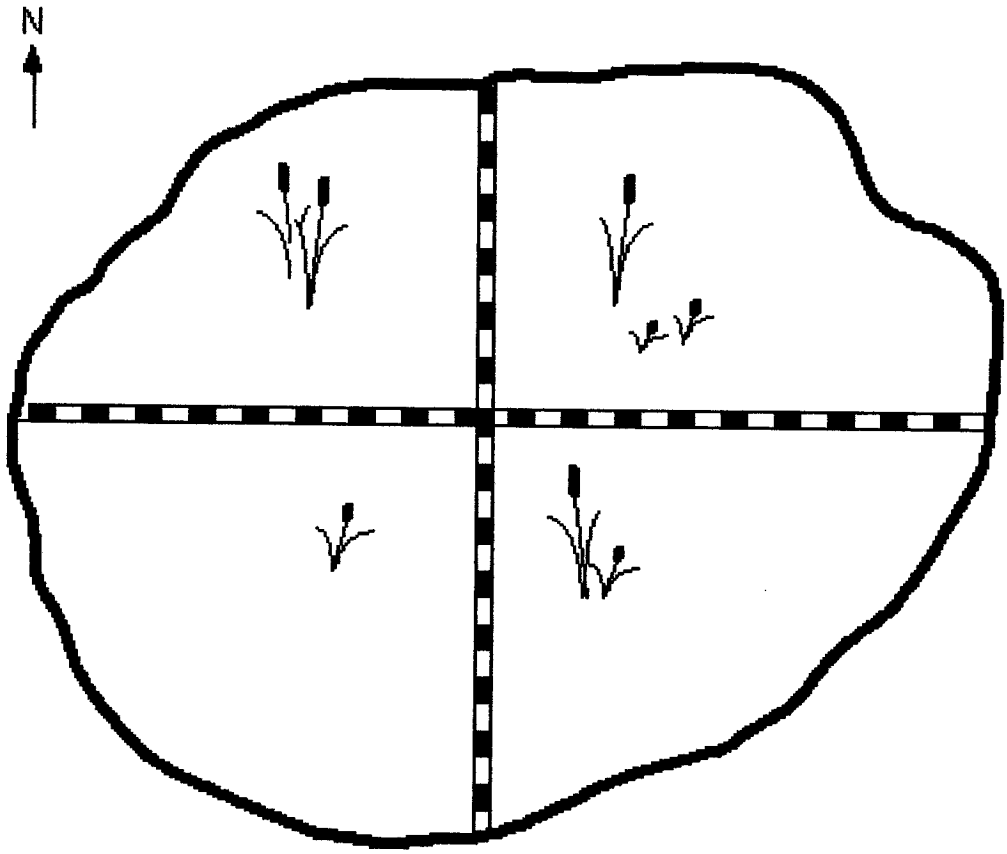

Figure 3-2. Transect and quadrat locations in a hypothetical depressional wetland.

side of the transect tape for each quadrat from the wetland edge to the center of the wetland. Unknown species were collected, labelled, and pressed for later identification by David Hall of the University of Florida. All collected species were stored in the Howard T. Odum Center for Wetlands herbarium.

Drought conditions during 1999 and 2000 field seasons forced the sampling period to be both delayed in its onset and extended in duration to ensure hydration of the sampled wetlands (Table 3-2). Thus, some species encountered lacked the fruiting bodies, flowers, and other parts requisite for identification due to seasonal vagaries in

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reproduction or growth. All macrophytes found were identified to the lowest level possible, generally species, and entered into the database for further use in metric development. This study focused on taxa identified to species.

Table 3-2. Sampling dates for each region.

Region	Earliest Date 1999	Latest Date 1999	Earliest Date 2000	Latest Date 2000
South	August 3	November 4	August 29	October 25
Central	July 23	August 17	September 9	October 26
North	July 28	November 11	October 16	November 1

Database Development

Field data sheets denoting presence of macrophytes within each 1m x 5m quadrat along each of the four transects were transcribed into Microsoft Excel spreadsheets and a Microsoft Access database and error-checked by two people: the person who originally transcribed the data into Microsoft Excel, and another person who reread the original datasheet back to the original transcriber who reviewed the Microsoft Excel output. A master plant list of all plants identified was developed, and ancillary species' characteristics from the literature, as well as Coefficient of Conservatism scores (described below), were added to the list. The plant database was linked in such a way that all characteristics could be analyzed by site, transect, or quadrat.

Species characteristics

Plant species characteristics (listed in Table 3-3) were taken from the literature for use in developing biological indicators. The primary source for these data was Wunderlin (1998), but other sources were also consulted, including Godfrey and Wooten (1979, 1981), Tobe et al. (1998), and the internet-based *Atlas of Florida Vascular Plants* (Wunderlin and Hansen 2000).

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Table 3-3. Ancillary data added to the database for each plant species.

Family
Annual or perennial (biennial plants were incorporated in the annuals)
Form: grass, sedge, rush, forbs, vine, fern, tree, shrub, aquatic
Deciduous or evergreen
Native or exotic
Coefficient of Conservatism score

Coefficient of Conservatism

Ten expert botanists (Anthony Arcuri, Keith Bradley, Kathy Burks, David Hall, Ashley O'Neal, Jim Poppleton, Nina Raymond, Bruce Tatje, John Tobe, and Wendy Zomlefer) were enlisted to assist in identifying species characteristics for use in developing a Coefficient of Conservatism score. The Coefficient of Conservatism (CC) was developed by Wilhelm and Ladd (1988) as part of the Floristic Quality Assessment Index, a method of assessing vegetation in the Chicago area based on expert botanists' opinion on the fidelity of a plant species to particular environments. Since its inception, this method has been utilized to assess wetland condition based upon species present in numerous studies (Herman et al. 1997, Fennessey et al. 1998, Mack 2001, Mushet et al. 2002, Cohen et al. *submitted*). To determine CC scores, the botanists were sent the full list of species encountered during the two sampling seasons (n=397), and were asked to score the plants using the coefficients and definitions given in Table 3-4. The botanists were also asked to weight the confidence of their answers on a 1-3 scale (3 being the most confident). Subsequently, each botanist's CC value for each plant was multiplied by each botanist's confidence weight for that plant, increasing the proportional representation for extremely confident scores. The score for each plant after confidence

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Table 3-4. Scoring Criteria for the Coefficient of Conservatism.

C of C		Description
0	-	Alien taxa and those native taxa that are opportunistic invaders or common components of disturbed communities.
1 – 3	-	Widespread taxa that are found in a variety of communities, including disturbed sites.
4 – 6	-	Taxa that display fidelity to a particular community, but tolerant of moderate disturbance to that community.
7 – 8	-	Taxa that are typical of well-established communities that have sustained only minor disturbances.
9 – 10	-	Taxa that exhibit a high degree of fidelity to a narrow set of ecological conditions.

weighting was summed and averaged across all botanists, and normalized to a 1-10 scale. See Cohen et al. (*submitted*) for additional information.

Soil and Water Physical-Chemical Sampling and Analysis

To relate the distribution of wetland macrophytes to environmental parameters, soil and water physical and chemical parameters were measured at each site as described in Chapter 2 (Methods: Field Data Collection, Sample Preparation and Laboratory Procedures) and Appendix B.

Data Analyses

Summary statistics

Summary measures of sample richness, Shannon diversity, Simpson diversity, beta, and gamma diversity were calculated for each site and provided information on species distributions vis-à-vis land use as described in Chapter 2: Data Analyses.

Macrophytes-compositional analysis

While wetlands throughout peninsular Florida were sampled, the sites were initially stratified into three peninsular wetland regions, or areas of similar climatic and physiographic conditions (after Lane 2000, see Figure 3-1). Through examination of data at the regional level, it was expected that variations in species assemblages due to

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climatic or physiographic differences between sites within a given region would be minimized (Gerritsen et al. 2000, Smogor and Angermeier 2001). To test the significance of the modeled wetland regions on species distribution, a compositional test, the multiple response permutation procedures (MRPP,) available in PCOrd (MjM Software, Gleneden Beach, Oregon, version 4.10) and described in Chapter 2 (Methods: Site Selection and Agricultural Development Gradient), was used.

Macrophyte composition and soil and water parameter correlates

The following soil variables were arcsine square root transformed to decrease measured skewness and kurtosis in the dataset: %TN, %OM, and %TC. Log_{10} transformations were completed on soil TP, color, specific conductivity, turbidity, ammonia, nitrate/nitrite, TKN, and water TP for similar reasons. Multivariate colinearity among the environmental variables, described as strong correlations between independent variables (Zar 1999), was tested for using the variance inflation factor and tolerance, described in Chapter 2 (Methods: Data Analyses). The non-parametric Mann-Whitney U-test was used to test the null hypothesis of equal medians between reference and impaired classes for each environmental variable (Zar 1999).

The relationship between measured environmental values (including LDI score) and species composition was examined with the Mantel test (Mantel 1967) and with non-metric multidimensional scaling (NMDS, Kruskal 1974, Mather 1976), both available in PCOrd and described in Chapter 2 (Methods: Data Analyses). A two-dimension NMDS solution was selected for macrophyte taxa as increased dimensionality only marginally improved the fit. Correlations of site scores with environmental parameters, including LDI score and latitude and longitude (decimal degrees), were calculated and a bi-plot of parameters with correlations (Pearson's $r^2 \geq 0.30$) to site scores was constructed.

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Metric Development

Macrophyte metrics were identified from the literature (e.g., Wilhelm and Ladd 1988, Adamus and Brant 1990, Andreas and Lichvar 1995, Adamus 1996, Kantrud and Newton 1996, Gernes and Helgen 1999, Cronk and Fennessy 2001, Mack 2001, Fennessy et al. 2001) or developed from iterative analyses of data response. Metrics were selected for inclusion in the preliminary Vegetative Index of Wetland Condition (VIWC) if they demonstrated a constant and predictable change with increasing Landscape Development Intensity (LDI) scores as measured by the Spearman correlation coefficient. Final metrics were those with significant ($p<0.05$) Spearman's correlations $\geq|0.50|$ with the LDI. Metrics were also tested for their ability to discern between the classification of sampled wetlands in impaired (LDI $\geq$2.0) and reference (LDI <2.0) landscapes using the nonparametric Mann-Whitney U-test. Additionally, to normalize wetlands of different sizes, metrics that incorporated the enumeration of species (such as the count of exotic species encountered at a site) were changed into abundance values by dividing the sum of the metric by the total number of plants identified to species at a site. All evaluations of potential metrics were conducted with SAS (SAS Institute, Cary N.C., version 8.02). Based on preliminary data analysis and the strength of the metric correlation with the LDI, the macrophyte metrics described below were selected for inclusion in each wetland region.

Sensitive and tolerant species

Indicator Species Analysis (ISA, Dufrene and Legendre 1997) was utilized to identify species with a significant association with LDI classification of impaired (LDI$\geq$2.0) or reference conditions (LDI<2.0) as described in Chapter 2 (Metric Development).

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Exotic species

The percent of total species exotic to Florida at a wetland site was calculated by dividing the number of exotic species encountered by the total number of species found at the site. Native or exotic plant status was determined using Tobe et al. (1998), Wunderlin (1998), Godfrey and Wooten (1979, 1981), Langeland and Burks (1998), and Wunderlin and Hansen (various dates 2000). In general, exotic species were those thought to have not been present in Florida before European settlement of North America. The abundance of exotic species was expected to increase with increasing development of the landscape surrounding the wetland (Fennessy et al. 2001).

Annual to perennial ratio

The ratio of annual to perennial (A:P) species was calculated for each wetland. The trend expected was that as development increased in a wetland landscape matrix, the proportion of "weedy," or annual species versus perennial species would increase (Edwards and Weakley 2001). Life-history status (i.e., annual/perennial) was determined from the literature for each species identified, and a ratio was determined for each wetland.

Average Coefficient of Conservatism

To obtain an average CC score for each wetland sampled, the sum of the confidence weighted CC values for each plant found at the sampled wetland was divided by the total number of plants sampled. The resultant CC score provided an estimate of the conservatism of the plants encountered. As development increased within the wetland landscape matrix, the CC score for the wetland was expected to decrease, indicating the presence of both plants with more general habitat requirements and exotics.

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Vegetative Index of Wetland Condition

To create a multi-metric index of wetland condition based on metrics that were strongly correlated with the LDI, it was necessary to score the proposed metrics. Scoring allows the disparate metrics (i.e., such as ratios and relative abundance metrics) to be summed to provide a numerical score for the condition of the wetland to compare vis-à-vis a set of reference wetlands within a given region (Karr and Chu 1997). Scoring methods are described in Chapter 2 (Methods: Constructing the Multimetric Index). Following the metric scoring, the Vegetative Index of Wetland Condition was determined for each wetland sampled by summing the scored metrics.

Results

Field Data

Seventy-five herbaceous depressional wetlands throughout peninsular Florida were sampled in 1999 and 2000. Twenty-three were sampled in the South region, thirty in the Central region, and twenty-two in the North region. Based on the predominant land use around the wetland, twelve of the sites sampled in the South region were *a priori* considered impaired, sixteen in the Central region, and eight in the North region.

Three hundred and ninety-seven different taxa from 207 plant genera representing 111 families were identified during the 1999 and 2000 field seasons. Two hundred twenty-six taxa were identified in the South region, 270 taxa were identified in the Central region, and 242 taxa were identified in the North region; many species were found in more than one region.

Summary measures of sample richness, Shannon diversity, and Simpson diversity were calculated for each site (Brower et al. 1990). Summary statistics are presented in Table 3-5. Mann-Whitney U-tests indicated no significant difference between reference

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and impaired sites for richness, Shannon diversity, or Simpson diversity (all tests Z = -0.005, $p = 0.996$). Two additional indices were also calculated from the macrophyte data: beta and gamma diversity (Ricklefs 1990, McCune and Grace 2002). Beta and gamma diversity were higher in impaired sites than in reference sites (impaired sites average richness: 31.60, beta diversity: 9.81, gamma diversity: 310; reference sites average richness: 31.66, beta diversity: 8.18, gamma diversity: 259).

Ancillary Data

A histogram of the distribution of CC scores for the species identified is given in Figure 3-3. As the data were normalized, the mean of the distribution is 5.0, while the median value is 5.56, and the standard deviation is 2.57. The plant with the highest conservatism score (10) was *Coelorachis tuberculosa*. In general, exotic species and invasive native species were given scores of zero. However, several exotic species, such as *Xyris jupicai* (CC = 3.51), were given higher scores by the botanists, indicating a perceived higher fidelity to a narrower set of ecological conditions. A list of species identified and their corresponding CC scores is given in Appendix D.

Compositional Analysis

A matrix of the co-occurrence of identified species in the wetland regions of Florida is presented in Table 3-6. The co-occurrence of species found in reference and impaired sites for the three wetland regions are also given in Table 3-6. The South and North regions shared the fewest species, slightly more than half, while approximately 60% of the plants found in the Central region were also found in the North or South regions. The Central region was also most similar to the North and the South regions for both impaired and reference plants.

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Table 3-5. Summary statistics of richness (S), Simpson's diversity index (H) and Shannon diversity index (D') for macrophytes.

Site Name	S	H	D`
ALPaynes	28	3.332	0.9643
Audobon	16	2.773	0.9375
Bear Scat	39	3.664	0.9744
Big Cow	34	3.526	0.9706
BRSebastian	24	3.178	0.9583
CabPatch	34	3.526	0.9706
Caravelle	22	3.091	0.9545
Chuluota	41	3.714	0.9756
CLBayard	20	2.996	0.95
CLCove	34	3.526	0.9706
CMWPast	39	3.664	0.9744
CMWRef	21	3.045	0.9524
COBurgle	16	2.773	0.9375
COHole	25	3.219	0.96
Crew	31	3.434	0.9677
DEMelon	41	3.714	0.9756
Deerfly	31	3.434	0.9677
GarberRanch	22	3.091	0.9545
GBarE	28	3.332	0.9643
GLDonut	24	3.178	0.9583
GLPont	20	2.996	0.95
Goethe	24	3.178	0.9583
GreenSwamp	34	3.526	0.9706
HaRare	33	3.497	0.9697
HagueI	27	3.296	0.963
HagueII	21	3.045	0.9524
Half Moon	37	3.611	0.973
HEBad	37	3.611	0.973
HEL2	18	2.89	0.9444
HEOKAY	46	3.829	0.9783
HighPast	16	2.773	0.9375
HighRef	23	3.135	0.9565
HillsRef	43	3.761	0.9767
Hunt Camp	26	3.258	0.9615
IFASI	26	3.258	0.9615
IFASII	27	3.296	0.963
Immokalee	26	3.258	0.9615
IRBlueCypress	42	3.738	0.9762

Site Name	S	H	D`
IRCanal	22	3.091	0.9545
IROJ	43	3.761	0.9767
JD6	33	3.497	0.9697
Kelly Park	27	3.296	0.963
LCork	48	3.871	0.9792
LLeeCounty	58	4.06	0.9828
LEGo	41	3.714	0.9756
LESuwan	42	3.738	0.9762
MALudy	54	3.989	0.9815
MASpray	24	3.178	0.9583
McArthur	29	3.367	0.9655
MNElmer	45	3.807	0.9778
MNErik	38	3.638	0.9737
MNOcala	38	3.638	0.9737
MRPepper	46	3.829	0.9783
Myakka	35	3.555	0.9714
OKCara	47	3.85	0.9787
OKKiss	37	3.611	0.973
OKPast	28	3.332	0.9643
PacificTom	37	3.611	0.973
Pall-Mar	17	2.833	0.9412
PBCorbett	33	3.497	0.9697
PBEnjay	43	3.761	0.9767
Penner	14	2.639	0.9286
POWales	18	2.89	0.9444
POWeowak	44	3.784	0.9773
PUPond	19	2.944	0.9474
RiceCreek	16	2.773	0.9375
SANorthMya	31	3.434	0.9677
SAOscer	38	3.638	0.9737
SandhillCrane	35	3.555	0.9714
Savannas	41	3.714	0.9756
STCow	40	3.689	0.975
SUVaca	37	3.611	0.973
SUWarhol	33	3.497	0.9697
UNHealthy	30	3.401	0.9667
Wekiva	15	2.708	0.9333

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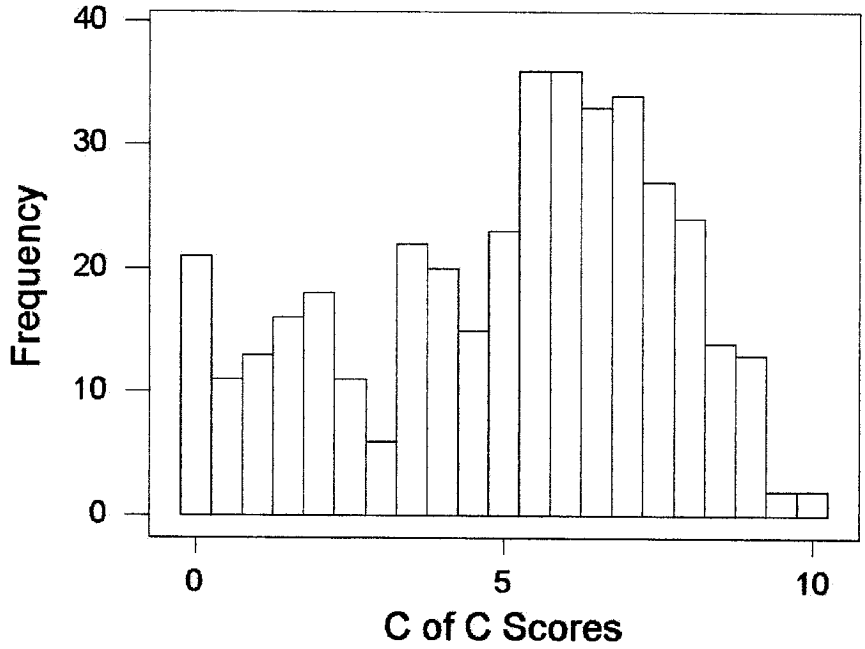

Figure 3-3. Normalized Coefficient of Conservatism Histogram for confidence weighted scores for wetland macrophytes.

Tests of compositional similarities between wetland regions (Lane 2000) were conducted using MRPP (Table 3-7). Significant differences in compositional similarity among wetland regions were identified for both simultaneous (i.e., South vs. Central vs. North) and iterative tests (i.e., South vs. Central, South vs. North, Central vs. North). When reference sites were compared, significant differences were also identified for both simultaneous and iterative tests. Tests of impaired sites indicated significant differences when simultaneously tested, however, iterative tests found only significant difference between impaired sites of the South and North regions at $p<0.05$. The iterative tests of impaired sites between all regions were significantly different at $p<0.10$.

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Table 3-6. Co-occurrence matrices of macrophytes by region and impairment condition.

Condition	Region	South	Central	North
All Sites (n=75)	South	100.0 %	61.9 %	52.3 %
	Central	73.4 %	100.0 %	66.9 %
	North	56.6 %	60.0 %	100.0 %
Reference (n=35)	South	100.0 %	54.4 %	47.8 %
	Central	58.1 %	100.0 %	65.7 %
	North	43.2 %	55.7 %	100.0 %
Impaired (n=40)	South	100.0 %	58.2 %	47.9 %
	Central	67.9 %	100.0 %	55.8 %
	North	56.2 %	56.1 %	100.0 %

Table 3-7. Simultaneous and iterative tests of community composition among regions.

Test	Comparison	*A*	*T*	*p*-value
Simultaneous Tests: All Sites	S vs. C vs. N	0.0211	-6.235	< 0.001
Simultaneous Reference Only	S vs. C vs. N	0.0502	-7.198	< 0.001
Simultaneous Impaired Only	S vs. C vs. N	0.0176	-3.137	0.005
Iterative Tests: All Sites	S vs. C	0.0120	-3.330	0.010
	S vs. N	0.0323	-8.066	< 0.001
	C vs. N	0.0075	-2.156	0.039
Iterative Tests: Reference Only	S vs. C	0.0300	-4.156	0.001
	S vs. N	0.0690	-7.492	< 0.001
	C vs. N	0.0204	-2.961	0.010
Iterative Tests: Impaired Only	S vs. C	0.0094	-1.703	0.060
	S vs. N	0.0022	-3.096	0.009
	C vs. N	0.0110	-1.873	0.050

Note: S = South region, C = Central region, N = North region

Community Composition and Environmental Gradients

Including the LDI, fourteen environmental variables were measured at each site. The measured water and soil parameters are given in the Appendix B. Significant differences between impaired and reference conditions, as measured by the Mann-

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Whitney U-test, were found for the following parameters: soil pH, soil TP, specific conductivity, water pH, ammonia, TKN, and water TP (Table 3-8). Water color was significant lower in reference conditions at $p<0.10$.

Colinearity among variables, which was identified as a VIF>5.0 and a tolerance of <0.20, was recognized for %TC, TKN, and water pH following regression analyses of the transformed data (SAS 1990). These variables were removed. The Mantel test was used to test the hypothesis of no relationship between the identified macrophytes and the measured environmental parameters, including LDI score. Results indicated a strong and significant relationship (Mantel's $r = 0.49$, $p=0.001$).

An NMDS ordination was completed to examine the underlying structure of the sites in ordination space and to ascertain the environmental variables driving the composition of sites sampled. Correlations for all measured environmental variables are presented in Table 3-9, and a biplot of site ordination overlaid with vectors representing the environmental variables is presented in Figure 3-4. The stress, or "goodness of fit" of the ordination was 21.11 and indicated a marginally adequate and representative decreased dimensionality of the dataset (Kruskal 1964, Clarke 1993). Likewise, the final instability after 400 iterations was 0.00399, which was higher than the ideal instability (<0.001) but nevertheless a generally acceptable instability value (McCune and Grace 2002). The ordination indicated that 74.5% of the variance in the dataset was captured by the two dimensional ordination (first axis: 42.5%, second axis:33.0%). The ordination scores for each site were linearly correlated (Pearson's r) with the measured environmental values, including LDI, latitude, and longitude. Correlations (Pearson's r^2)

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Table 3-8. MRPP comparison of the median values of water and soil variables between reference and impaired sites.

Water Values	Statistic (Z)	*p*-value
Color	-1.868	0.066
Specific Conductivity	-3.919	< 0.001
Turbidity	-1.524	0.127
Water pH	-4.700	< 0.001
Ammonia	-2.707	0.007
Nitrates/Nitrites	0.782	0.437
TKN	-2.932	0.003
TP	-4.836	< 0.001
Soil Values	**Statistic (Z)**	***p*-value**
Soil pH	-2.894	0.004
%TC	-0.616	0.538
%OM	-1.105	0.269
%TN	-0.924	0.356
%TP	-2.719	0.008

Table 3-9. Correlations of environmental variables and ordination scores.

	Axis 1 (42.5%)		Axis 2 (33.0%)	
Parameter	r^2	**tau**	r^2	**tau**
Soil pH	0.14	0.26	0.57	0.57
% TN	<0.01	0.14	0.16	-0.21
TP (mg/kg)	0.20	0.32	0.05	-0.13
% OM	0.02	0.14	0.22	-0.27
Color (PCU)	0.11	0.21	0.08	-0.17
Spec. Cond. (umhos/cm)	0.33	0.40	0.10	0.23
Turbidity (NTU)	0.07	0.15	0.01	-0.05
Ammonia (mg/L)	0.07	0.24	0.03	-0.07
Nitrate/Nitrite (mg/L)	<0.01	-0.05	0.05	-0.20
Water TP (mg/L)	0.48	0.52	<0.01	-<0.01
LDI Score	0.66	0.56	0.11	0.22
Latitude (decimal degrees)	0.01	0.07	0.39	-0.45
Longitude (decimal degrees)	0.01	0.04	0.03	-0.14

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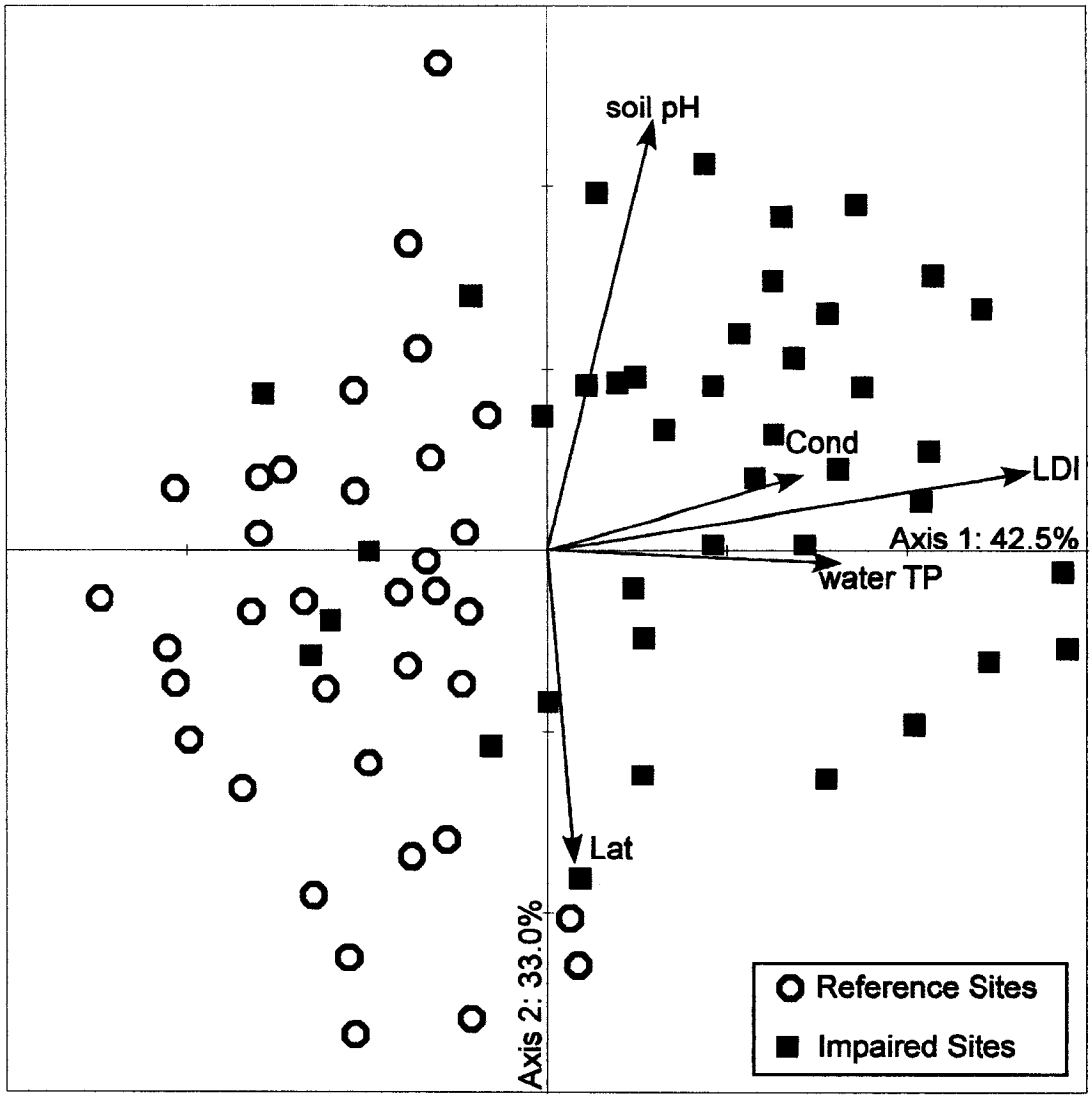

Figure 3-4. Biplot of macrophyte NMDS ordination scores for each site and strongly correlated environmental variables. The vectors are shown at 150% of original length for clarity. The percent variance explained by each axis is noted on the axis. The length of the vectors represents the strength of the correlation (all >|0.30|) and the angle represents the direction of maximum change. Five variables are shown: soil pH, Cond (specific conductivity), water TP, latitude, and LDI.

> 0.30 were found for LDI: 0.66, soil pH 0.57, water TP: 0.48, latitude: 0.39, and specific conductivity: 0.33, indicating their importance in driving the structure of the wetland macrophyte community.

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Metric Development

Significant regional differences were identified in species composition, hence initial metric development focused on identifying regionally specific bioindicators with strong correlations with the LDI. It was anticipated that due to the regionally distinct composition of the isolated depressional herbaceous wetlands, the final metrics would likewise vary for each region. However, though the composition of the wetlands varied between regions, the suite of final metrics that had significant correlations with *p*-values <0.05 and Spearman's correlation coefficients of $r > |0.40|$ with the LDI gradient (% Sensitive, % Tolerant, % Exotic, Average CC, and A:P ratio) did not vary, indicating a common response of wetland systems to increases in land use development.

Sensitive and tolerant species

Indicator species analysis (ISA) identified 39 taxa as indicative of reference conditions and 26 taxa as indicative of impaired conditions (Tables 3-10 and 3-11, respectively). Thirty-four were identified in the South region (22 reference, 12 impaired), 45 in the Central region (27 reference, 18 impaired), and 8 in the North region (2 reference, 6 impaired). Correlations of the abundance of species indicative of reference conditions (% Sensitive) and impaired conditions (% Tolerant) with the LDI were significant ($p<0.001$) and $>|0.70|$ for all regions (Table 3-12). There was moderate overlap of indicator species between regions, with 33.9% (22/65) occurring in more than one region.

ISA was also used to identify species with specificity and fidelity to reference and impaired conditions using the peninsular dataset ($n=75$). While compositional differences existed between regions, it was hypothesized that some taxa would be robust indicators throughout peninsular Florida. It was also hypothesized that increasing the *n*

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Table 3-10. Regionally derived species indicative of reference conditions.

Reference Indicator Species (Sensitive Species)	South	Central	North
Amphicarpum muhlenbergianum		(50.1, 0.074)	
Andropogon virginicus	(49.9, 0.010)	(61.1, 0.019)	
Aristida purpurascens		(38.5, 0.007)	
Blechnum serrulatum	(47.3, 0.026)		
Cassytha filiformis	(27.3, 0.095)		
Cladium jamaicense	(38.4, 0.070)		
Eriocaulon decangulare	(41.8, 0.086)	(69.2, <0.001)	
Eupatorium leptophyllum	(36.4, 0.039)		
Fuirena scirpoidea	(62.7, <0.001)		
Gratiola ramosa	(45.5, 0.015)	(38.5, 0.011)	
Hedyotis uniflora		(23.1, 0.074)	
Hypericum brachyphylum		(38.5, 0.008)	
Hypericum fasciculatum	(41.8, 0.088)	(61.5, <0.001)	
Ilex glabra	(27.3, 0.087)	(38.5, 0.009)	
Ipomoea sagittata	(27.3, 0.090)		
Iva microcephala	(27.3, 0.096)		
Lachnanthes caroliniana		(44.5, 0.062)	(49.5, 0.084)
Ludwigia linifolia		(30.8, 0.027)	
Lycopodium appressum	(27.3, 0.087)		
Lyonia lucida		(23.1, 0.071)	
Oxypolis filiformis	(38.4, 0.069)		
Panicum erectifolium	(85.7, <0.001)	(48.5, 0.005)	
Panicum rigidulum	(38.4, 0.069)	(30.8, 0.026)	
Panicum spretum		(23.1, 0.073)	
Panicum tenerum		(48.5, 0.005)	
Paspalum monostachyum	(27.3, 0.090)		
Pinus elliottii	(54.5, 0.003)		(73.6, 0.002)
Proserpinaca pectinata		(66.7, <0.001)	
Rhynchospora fascicularis		(23.1, 0.070)	
Rhynchospora filifolia	(36.4, 0.037)	(30.8, 0.028)	
Rhynchospora tracyi	(50.4, 0.037)	(40.9, 0.027)	
Scleria baldwinii		(23.1, 0.077)	
Serenoa repens		(53.8, 0.001)	
Stillingia aquatica	(45.5, 0.015)		
Syngonanthus flavidulus		(33.4, 0.056)	
Utricularia purpurea		(23.1, 0.072)	
Woodwardia virginica		(58.9, 0.007)	
Xyris elliottii		(23.1, 0.068)	
Xyris jupicai	(38.4, 0.066)	(38.5, 0.009)	

Note: Calculated indicator values and the significance of the indicator value for each species, respectively, are given in parentheses.

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Table 3-11. Regionally derived species indicative of impaired conditions.

Impaired Indicator Species (Tolerant Species)	South	Central	North
Alternanthera philoxeroides			(36.4, 0.090)
Axonopus furcatus	(33.3, 0.087)		
Bacopa monnieri	(41.7, 0.036)		
Centella asiatica		(64.3, 0.002)	
Commelina diffusa	(50, 0.014)	(41.2, 0.01)	
Cuphea carthagenensis	(50, 0.013)	(41.2, 0.010)	
Cyperus haspan		(40.4, 0.036)	
Cyperus odoratus		(41.2, 0.009)	
Cyperus polystachyos		(58.8, 0.001)	(45.5, 0.034)
Cyperus surinamensis		(29.4, 0.050)	
Diodia virginiana		(70.6, <0.001)	(46.8, 0.062)
Eclipta prostrata		(52.9, 0.003)	
Eupatorium capillifolium		(42.2, 0.072)	
Hydrochloa caroliniensis	(33.3, 0.093)		
Juncus effusus		(75.3, <0.001)	(54.5, 0.011)
Lindernia grandiflora	(33.3, 0.090)		
Ludwigia octovalvis	(33.3, 0.092)		
Ludwigia peruviana	(33.3, 0.095)	(41.2, 0.010)	
Panicum repens		(41.2, 0.009)	
Paspalum notatum		(41.2, 0.010)	
Paspalum urvillei		(41.2, 0.010)	(36.4, 0.086)
Phyla nodiflora	(42.3, 0.070)	(47.1, 0.010)	
Polygonum hydropiperoides			(52.9, 0.088)
Polygonum punctatum	(47.3, 0.099)	(41.2, 0.010)	
Pontederia cordata	(68.7, 0.005)		
Setaria parviflora	(41.7, 0.040)	(29.4, 0.050)	

Note: Calculated indicator values and the significance of the indicator value for each species, respectively, are given in parentheses.

Table 3-12. Spearman correlations of the adundance of regional sensitive and tolerance macrophyte species with LDI scores for each site.

Region	Comparison	Spearman's *r*	*p*-value
South	% Sensitive Spp.	-0.75	< 0.001
	% Tolerant Spp.	0.72	< 0.001
Central	% Sensitive Spp.	-0.77	< 0.001
	% Tolerant Spp.	0.78	< 0.001
North	% Sensitive Spp.	-0.73	< 0.001
	% Tolerant Spp.	0.86	< 0.001

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Table 3-13. Indicator species of reference conditions derived from the peninsular dataset.

Reference Indicator Species (Sensitive Species)	Observed Ind. Val.	*p*-value	Reference Indicator Species (Sensitive Species)	Observed Ind. Val.	*p*-value
Amphicarpum muhlenbergianum	37.5	0.023	*Panicum rigidulum*	27.1	0.004
Andropogon virginicus	56.2	<0.001	*Panicum tenerum*	29.3	0.007
Aristida purpurascens	22.6	0.034	*Paspalum monostachyum*	8.6	0.097
Brasenia schreberi ‡	11.4	0.043	*Paspalum praecox* ‡	8.6	0.100
Drosera capillaris ‡	11.4	0.042	*Pinus elliottii*	47.7	<0.001
Eriocaulon decangulare	39.3	<0.001	*Proserpinaca pectinata*	47.6	<0.001
Fuirena scirpoidea	39.8	0.007	*Rhexia mariana* ‡	30.5	0.009
Gratiola ramosa	28.6	<0.001	*Rhexia nashii* ‡	8.6	0.097
Hypericum brachyphylum	24.3	0.011	*Rhynchospora filifolia*	22.9	0.002
Hypericum fasciculatum	44.0	<0.001	*Rhynchospora rariflora* ‡	14.3	0.019
Ilex glabra	29.1	<0.001	*Rhynchospora tracyi*	29.3	0.005
Iva microcephala	11.4	0.046	*Rhynchospora wrightiana* ‡	8.6	0.098
Lachnocaulon anceps ‡	8.6	0.094	*Scleria baldwinii*	12.2	0.089
Lachnanthes caroliniana	37.0	0.007	*Scleria reticularis* ‡	30.4	0.024
Lachnocaulon minus ‡	11.4	0.040	*Serenoa repens*	30.9	0.002
Ludwigia linifolia	18.8	0.036	*Stillingia aquatica*	14.3	0.019
Lycopodium appressum	11.4	0.040	*Syngonanthus flavidulus*	19.9	0.059
Nymphaea odorata ‡	19.9	0.089	*Utricularia purpurea*	12.2	0.094
Nyssa biflora ‡	16.0	0.076	*Viola lanceolata* ‡	8.6	0.099
Oxypolis filiformis	22.6	0.029	*Woodwardia virginica*	38.4	0.002
Panicum chamaelonche ‡	12.2	0.094	*Xyris elliottii*	17.8	0.025
Panicum erectifolium	45.3	<0.001	*Xyris jupicai*	27.1	0.005
Panicum hemitomon ‡	52.5	0.027	*Xyris smalliana* ‡	16.0	0.073

Note: Taxa marked (‡) were not significant indicator species at the regional level. Calculated indicator values and the significance of the indicator value for each species are also presented.

of the dataset would provide additional power in identifying indicator species. Forty-six species were identified as reference indicator species and 36 were identified as impaired indicators using the peninsular dataset (Tables 3-13 and 3-14, respectively). There was substantial overlap between regional and peninsular indicator species. Three regional impaired indicator species (South Region: *Axonopus furcatus*, Central Region: *Eupatorium capillifolium*, and North Region: *Polygonum hydropiperoides*), all of which have a statewide distribution (Tobe et al. 1998), were not calculated to be peninsular-wide indicator species. Nine regional reference indicator species (South Region:

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Blechnum serrulatum, Cassytha filiformis, Cladium jamaicense, Eupatorium leptophyllum, and *Ipomea sagittata,* Central Region: *Hedyotis uniflora, Lyonia lucida,Panicum spretum,* and *Rhyncospora fascicularis*), all of which have statewide distributions (with the exception of *Cassytha filiformis,* which is limited to south and central Florida – Wunderlin 1998, Tobe et al. 1998), were not significant indicator species using the peninsular dataset. However, 88.5% (23 of 26) of the regional impaired indicator and 76.9% (30 of 39) of the regional reference indicator species were also indicator species with the peninsular dataset. The additional power from combining the regional data into a peninsular dataset also allowed for an additional 17 species to be

Table 3-14. Indicator species of impaired conditions derived from the peninsular dataset.

Impaired Indicator Species (Tolerant Species)	Observed Ind. Val.	*p*-value	Impaired Indicator Species (Tolerant Species)	Observed Ind. Val.	*p*-value
Alternanthera philoxeroides	20.0	0.007	*Kyllinga brevifolia* ‡	12.5	0.058
Aster subulatus ‡	27.4	0.002	*Lindernia grandiflora*	20.0	0.007
Bacopa monnieri	21.7	0.086	*Ludwigia octovalvis*	24.9	0.005
Carex albolutescens ‡	12.5	0.059	*Ludwigia peruviana*	35.0	<0.001
Centella asiatica	43.4	0.032	*Ludwigia repens* ‡	33.4	0.014
Commelina diffusa	39.8	<0.001	*Melochia corchorifolia* ‡	12.5	0.058
Cuphea carthagenensis	37.3	<0.001	*Panicum repens*	32.4	0.006
Cynodon dactylon ‡	12.5	0.057	*Paspalum acuminatum* ‡	20.0	0.015
Cyperus haspan	24.0	0.052	*Paspalum notatum*	30.0	0.004
Cyperus odoratus	20.0	0.006	*Paspalum urvillei*	24.9	0.003
Cyperus polystachyos	45.0	<0.001	*Phyla nodiflora*	37.3	<0.001
Cyperus retrorsus ‡	12.5	0.055	*Polygonum punctatum*	38.3	<0.001
Cyperus surinamensis	20.0	0.006	*Pontederia cordata*	43.4	0.057
Diodia virginiana	53.8	<0.001	*Sacciolepis striata* ‡	26.7	0.080
Eclipta prostrata	30.0	<0.001	*Sesbania herbacea* ‡	15.0	0.029
Galium uniflorum ‡	12.5	0.060	*Setaria parviflora*	25.0	<0.001
Hydrochloa caroliniensis	24.0	0.050	*Thelypteris interrupta* ‡	12.5	0.058
Juncus effusus	52.3	<0.001	*Typha latifolia* ‡	12.5	0.056

Note: Taxa marked (‡) were not significant indicator species at the regional level. Calculated indicator values and the significance of the indicator value for each species are also presented.

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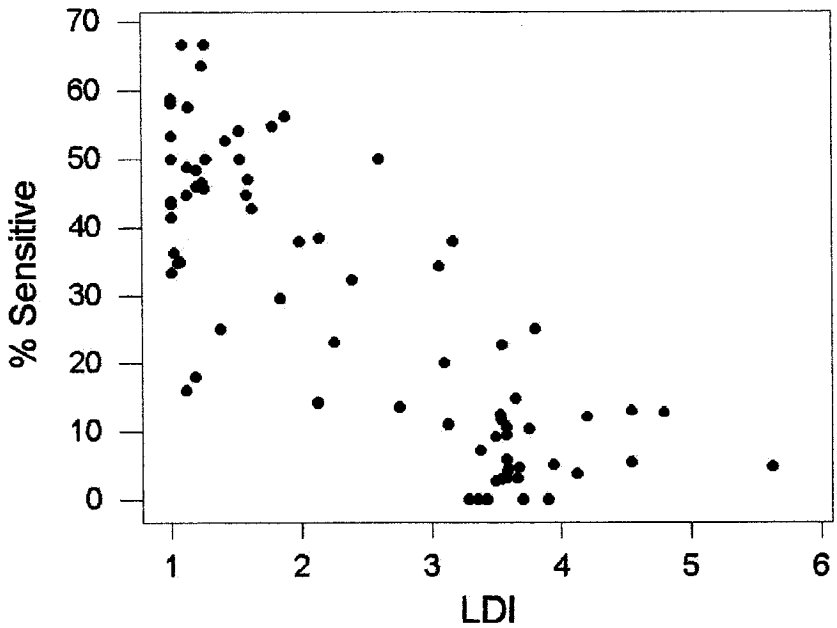

Figure 3-5. Abundance of sensitive species along the disturbance gradient.

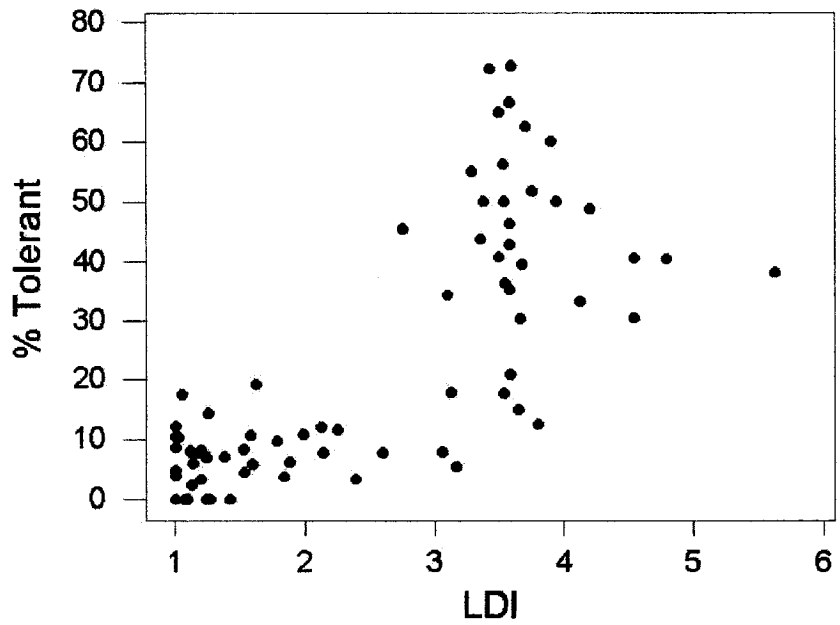

Figure 3-6. Abundance of tolerant species along the disturbance gradient.

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identified as indicator species of reference (7 species) or impaired (10 species) conditions (noted in Tables 3-13 and 3-14).

Using the peninsular indicator species (n=82 species), the abundance of indicator species for both reference (% Sensitive) and impaired (% Tolerant) conditions was calculated for each site and correlated with the LDI. A plot of the relationship between LDI score and % Sensitive and % Tolerant species are presented in Figures 3-5 and 3-6, respectively. The Spearman's correlation coefficients were significantly and highly correlated with the LDI (% Sensitive r = -0.76, p<0.001; % Tolerant r = 0.75, p<0.001). The abundance of sensitive taxa plotted in Figure 3-5 suggested a relatively accurate and precise distribution, with fair goodness of fit. There was substantial variation in the data for sites with LDI scores of approximately 1.0, but a noticeable and linear decreasing trend with increasing LDI values was evident. Distribution of the % Tolerant data indicated a low correlation between the LDI and the % Tolerant until LDI scores of approximately 3.5. At that point, substantial variation in the dataset was evident, with abundance values ranging from approximately 10% to 75%.

The peninsular dataset was utilized to identify the proposed bioassessment metrics. The apparent advantage of utilizing the peninsular dataset lay in the higher number of significant indicator species identified for community condition assessment and the strength and significance of the correlations with the LDI. The combined dataset provided 48 additional indicator species in the South region, 37 additional indicators in the Central region, and 74 additional significant indicators in the North region. However, as the number of reference and impaired sites sampled was not equal for the regions (South reference = 11, Central reference = 13, North reference = 11; South impaired =

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12, Central impaired = 17, North reference = 11), Kruskal-Wallis tests were employed to ensure that the median abundance of sensitive and impaired indicators were equal for each region. The Kruskal-Wallis test null hypothesis was equal medians for all groups. Unequal medians would indicate a regional bias in the distribution of indicator species and likely predispose a reference or impaired site in a particular region to lower assessment values vis-à-vis reference or impaired sites in the other regions. No significant difference in median values between regions for either % Sensitive or % Tolerant species were identified using the Kruskal-Wallis test (% Sensitive H=0.076, p=0.963, % Tolerant H=1.598, p=0.450). Thus, the abundance of sensitive and tolerant species identified using ISA and the peninsular dataset were selected as metrics.

Percent exotic

Fifty-one exotic species were identified throughout peninsular Florida (Table 3-15). Exotics, which included pasture grasses planted for forage, were significantly more abundant in impaired conditions than in reference conditions (peninsular dataset, Mann-Whitney U-test Z= -5.379, p<0.001). Median values between regions did not differ (Kruskal-Wallis H = 0.740, p=0.691), nor were significant differences identified when the abundance of exotic species was compared between regions for both impaired sites (Kruskal-Wallis H=0.247, p=0.884) and reference sites (Kruskal-Wallis H=0.998, p=0.607). The abundance of exotic species was significantly correlated with the LDI in each region (Spearman's r: South = 0.71, p=<0.001; Central = 0.54, p=0.002; North = 0.74, p<0.001). The Spearman correlation coefficient was also calculated for the abundance of exotic species and the LDI using the peninsular data set (Spearman's r = 0.67, p<0.001, Figure 3-7).

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Table 3-15. Exotic taxa encountered and region(s) of occurrence.

Exotic Species	S	C	N	Exotic Species	S	C	N
Aeschynomene indica		●		*Macroptilium lathyroides*		●	
Alternanthera philoxeroides	●	●	●	*Melaleuca quinquenervia*	●		
Alternanthera sessilis	●	●		*Melochia corchorifolia*	●	●	●
Alternanthera tenella	●			*Mollugo verticillata*			●
Amaranthus blitum		●	●	*Murdannia keisak*			●
Amaranthus spinosus			●	*Murdannia nudiflora*		●	●
Caperonia palustris	●			*Panicum maximum*			●
Chenopodium ambrosioides			●	*Panicum repens*	●	●	●
Cuphea carthagenensis	●	●	●	*Paspalum acuminatum*		●	●
Cynodon dactylon	●	●	●	*Paspalum notatum*	●	●	●
Cyperus lanceolatus		●		*Paspalum urvillei*	●	●	●
Desmodium triflorum	●			*Phyllanthus urinaria*			●
Digitaria bicornis	●			*Polygonum lapathifolium*		●	
Echinochloa colona			●	*Portulaca amilis*			●
Echinochloa crusgalli			●	*Richardia scabra*	●		
Eichhornia crassipes			●	*Sacciolepis indica*	●	●	
Eleusine indica		●	●	*Salvinia minima*	●	●	●
Eragrostis atrovirens	●	●		*Schinus terebinthifolius*	●	●	
Fimbristylis miliacea		●		*Scleria vaginata*		●	
Gomphrena serrata		●		*Senna occidentalis*			●
Hedyotis corymbosa		●		*Solanum viarum*	●		●
Hymenachne amplexicaulis	●	●		*Sporobolus indicus*	●	●	●
Ipomoea quamoclit			●	*Urena lobata*	●	●	
Kyllinga brevifolia	●	●	●	*Verbena bonariensis*			●
Ludwigia peruviana	●	●	●	*Xyris jupicai*	●	●	●
Lygodium microphyllum	●						

The abundance of exotic taxa appeared to not be strongly correlated with increases in LDI value until a threshold of approximately 3.5 was reached. Many sites before LDI values of approximately 3.5 appeared to have "background" values for exotic taxa of less than 10%, suggesting a nearly universal distribution of exotic taxa throughout Florida. Near LDI values of 3.5, however, marked increases in the abundance of exotic taxa suggested a threshold value has been crossed, although substantial variation in the dataset remained.

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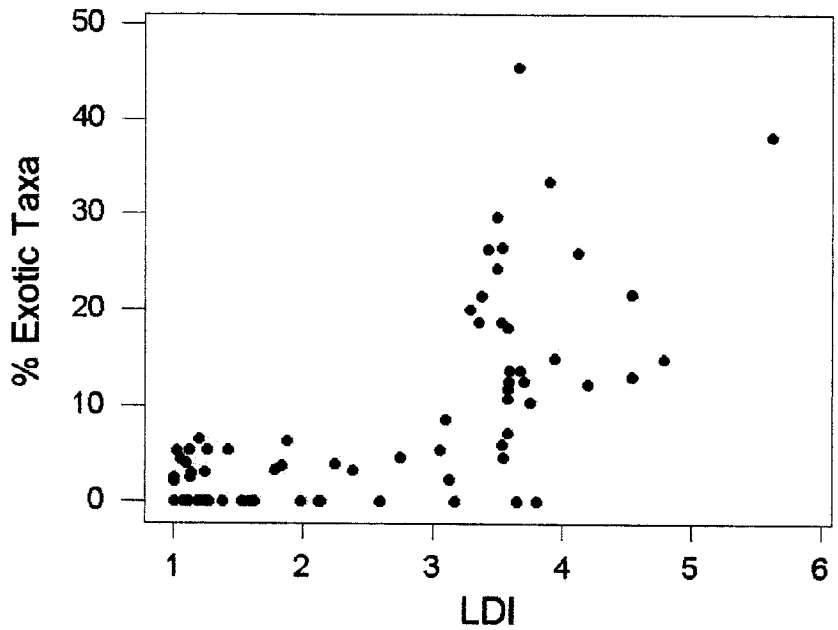

Figure 3-7. Abundance of exotic taxa at each site along the disturbance gradient.

Annual to perennial ratio

The median annual to perennial ratio was significantly higher in impaired conditions versus reference conditions for the peninsular dataset (Mann-Whitney U-test Z = -5.122, $p<0.001$). The correlation between the A:P ratio and the LDI was significant when the peninsular dataset was examined (Spearman's $r = 0.65$, $p< 0.001$, Figure 3-8). Spearman's correlation coefficient between the A:P ratio and the LDI was also significant for each region: South $r = 0.60$, $p=0.002$; Central $r = 0.69$, $p<0.001$; North $r = 0.65$, $p=0.001$. The median value for the A:P ratio did not differ between regions (Kruskal-Wallis H = 0.938, $p=0.626$), nor did median values differ between regions for reference conditions (Kruskal-Wallis H = 2.130, $p=0.344$) or impaired conditions (Kruskal-Wallis H = 0.114, $p=0.945$).

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Variance in the A:P ratio dataset, as well as sensitivity and accuracy appeared to be minimal until a threshold value of approximately 3.2 was passed. At that point, the ratio increased, as did the variance in the data.

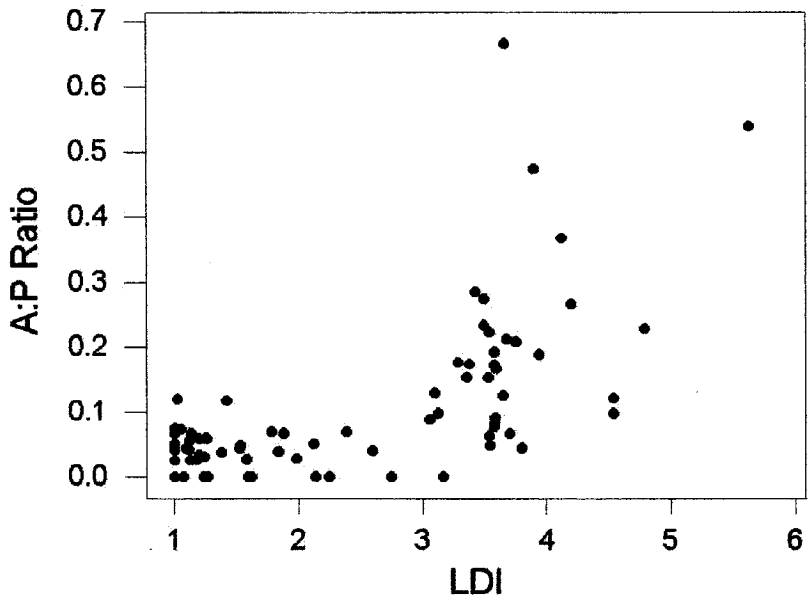

Figure 3-8. Annual to perennial ratio at each site along the disturbance gradient.

Average Coefficient of Conservatism

Significant differences in the median CC value between reference and impaired conditions using the peninsular dataset were identified (Mann-Whitney U-test Z = 6.510, $p < 0.001$). A significant correlation was calculated between the peninsular dataset average CC and the LDI score (Spearman's $r = -0.78$, $p < 0.001$, Figure 3-9). Median values between regions were not significantly different (Kruskal-Wallis H = 0.501, $p = 0.778$). Kruskal-Wallis tests also indicated that the median average CC value did not differ between regions for reference sites (H = 0.501, $p = 0.778$) or impaired sites (H = 0.140, $p = 0.932$). Significant (Spearman's r) correlations between the LDI and the

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average CC were identified for each region (South r = -0.74, p< 0.001; Central r = -0.75, p< 0. 001, North r = -0.76, p< 0.001). The average CC appeared to be sensitive to increases in LDI value once a threshold of approximately 3.0 was passed. Marked increases in variance within the dataset followed, although in general a trend of decreasing average CC values continued.

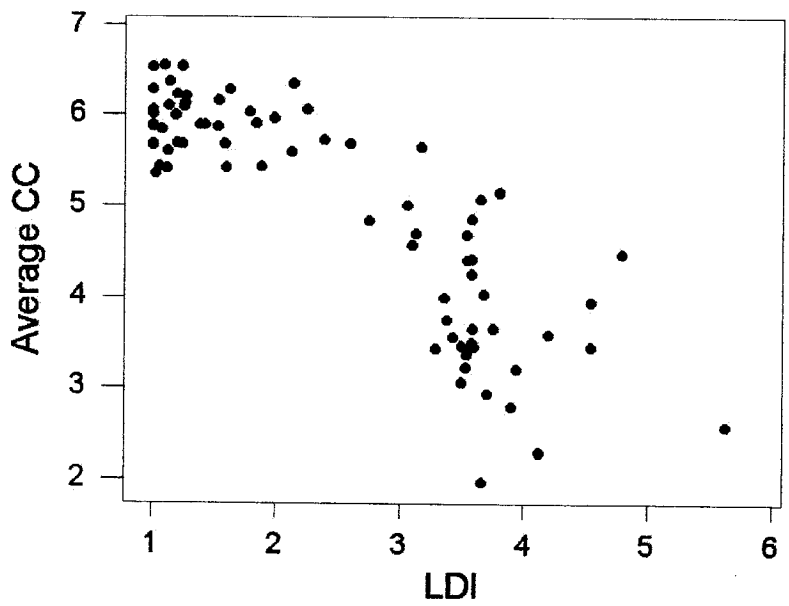

Figure 3-9. Average Coefficient of Conservatism value for each site along the disturbance gradient.

Vegetative Index of Wetland Condition

The Vegetative Index of Wetland Condition (VIWC) was calculated for each site by scoring the 95th percentile of each metric based on the peninsula dataset (n=75). The peninsular dataset was utilized as no significant regional effects were found in the distribution of the five metrics between reference and impaired conditions, as previously described. The 95th percentile and the scoring breaks for each metric are given in Table

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3-16, and the scores for each site and metric are given in Table 3-17. The scored metrics were summed to calculate the VIWC. The relationship between the LDI and the VIWC for each site is presented in Figure 3-10. The VIWC ranged from 0 (the lowest score

Table 3-16. Quadrisect values and 95th percentile for metric scoring.

Metric	95th Percentile	0 Scores	3 Scores	7 Scores	10 Scores
% Sensitive Spp.	0.5834	< 0.0820	0.0820 – 0.2308	> 0.2308 – 0.4474	> 0.4474
% Tolerant Spp.	0.6321	> 0.3723	> 0.1081 – 0.3723	0.0597 - 0.1081	< 0.0597
% Exotic Taxa	0.2742	> 0.1198	> 0.0385 – 0.1198	0.0001 – 0.0385	0
A:P Ratio	0.3105	> 0.1231	> 0.0625 – 0.1231	0.0352 – 0.0625	< 0.0352
Average CC	6.352	< 3.7015	3.7015 – 5.4085	> 5.4085 – 5.8874	> 5.8874

possible) to 50 (the highest possible score), with 9 sites scoring 0 and 3 scoring 50. The nine low-scoring sites were all located in cattle pastures with high densities (C. Lane, *personal observation*) in the South (3 sites), Central (3 sites), and North (3 sites) regions. The three high-scoring sites were also evenly distributed with one in each region: South (on conservation lands owned by the South Florida Water Management District), Central (in a state park), and North (in a national forest). The VIWC was significantly correlated to the LDI score (Spearman's $r = -0.75$, $p < 0.001$) and was significantly different between reference and impaired classes (Mann-Whitney U-test $Z = 6.496$, $p < 0.001$). No difference between regions was found for the summed VIWC (Kruskal-Wallis $H = 0.865$, $p = 0.649$), nor between regions and impaired sites (Kruskal-Wallis $H = 0.438$, $p = 0.804$) or between regions and reference sites (Kruskal-Wallis $H = 4.534$, $p = 0.104$).

As in the metrics that comprised the VIWC, variance within the dataset appeared to be manifested most strongly at approximate LDI values of 3.5. In addition, higher

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Table 3-17. Site scores for each metric and the summed VIWC.

Site	% Sensitive	% Tolerant	% Exotic Spp.	Average CC	A:P Ratio	VIWC
ALPaynes	7	7	10	10	7	41
Audobon	3	0	0	0	0	3
Bear Scat	3	7	10	10	10	40
Big Cow	0	3	3	3	0	9
BRSebastian	10	10	3	10	7	40
CabPatch	3	3	3	3	7	19
Caravelle	3	3	3	3	7	19
Chuluota	7	10	7	10	7	41
CLBayard	7	10	10	7	10	44
CLCove	0	0	0	0	0	0
CMMPas	7	7	10	10	10	44
CMWRef	7	3	10	10	10	40
COBurgle	0	0	0	3	0	3
COHole	3	7	10	3	7	30
Crew	10	7	7	10	3	37
DEMelon	3	0	0	0	0	3
Deerfly	7	10	7	7	3	34
Garber	3	0	3	3	10	19
GbarE	0	0	0	3	0	3
GLDonut	0	3	0	0	3	6
GLPont	0	0	0	0	0	0
Goethe	10	7	10	7	7	41
GreenSwamp	10	10	10	7	10	47
HaRare	0	0	0	0	0	0
HagueI	0	3	0	0	0	3
HagueII	0	0	0	0	0	0
HalfMoon	7	7	10	10	10	44
HEBad	0	0	0	0	0	0
HEL2	0	0	0	0	0	0
HEOKAY	7	3	3	7	3	23
HighRef	7	7	10	7	7	38
HighPast	0	0	0	0	3	3
HillsRef	10	7	10	7	10	44
Hunt Camp	10	10	10	7	7	44
IFAS I	3	3	7	10	10	33
IFAS II	7	10	7	10	7	41
Immokalee	10	7	10	7	7	41
IRBlueCypress	3	0	3	3	3	12
IRCanal	0	0	0	0	0	0
IROJ	0	0	0	3	0	3
JD6	10	10	7	10	10	47

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Table 3-17. Continued.

Site	% Sensitive	% Tolerant	% Exotic Spp.	Average CC	A:P Ratio	VIWC
Kelly Park	3	3	10	3	0	19
Lcork	7	7	7	7	3	31
LLeeCounty	7	7	3	3	3	23
LEGo	7	3	10	7	10	37
LESuwan	3	3	10	7	7	30
MALudy	3	0	0	0	0	3
MASpray	7	3	10	3	7	30
McArthur	3	0	3	0	0	6
MNElmer	3	3	7	3	3	19
MNErik	7	7	3	3	3	23
MNOcala	7	7	10	7	10	41
MRPepr	3	3	0	3	3	12
Myakka	10	3	10	10	7	40
OKCara	3	0	0	3	0	6
OKKiss	10	7	10	10	7	44
OKPast	3	0	3	3	3	12
PacTom	0	0	0	0	3	3
Pall-Mar	10	10	10	10	10	50
PBCorbett	10	7	7	10	3	37
PBEnjay	10	10	7	10	3	40
Penner	10	10	10	10	10	50
POWales	10	10	3	10	7	40
POWeowak	10	10	10	10	7	47
PUPond	10	10	3	10	3	36
RiceCreek	10	7	3	7	3	30
SANorthMya	10	10	3	7	10	40
SAOscer	7	7	3	7	7	31
SandhillCrane	3	3	3	3	0	12
Savannas	10	10	7	10	10	47
STCow	0	0	0	0	0	0
SUVaca	7	10	10	7	10	44
SUWarhol	0	3	0	0	0	3
UNHealthy	0	0	0	0	0	0
Wekiva	10	10	10	10	10	50

variance than was expected was also evident at LDI values of approximately 1.0, based on the distribution of the metric values that comprised the VIWC.

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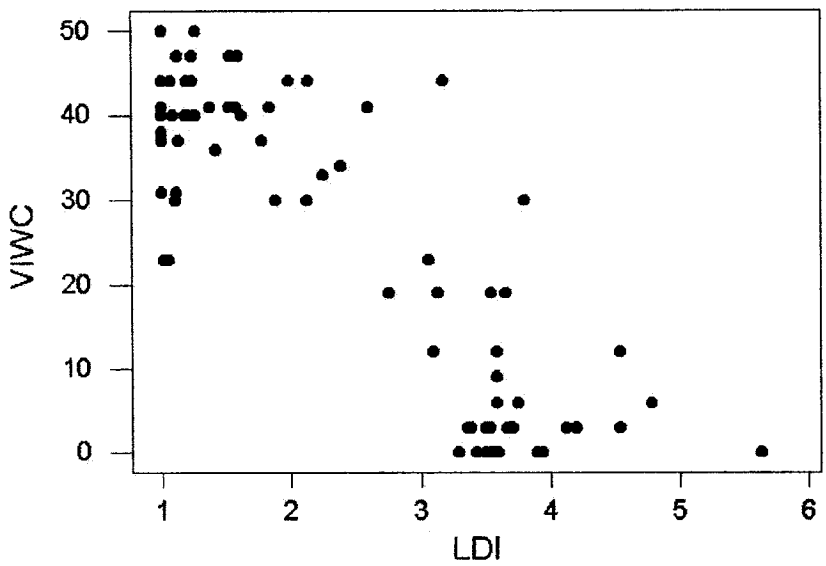

Figure 3-10. The relationship between the VIWC and the LDI.

Discussion

Correlations between metrics and LDI scores suggested landscape modification affected that the composition and structure of the wetland flora, although the variance in the dataset was substantial, especially for LDI values near 3.5. Five metrics were correlated >|0.50| with the LDI: abundance of sensitive and tolerant taxa, abundance of exotic taxa, annual to perennial ratio, and average coefficient of conservatism score. The VIWC, which combines the macrophyte metrics into a single value, defined the condition of each wetland such that the lowest scoring wetlands tended to have the highest landscape development intensity scores, while the wetlands with the highest VIWC scores were in areas with lower LDI values.

Environmental Gradients and Species Distributions

Macrophytes respond strongly to anthropogenic modifications of wetland ecosystems and surrounding landscapes (Doherty et al. 2000, Adamus et al. 2001), including alterations in community composition due to hydrology (van der Valk 1981,

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David 1996), nutrient loading (Koch and Reddy 1992, Doren et al. 1997, Bedford et al. 1999), physical/chemical alteration (Latham et al. 1994, Adamus et al. 2001), sedimentation (Waldrop and Brooks 1998), and other factors such as selective herbivory (Reader and Craft 1999), trampling (Blanch and Brock 1994), or modification of fire regime (Bayley and Odum 1976, Winchester et al. 1985, Newman et al. 1998). The wetlands sampled in this study were subjected to a myriad of anthropogenic modifications, with the strongest gradient being of increased agricultural land use intensity and associated nutrient loading and chemical modification, along with hydrologic alterations and herbivory/trampling. These vectors of disturbance were manifested by significant differences between the constituents measured (Table 3-8). Five measured constituents or calculated values were of particular importance in driving community structure based on the relationship (Pearson's $r^2 > 0.30$) between the NMDS ordination scores and the LDI, site soil pH, water TP, specific conductivity, and latitude (Table 3-9).

Macrophyte composition was most strongly correlated with the LDI score, which is an agglomerative disturbance index capturing landscape modification due to altered hydrology, nutrient loading, and predicted agrochemical application and other disturbances associated with various land uses (Brown and Vivas *submitted*). As the LDI captures many disturbance vectors, the strength of the relationship between macrophyte distribution and LDI score was not surprising. The ordination results buttress the use of GIS-based independent measures of general impairment, such as the LDI, in identifying modifications to landscapes and predicting wetland community condition (Johnson 1994, Adamus and Bergman 1995, Roth et al. 1996, Miller et al. 1997, Cuffney et al. 2000,

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Carpenter and Waite 2000, O'Conner et al. 2000, Munn et al. 2002, Fore and Grafe 2002, Fore 2003). The strength of the relationship between site ordination structure and the LDI also supported the metrics developed with the LDI as the abscissa, or measured disturbance gradient.

The ordination scores were also correlated with soil pH, specific conductivity, and water TP. Wetlands are generally low-lying landscape features, which predisposes these ecosystems to down-slope accumulations of nutrients from cattle grazing (Reddy et al. 1998) and crop and citrus agrochemical application and drift (Euliss and Mushet 1999, Anderson and Vondracek 1999). In a south-central Florida study by Graetz and Nair (1995), nutrient augmentation through fertilizers and cattle wastes in pastureland soils augmented background levels, such that approximately 40% of the phosphorous became bioavailable (labile), whereas only 2% was bioavailable in native areas. These massive loading rates may allow nutrients to be moved directly or indirectly into wetland systems from overland or interstitial flow, and can be manifested by drastic alterations in wetland community composition (Craft et al. 1995, Newman et al. 1998, Mitsch and Gosselink 2000). Beyond altering species composition in wetland systems directly down-slope of deleterious anthropogenic land uses, sufficient nutrient loading of wetland systems can occur such that wetlands, rather than being nutrient sinks (Flaig and Reddy 1995), may end up as nutrient sources for water bodies farther down-slope including wetlands, streams, and lakes (Agami et al. 1990). Alterations in soil pH, specific conductivity, and available nutrients, among other changes, may be indicative of wetland loading from agrochemical fertilizers and cattle wastes (Fore and Grafe 2002, U.S. EPA 2002e).

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In addition to the LDI and physical/chemical determinants of community composition from the NMDS ordination, a latitudinal gradient affecting species composition was also correlated with ordination scores. The Florida peninsula, which juts into the Gulf of Mexico and Atlantic Ocean, is tempered from climatic extremes by the heat absorbing/desorbing nature of water. This protects many species that have evolved in south Florida's sub-tropical environment. Northern Florida, however, is somewhat exposed to the temperate climate of mainland North America, with many freezing days and different precipitation patterns than southern Florida (Fernald and Purdum 1998). As suggested by significant compositional differences (Table 3-7) between wetland macrophytes of northern, central, and southern Florida, different species have autecological requirements associated with latitudinal changes.

Metric Development

The results suggested that while macrophytes responded to increased development intensity, as measured by increased LDI values, substantial variance was evidenced by the five metrics that comprised the VIWC. In addition, the metrics appeared to be relatively insensitive to changes in LDI values until an LDI score of approximately 3.5 was reached. Throughout peninsular Florida, the metrics appeared able to approximate the condition of the sampled wetlands along increases in landscape modification, however, the substantial variance within each of the metric values, especially after LDI values of approximately 3.5, indicated that additional information relating the plant metrics to the landscape metrics was required to more fully understand the relationship between plant metrics and landscape development. The results of the analysis for each of the final metrics are described below.

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Sensitive and tolerant species

It is generally accepted that most organisms have autecological optima, or environmental conditions most suited for the highest possible productivity and/or fitness (Ricklefs 1990, Gaiser et al.1998, Stevenson et al. 1999, U.S. EPA 2002e). These conditions can include adequate water, nutrients, photoperiod, and temperature. A hypothetical gradient (Figure 3-11) is thus likely to exist for each species. As optima exist, there are likewise extremes where conditions are unfavorable for growth, let alone

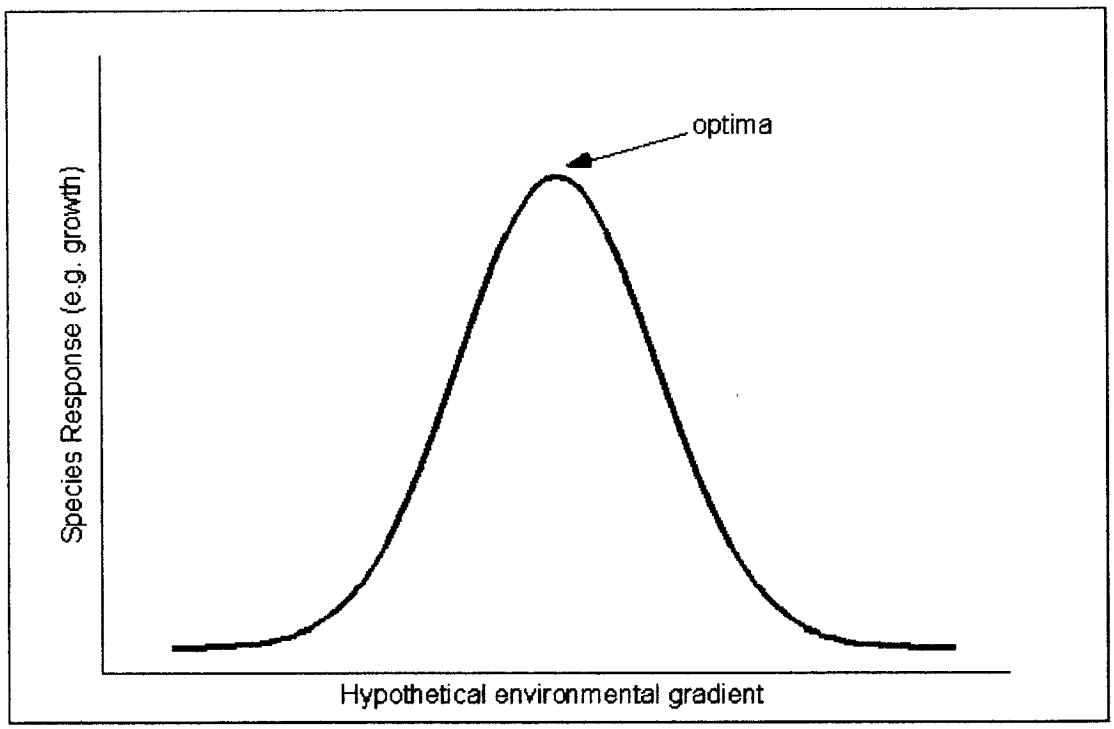

Figure 3-11. Environmental optima along a hypothetical environmental gradient.

survival, for species not adapted to the environment. Environmental extremes can include altered salinity, pH, hydropatterns, nutrients, physical disturbances, chemical disturbances, anoxia, or photoperiod. In this study, the environmental conditions generally found at reference sites were significantly different in many physical/chemical constituents than those found in impaired sites (Table 3-8). A review of the literature

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suggests that other constituents of the soil and water, such as metals or pesticides/herbicides, likely differ between reference and impaired conditions (Purvis et al. 1991, Euliss and Mushet 1999, Mitsch and Gosselink 2000). Likewise, though not quantified, pressures associated with browsing and selective grazing were likely significantly different in reference than in impaired conditions. The abiotic and biotic factors listed above could be considered to define the range of environmental conditions acceptable for the plants identified in this study. The LDI was utilized in Indicator Species Analysis as an analog for the acceptable environmental conditions of various species. Species with significant fidelity and specificity to low LDI scores were thus considered to have optima within the range of environmental conditions often found in reference sites. Those species indicative of higher LDI values were conversely considered to have optima within the range of conditions expressed by impaired sites. It is beyond the scope of this research to explore the autecologies of the sensitive and tolerant plants listed in Tables 3-13 and 3-14 further, but the significance of the relationship suggests proclivities towards particular environmental conditions for those indicator macrophytes.

In this study, Indicator Species Analysis was able to discern 82 plants with fidelity and specificity to a particular landscape development condition throughout peninsular Florida. The abundance of these sensitive and tolerant plants was correlated with the LDI (Spearman's $r > |0.70|$), and not significantly affected by regional vagaries in species composition. However, the highly variable relationship between the abundance of sensitive and tolerant plants and the LDI indicated limitations to conclusions drawn. The abundance of sensitive plants demonstrated high variance throughout the plot along the

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LDI, although lower values appeared near LDI scores of approximately 3.5. Tolerant plants, which expressed a minimal relationship with the LDI until a threshold of approximately 3.5 was reach, were also highly variable, although not as sensitive to changes in LDI values for the low scores.

Percent exotic

Increased development has the potential of promoting the spread of exotic species into wetlands where they can alter community composition, patterns, processes, and even functioning (Leibowitz and Brown 1990, Hobbs and Huenneke 1992, Cronk and Fennessy 2001). Disturbance within the wetland itself from activities such as cattle grazing provides a pathway for exotic propagules through cattle feces or by seeds adhering to the coats of domestic herbivores. The heavy machinery used in typical cropping or citrus operations can also provide a pathway for propagules. The trampling associated with cattle grazing can provide patches of bare ground for colonization of exotic species. Drift of herbicides into wetlands from agricultural practices can alter the composition or fitness of wetland flora and create patches of bare ground, which can subsequently be colonized by exotic species. Drainage of landscapes for development can provide direct conduits to wetlands for exotic species, if those drainages directly link to the wetland (Hobbs and Huenneke 1992, Galatowitsch et al. 1999b). Altering the natural sheet flow pattern through landscape level drainages can also affect the species composition of wetlands by providing drier conditions conducive to colonization by exotic annual species (David 1999). Increased development that affects the landscape surrounding the wetland may also cause a potential increase in exotic propagules entering the wetland system through the increase in the proportion of the perimeter of the wetland that is subjected to seed sources of exotic species.

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The results, however, suggested that the abundance of exotic taxa was not sensitive to increases in land development intensity until a threshold had been crossed, approximately 3.2. Once that threshold had been passed, substantial variance was manifested in the dataset, which suggested additional processes not captured by the LDI were affecting the distribution of exotic taxa throughout peninsular Florida. These processes may be further understood through the collection of additional data (i.e., requesting planting history from land owners) or through additional observations (i.e., the distance from the nearest road to the wetland, or the existence of perches within the site; both would potentially increase the abundance of propagules).

Several species found in the wetlands sampled are on the Florida Exotic Pest Plant Council's list of "Category I" exotics (*Eichornia crassipes, Hymenachne amplexicaulis, Lygodium microphyllum, Melaleuca quinquenervia, Panicum repens, Schinus terebinthifolius*, and *Solanum viarum*). Category I exotic species are those considered to alter native plant communities by directly displacing native species, changing the structures or ecological functions within a community, or hybridizing with native species (FLEPPC 2003). Two plants identified were FLEPPC Category II exotic species (*Alternanthera philoxeroides* and *Urena lobata*). These plants are increasing in abundance throughout Florida but have not yet affected the natural areas, as have Category I exotics (FLEPPC 2003). It is interesting that not all of the exotic species found appear to possess such damning traits. Richard's yellow-eyed grass, *Xyris jupicai,* native to French Guiana (Wunderlin and Hansen 2002), is one such species. This species was found frequently enough in reference conditions to be considered a sensitive indicator species.

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Annual to perennial ratio

As hydrologic conditions in wetlands are dynamic, many wetland plants have evolved life-history traits to maintain themselves during periods of endogenous environmental perturbations. In an adaptation that enables wetland plants to combine reproductive structures with storage devices, most wetland plants are vegetatively reproducing perennials (Galatowitsch et al. 1999b, Cronk and Fennessy 2001). Perennial plants, by dint of their storage/reproductive structures such as rhizomes, tubers, and corms, are thus better able to pass through the Gleasonian sieve that regulates the presence and abundance of species within the confines of the wetland system (van der Valk 1981). However, exogenous perturbations that disrupt natural processes, such as hydroperiod alterations, nutrient loading, and trampling, may provide opportunities for the generally faster-growing annual species to out-compete perennial species for nutrients, water, and light. Annual species (often considered "weedy" species) increase in wetlands undergoing anthropogenic perturbations, while perennial species decline (Hobbs and Huenneke 1992, Galatowitsch et al. 1999b). The results of the annual to perennial ratio metric generally support the conclusions from the literature, as the ratio of annuals to perennials increases with increasing development.

However, at low levels of measured disturbance (LDI scores), the results suggested that the A:P ratio was not sensitive to change. Only once an LDI threshold of approximately 3.2 was passed were changes evident in A:P ratio. This suggests that wetland processes maintain A:P ratios until development intensity increases sufficiently to alter those processes, for instance through decreased hydroperiod as a result of ditching and draining associated with higher LDI values.

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Average Coefficient of Conservatism

Since its inception by Wilhelm and Ladd (1988) as a method to measure the "quality" of the vegetation in the Chicago area, the Floristic Quality Assessment Index (FQAI, originally called the Natural Area Index), and the associated Coefficient of Conservatism have found widespread use in a variety of different ecosystems (Southern Ontario Woodlands: Francis et al. 2000; Illinois uplands, flatwoods, and prairies: Taft et al. 1997; North Dakota wetlands: Mushet et al. 2002). Many authors have utilized the FQAI, calculated as the average CC value for the site multiplied by the square root of the total number of species at a site. This inherently rewards systems richer in species and assumes that such a system is more ecologically desirable (Fennessy et al. 1998, Cohen et al. *submitted*). Like Francis et al. (2000), in this study the average CC score was utilized as a metric. This decreased the influence of species richness on the site score and more accurately reflected the ecological data collected as the average species richness for sites with an LDI score of less than 2.0 (31.70 +/- 10.80) was not significantly different (Mann-Whitney U-test Z = -0.005, p= 0.990) than those sites scoring greater than 2.0 (31.68 +/- 9.51). The lack of richer systems in reference wetlands may be a function of the strong hydrological driving forces maintained on the minimally developed landscape. Whereas developed landscapes often drain the wetland area, which can allow species into the wetland proper that would otherwise be excluded due to anoxic soil conditions, the wetlands located in reference landscapes generally have zones where species tolerant of different hydroperiods and anoxia can thrive. Thus the richness found in developed sites may be an artifact of the drainage associated with development.

Landscape drainage may have also affected the highly variable distribution of CC values associated with LDI scores of approximately 3.5. As noted, drainage increases the

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area available for colonization by upland and facultative plants due to decreases in soil anoxia. However, the slope and additional site-specific factors (e.g., the presence of perches, as discussed in the % Exotic metric) may have decreased the opportunity for plants with low CC scores to colonize wetland zones.

Vegetative Index of Wetland Condition

The VIWC, developed for isolated depressional herbaceous wetlands in peninsular Florida, combined five biological measurements into a single score to ascertain the relative condition of a sampled wetland vis-à-vis a suite of reference wetlands located in low development landscapes (Karr and Chu 1999). While significantly correlated with the disturbance gradient, the VIWC appeared to be highly variable for any given LDI score, especially scores located near LDI values of 1.0 and 3.5. The high variance in each of the metrics, as well as in the final correlation between the LDI and VIWC may have been in locations with a time delay between impairment activity and the manifestation of that impairment in the wetland macrophyte community (i.e., a field, long held fallow, that is now in crop rotation). Conversely, LDI scores, based on landscape values, may not have accurately quantified the condition of a site that was in rotation or grazed that was sampled months or years after such deleterious activities had ceased. While ground-verification occurred, in some cases accurate land use maps or site histories could not be obtained, and best scientific judgment was employed in spatial off-site GIS analysis. While a few outliers appeared to exist in the relationship between the LDI and VIWC, the focus of this analysis was on the utility of macrophytes as indicators of wetland condition. An assumption inherent in this study, as in most analyses utilizing bioindicators, was that should environmental conditions be such that the macrophytic community was characteristic of sites in reference conditions, then the integrity (c.f. PL

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92-500, Clean Water Act, §101(a)) of the site was not suspect. On the other hand, sites with vegetation characteristic of impaired conditions were assumed to be under various degrees of human modification of deterministic, driving variables. In this manner, the VIWC, which directly compares wetland vegetation, may be best employed as an accurate assessment tool for isolated depressional herbaceous wetlands in Florida.

Field Data Collection

Florida was in the midst of a significant drought during the study period (Lawrimore et al. 2001), which was manifested by decreased hydroperiod, extent, and depth of inundation in many of the sampled wetlands. Many wetlands identified as potential sites were not sampled during the study period due to the lack of standing water – originally a prerequisite for inclusion that was slackened as the sampling season waned in 2000. Five of the ephemeral wetlands sampled in this study (all in the North region) were not hydrated during the sampling window, and may not have been hydrated to the point of standing water at any point during the study period. In addition, the hydroperiod may have been significantly decreased in many, if not all, of the wetlands sampled. This may have affected the species composition in both reference and impaired wetlands – for instance through the increased abundance of weedy annuals such as *Eupatorium capillifolium*. The effect of the drought on the macrophytes identified would likely have been most pronounced on the wetland fringe, as macrophytes typically found in more upland conditions may have colonized the non-hydrated wetland fringe and out-competed typical (i.e., obligate or facultative) wetland plants. As the drought was endogenous in nature and pervasive throughout Florida, the effects were likely evenly distributed in both impaired and reference sites. It was noted, however, that sites in impaired landscapes often expressed modified hydrological functioning due to drainage ditches (C. Lane,

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personal observation) that may have caused a greater response to the effects of the drought.

As mentioned, the sampling protocol was modified between sampling years. The slight changes in the sampling protocols between 1999 and 2000 were not expected to have had a noticeable effect on the accurate characterization of the 2% of sampled wetlands without 4 full transects from the wetland edge to the center. No wetland had all 4 transects halted before the center, and the additional information possibly gathered from the extended transects after $15m^2$ had been sampled without a change in species composition would likely be minimal.

Conclusions and Recommendations

This chapter of the study quantified the condition of sampled wetlands through an assessment of the structure and composition of the wetland macrophyte community. However, the VIWC, should be seen as a model that must be verified and calibrated to decrease the variability between metric response and the LDI. A recommended calibration for the proposed metrics and the VIWC that is currently underway (C. Lane, *unpublished results*) includes re-sampling a number of study wetlands to ascertain yearly fluctuations in species assemblages and the subsequent effect on the VIWC. To sample study wetlands twice within a given year but during different seasons would provide the opportunity to examine seasonal effects on the composition and structure of wetlands and the corresponding alterations to the individual metrics and VIWC. Model verification would also increase the value of the proposed VIWC. Florida, by nature of rainfall and topography, is a state rich in wetland resources. Increasing the number of sampled wetlands across all landscape types would increase the power, precision, and accuracy of the individual metrics as well as the multi-metric VIWC. The addition of larger isolated

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herbaceous wetland systems into the calibration and verification of the VIWC would also permit the expression of the VIWC across wetlands of different scales.

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CHAPTER 4
MACROINVERTEBRATES AS BIOINDICATORS

Introduction

Shallow, isolated depressional wetlands typically support a variety of aquatic, semi-aquatic, and terrestrial invertebrates that are important components of wetland and local terrestrial food webs (Kushlan 1990, Evans 1996, Sharitz and Batzer 1999, Evans et al. 1999, Leslie et al. 1999). Because of the nature of depressional wetlands, invertebrates associated with these ecosystems are regularly subjected to endogenous environmental stresses such as anoxia, wide salinity fluxes, and hydroperiod fluctuations. Invertebrates in depressional wetlands may be further stressed by exogenous (human-induced) factors such as nutrient and organic loading, contaminant toxicity, acidification, salinization, sedimentation, changes in vegetation cover, thermal alteration, dehydration, hydrologic alterations, and habitat fragmentation (Adamus and Brandt 1990, Adamus 1996, Danielson 1998, Doherty et al. 2000). Measured changes in invertebrate community structure and function relative to these exogenic perturbations may be used to assess the ecological condition of the depressional wetland ecosystem through development of a multimetric index of biologic integrity (Adamus 1996, Danielson 1998, Galatowitsch et al. 1999a, Karr and Chu 1999, Kashian and Burton 2000).

Multimetric indices utilizing macroinvertebrates have been used in biological monitoring to determine the relative quality of lentic and lotic aquatic ecosystems throughout the country (Hellawell 1986, Plafkin et al. 1989, Cairns and Pratt 1993, Kerans and Karr 1994, Rosenberg and Resh 1996, Karr and Chu 1999). As of 1997,

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forty-eight states, including Florida, have implemented or have begun developing macroinvertebrate assessment methods for lakes or streams (Karr and Chu 1999). In Florida, the Florida Department of Environmental Protection (FDEP) combined metrics that measure diversity, composition, and functional feeding groups in the development of the Stream Condition Index (SCI) for the evaluation of lotic systems (Barbour et al. 1996). Metrics were likewise identified by the FDEP to assess the biological integrity of lakes in the state (Gerritsen and White 1997, Gerritsen et al. 2000). Macroinvertebrate assemblages in Florida have also been utilized to develop a bio-monitoring program in southern Florida canal systems (Snyder et al. 1998, in Doherty et al. 2000), for evaluating the success of restoration projects (e.g., Merritt et al. 1999) constructed wetlands (e.g., Erwin et al. 1997, Crisman et al. 1997), and for assessing the impact of human-induced disturbances on cypress domes and the Everglades (i.e., Brightman 1984, Leslie et al. 1999, Rader 1999).

Multimetric assessments of wetland condition using macroinvertebrates as indicator organisms have been conducted in a growing number of states and agencies (e.g., Yoder and Rankin 1995, Danielson 1998, Burton et al. 1999, Gernes and Helgen 1999, Ludden and Hauer 2000). Macroinvertebrates are well suited for the effective measurement of the spatial and temporal impacts of human-induced disturbances (Table 4-1). Macroinvertebrates are typically abundant in wetlands, easily sampled, and relatively easily identified (Rosenberg and Resh 1993). Additionally, there is ample literature on various sampling methods (Cheal et al. 1993, Adamus 1996), taxonomic keys (i.e., Merrit and Cummins 1996), and macroinvertebrate tolerance values for pollutants in lotic environments (Hilsenhoff 1987, Lenat 1993, Barbour et al. 1996), as

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Table 4-1. Advantages and disadvantages of using macroinvertebrates in wetland bioassessment.

Advantages	Disadvantages
• Widely distributed in wetlands • Easily sampled and generally abundant • Some organisms have known response to perturbations • Many are sedentary within a single wetland • Important components of trophic webs • Respond quickly to perturbations • Some are long-lived	• Some are extremely difficult to identify • Many short-lived • Identification is labor intensive • May be sparse in some wetlands or seasonally abundant • Hydrated wetlands generally required • Poor taxonomic knowledge of some groups • Some taxa migrate from other water bodies

Sources: Danielson (1998), Doherty et al. (2000), U.S. EPA (2002c)

well as a growing array of metrics that have responded to the effects of episodic or constant human disturbances on aquatic systems (see Yoder and Rankin 1995, Barbour et al. 1996, Gerritsen and White 1997, Galatowitsch et al. 1999a, Karr and Chu 1999, Gernes and Helgen 1999, Kashian and Burton 2000, Ludden and Hauer 2000, Doherty et al. 2000, U.S. EPA 2002c).

While the advantages of macroinvertebrates have dictated their use in biological assessments throughout the country, disadvantages to using them in biological assessments exist (see Table 4-1). Detailed taxonomic keys are necessary to identify most organisms to genus or species levels, and often the keys do not address the diagnostic features of larvae or instars. Sample processing time is large with macroinvertebrates (e.g., sorting, subsampling, enumeration and identification) and some families (e.g., Chironomidae) require mounting specimens on slides for identification. Most wetland macroinvertebrates emerge after the wetland has hydrated, thus requiring adequate rainfall and/or standing water, which typically is seasonal in Florida. The time

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since the wetland was hydrated will also affect the sampled community composition (e.g., predators typically emerge later than their prey). Additionally, many macroinvertebrate responses to perturbations in the literature are from lotic or lentic systems as data on macroinvertebrate responses to perturbations in wetland systems are sparse, but growing (Danielson 1998, Adamus et al. 2001, U.S. EPA 2002c).

In this chapter, results of testing and evaluating community structural and functional measurements for macroinvertebrates (insect and non–insect taxa) of isolated herbaceous depressional wetlands in peninsular Florida are given. The goal was to identify metrics, or measurable components of the wetland macroinvertebrate community, that showed an empirical and predictable change in value along increases in a landscape-level measure of human disturbance, the Landscape Development Intensity index (Brown and Vivas *submitted*). Those metrics were selected for use in the creation of the multimetric Macroinvertebrate Index of Wetland Condition, or MIWC. The variation between LDI values and developed metrics was examined to address the sensitivity and precision of macroinvertebrate metrics and the relationship of the metrics to the LDI. Additionally, analyses of environmental parameters measured (both abiotic and biotic) were conducted to assess the environmental variables that affect the distribution of macroinvertebrate taxa within wetlands.

Methods

Site Selection and Agricultural Development Gradient

Seventy isolated depressional marshes were sampled for macroinvertebrate community composition throughout peninsular Florida in the summers of 1999 and 2000. Site condition was independently assessed using geographic information systems (GIS) and the Landscape Development Intensity index (LDI, Brown and Vivas *submitted*).

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Additional information on the sites sampled and the LDI may be found in Chapter 2 (Methods: Site Selection and Agricultural Development Gradient).

Macroinvertebrate Collection and Identification

Macroinvertebrates were collected using standard U.S. 30-mesh D-frame dip-nets. Twenty sweeps were collected at each site, proportionally divided according to the different vegetation/habitat zones present. Vegetation zones (such as a fringing *Panicum* spp. zone or a deep water *Pontederia* sp. zone) were determined from an on-site assessment of dominant wetland plants. A typical marsh had between one and four primary vegetation zones. A single dip-net sweep was one net width and two net lengths covering an area of $0.5m^2$. The contents of each sweep were deposited into a labeled 3.8L plastic composite jar. When all 20 sweeps were completed, the sample was preserved with buffered formalin at a rate of approximately 10% of the sample volume. The preserved macroinvertebrate samples were stored at the Howard T. Odum Center for Wetlands and delivered to the FDEP for enumeration and identification by FDEP personnel. At FDEP Central Laboratories, the material was sieved and washed following DEP Standard Operating Protocols and placed on a pan with twenty-four numbered cells. Eight random cells were selected (a third of the sample) and placed in another numbered tray. From the second numbered tray a single sub-sample was taken and all organisms enumerated and identified. If fewer than 100 organisms were encountered, a second randomly chosen cell from the second numbered tray was selected and all organisms within that cell enumerated and identified and added to the total of the first count. Identifications by FDEP were made to the lowest taxonomic level possible following FDEP Standard Operating Procedure #IZ-06. Data were error-checked following FDEP protocols and entered into the FDEP database for analysis.

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Water and Soil Physical-Chemical Sampling and Analysis

To relate the distribution of wetland diatoms to environmental parameters, soil and water physical and chemical parameters were measured at each site as described in Chapter 2 (Methods: Field Data Collection, Sample Preparation and Laboratory Procedures) and Appendix B.

Soil variables were transformed to decrease measured skewness and kurtosis in the dataset as described in Chapter 2 (Methods: Data Analyses). Multivariate colinearity among the environmental variables was also examined as described in Chapter 2 (Methods: Data Analyses). In addition, the non-parametric Mann-Whitney U-test was used to test the null hypothesis of equal medians between reference and impaired classes for each environmental variable (Zar 1999).

Macroinvertebrate Composition and Environmental Correlates

The relationship between measured environmental values (including LDI score) and wetland macroinvertebrate community composition was examined with the Mantel test (Mantel 1967) and with non-metric multidimensional scaling (NMDS, Kruskal 1974, Mather 1976) as described in Chapter 2 (Methods: Data Analyses). A three-dimension NMDS solution was selected for macroinvertebrate taxa as increased dimensionality only marginally improved the fit. Correlations of site scores with environmental parameters, including LDI score and latitude and longitude (decimal degrees), were calculated and a bi-plot of parameters with Pearson's correlations ($r^2 \geq 0.30$) to site scores was constructed.

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Macroinvertebrate Compositional Analyses

Summary statistics

Summary measures of sample richness, Shannon diversity, Simpson diversity, beta, and gamma diversity were calculated for each site and provided information on species distributions vis-à-vis land use as described in Chapter 2: Data Analyses.

Regional composition of macroinvertebrates

As isolated depressional marshes are not uniformly distributed throughout Florida, the sites were likewise generally located in counties with the necessary hydrology and geomorphic setting (Lane 2000, see Figure 2-1). The sites were initially stratified into three peninsular wetland regions, or areas of similar climatic and physiographic conditions after Lane (2000). Two compositional tests, the multiple response permutation procedures (MRPP) available in PCOrd and the Percent Similarity Index (PSI, Wolda 1981), were used to identify the ecological significance of the modeled wetland regions for macroinvertebrate distribution and are described further in Chapter 2 (Methods: Data Analyses).

Macroinvertebrate Metric Development

Metrics based on richness, taxonomic composition, and structural/feeding guilds were identified from literature sources and iterative tests of the dataset (e.g., Barbour et al. 1996, Gerritsen and White 1997, Burton et al. 1999, U.S. EPA 2002c). Metrics were tested for the strength of Spearman's coefficient of correlation (Spearman's r) with the LDI. Final metrics were selected as those with significant ($p<0.05$) correlations and Spearman correlation coefficients with the LDI of at least $|0.30|$. All evaluations of potential metrics were conducted with SAS (SAS Institute, Cary N.C., version 8.02).

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Categorical metrics

Categorical metrics were based on the relative abundance of a specific taxonomic or functional group. The categorical metrics tested provided information on the composition and trophic relationships of the assemblages of macroinvertebrates along the LDI gradient and between development classes. Categorical metrics tested were initially based on studies of Florida macroinvertebrates by Barbour et al. (1996) and Gerritsen and White (1997). As these authors examined macroinvertebrates within lentic and lotic environments, additional categorical metrics focusing on macroinvertebrates common in wetland environments were tested for their correlation with the LDI.

Composition measures (Table 4-2) provide information regarding the relative contribution of a population to the community structure of the wetland system (Barbour et al. 1996, Barbour et al. 1999). To compensate for species rich or species poor sites where richness may not be a reflection of the condition of the wetland (King and Richardson 2002), relative rather than absolute values were used for this measurement. In general, wetland systems without landscape modification will remain relatively consistent in proportional representation vis-à-vis other unperturbed wetlands, although individual abundance may vary in magnitude (Barbour et al. 1999). Variations in the representational proportion of taxonomic groups may indicate the encroachment of exogenous perturbations (Barbour et al. 1999). In addition to the measures listed in Table 4-2, compositional measures of each order, family, and genus were examined along the disturbance gradient and within each development classification for each site.

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Table 4-2. Compositional metrics, definition, and expected response.

Composition Metrics	Definition	Response	Source
Dominant taxon (%)	Abundance of the single most abundant taxon	Increase	1
% Diptera	% of dipterans	Increase	1
% Coleoptera	% of beetles	Decrease	1
% Odonata	% of dragonflies and damselflies	Variable	1
% Ephemeroptera	% of mayflies	Decrease	1
% Oligochaeta	% of aquatic worms	Increase	4
% Hemiptera	% of true bugs	Variable	3
% Gastropoda	% of snails	Decrease	1
% Decapoda	% of individuals classed as crustaceans	Decrease	2
% Tricoptera	% of caddis flies	Decrease	1
% Trombidiformes	% of mites	?	
% Amphipoda	% of amphipods	Decrease	1
Florida Index	Weighted sum of intolerant taxa, which are classed as 1 (least tolerant) or 2 (intolerant).	Decrease	1

Sources: 1=Barbour et al. (1996); 2=Barbour et al. (1999); 3=Gernes and Helgen (1999); 4=Fore et al. (1996).

Trophic categorical measures provide information on trophic relations, production, and feeding strategies (Resh and Jackson 1993, Barbour et al. 1999). A list of trophic measures examined, and the expected response to exogenous perturbations are given in Table 4-3. As in compositional measures, the trophic relationships in wetland systems with low LDI scores are expected to remain relatively stable vis-à-vis other wetlands in reference landscapes (Barbour et al. 1999). Increased landscape modification to the system was expected to alter the relative proportions of different trophic classes.

Ancillary data

Ancillary data for categorical analyses described above were obtained from the literature and the United States Department of Agriculture's Integrated Taxonomic Information System on-line database (United States Department of Agriculture 2003). The feeding strategy or trophic position of each organism was determined through literature reviews, particularly the text by Merritt and Cummins (1996) on aquatic invertebrates.

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Table 4-3. Trophic metrics, definition, and expected response.

Trophic Metrics	Definition	Response	Source
Predator %	% of the predator functional feeding group	Variable	4
Shredder (macr) (fw) %	% of the plant shredders functional feeding group	Decrease	2
Scraper (peri) (fw) %	% of the periphyton scrapers functional feeding group	Decrease	2
Col-fltr/susp fdr %	% collector-filterers/suspended feeders functional group	Variable	1
Plant piercer %	% of the macrophyte piercing functional feeding group	Decrease	1
Sub col-gthr/dep fdr %	% collector-gatherers/deposit feeders functional group	Variable	1
Epi col-gthr/dep fdr %	% collector-gatherers/deposit feeders functional group	Variable	1
Scavenger (animals) %	% of macrofauna that feed on dead animals	?	
Browser-grazer %	% of macrobenthos that browse/graze upon periphyton	Decrease	3
Parasite %	% of the parasite functional feeding group	?	2

Sources: 1=Barbour et al. (1996); 2=Gerritsen and White (1997); 3=Barbour et al. (1999); 4=Kerans and Karr (1994).

Sensitive and tolerant species

Indicator Species Analysis (ISA, Dufrene and Legendre 1997) was utilized to identify macroinvertebrate taxa having significant association with LDI classification of impaired (LDI≥2.0) or reference conditions (LDI<2.0). ISA is described in Chapter 2 (Methods: Metric Development). The abundance of species with significant specificity and fidelity to reference or impaired conditions was assessed for each site and correlated (Spearman's *r*) with the LDI.

Macroinvertebrate Index of Wetland Condition

To create a multimetric index of wetland condition using macroinvertebrates, it was necessary to score the proposed metrics. The scoring was completed by taking the quartile of the 95th percentile of the measured results for each metric. Doing this decreases the influence of outliers on the distribution of metric scores (Mack 2001). For metrics decreasing with increasing LDI scores, the lowest quartile was given a score of 0, the second quartile 3, the third quartile 7, and the highest quartile 10. Score values were switched for metrics that decreased with increasing LDI scores. A hypothetical example of this scoring method is presented in Figure 2-2. The Macroinvertebrate Index of

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Wetland Condition was determined for each wetland by summing the scores of the metrics.

Results

Wetland Macroinvertebrate Composition

Seventy herbaceous depressional wetlands throughout peninsular Florida with standing water were sampled in 1999 and 2000 (see Figure 2-1). Twenty-three were sampled in the South region, thirty in the Central region, and seventeen in the North region. Based on the dominant land use around the wetland, twelve of the sites sampled in the South region were *a priori* considered impaired, sixteen in the Central region, and eight in the North region.

The organisms identified in the samples from the studied wetlands represent 24 orders, 81 families, 182 different genera, and 120 different species. Of the organisms sampled and enumerated, 82% were identified to at least the genus level, but identification to the species level was possible for only 48% of the organisms due to lack of diagnostic features. While each site averaged 23.9 (+/- 5.7) taxa identified at least to genus, on average only 10.4 taxa (+/- 3.7) were identified to species. Analyses to develop indicators of wetland condition using macroinvertebrates and all subsequent analyses were therefore completed at the genus level.

Summary measures of sample richness, evenness, Shannon diversity, and Simpson diversity using the genus-level data were calculated for each site (Table 4-4). Mann-Whitney U-tests indicated no significant difference between reference and impaired sites for richness, evenness, Shannon diversity, or Simpson diversity (Table 4-5). No significant differences were found between wetland regions (e.g., South, Central,

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Table 4-4 Summary statistics of richness (S), evenness (E), Simpson's diversity index (H) and Shannon diversity index (D') for macroinvertebrates.

Name	S	E	H	D`
Audubon	8	0.602	1.253	0.5789
BearScat	22	0.892	2.757	0.9106
BigCow	25	0.794	2.557	0.8695
BRSebastian	26	0.923	3.006	0.9383
CabPatch	21	0.802	2.443	0.8704
Caravelle	23	0.758	2.377	0.8446
Chuluota	28	0.759	2.528	0.8702
CLBayard	10	0.727	1.675	0.7653
CLCove	11	0.467	1.121	0.4518
CMWPast	27	0.903	2.975	0.9351
CMWRef	29	0.828	2.790	0.8989
COBurgle	25	0.730	2.350	0.8384
COHole	24	0.803	2.551	0.8651
Crew	21	0.773	2.353	0.8479
Deerfly	21	0.832	2.532	0.8843
DEMelon	14	0.798	2.107	0.8269
Garber	20	0.875	2.620	0.9023
GbarE	27	0.782	2.578	0.8747
GLDonut	18	0.709	2.050	0.7880
GLPont	23	0.790	2.478	0.8615
Goethe	23	0.631	1.979	0.7073
GreenSwamp	24	0.870	2.764	0.9147
HagueI	20	0.721	2.161	0.7877
HagueII	12	0.760	1.888	0.8100
HalfMoon	20	0.866	2.593	0.8974
HARare	25	0.818	2.632	0.8915
HEBad	25	0.756	2.435	0.8637
HEL2	18	0.506	1.464	0.6243
HEOkay	14	0.552	1.458	0.5600
HighPast	25	0.849	2.733	0.9209
HighRef	19	0.838	2.467	0.8674
HillsRef	18	0.887	2.564	0.9020
HuntCamp	31	0.728	2.500	0.8720
IFASI	21	0.483	1.471	0.5149
IFASII	19	0.785	2.311	0.8541
Immokalee	15	0.761	2.060	0.8198
IRBlueCypress	26	0.689	2.246	0.7960
IRCanal	22	0.685	2.117	0.8146
IROJ	29	0.892	3.003	0.9365
JD6	11	0.907	2.175	0.8685
KellyPark	16	0.783	2.170	0.8219
LCork	21	0.656	1.996	0.7086
LEGo	16	0.722	2.003	0.8031
LESuwan	22	0.814	2.518	0.8812
LLeeCounty	30	0.879	2.989	0.9321
MALudy	20	0.781	2.339	0.8697
MASpray	20	0.776	2.326	0.8475
McArthur	17	0.646	1.829	0.6840
MNElmer	30	0.905	3.077	0.9354
MNOcala	22	0.842	2.601	0.8832
MRPepper	19	0.790	2.326	0.8498
Myakka	18	0.688	1.988	0.7861
OKCara	18	0.736	2.128	0.8230
OKKISS	18	0.736	2.128	0.8277
OKPast	25	0.773	2.487	0.8582
PacificTom	27	0.784	2.583	0.8568
PallMar	21	0.777	2.367	0.8256
PBCorbett	23	0.748	2.344	0.8296
PBEnjay	16	0.885	2.455	0.8953
Penner	17	0.725	2.053	0.7967
POWales	25	0.814	2.621	0.8831
POWeowak	27	0.701	2.311	0.7625
RiceCreek	18	0.860	2.485	0.8930
SandhillCrane	30	0.782	2.658	0.8710
SANorthMya	24	0.705	2.241	0.7867
SAOscer	22	0.865	2.673	0.9067
Savannas	15	0.793	2.146	0.8178
STCow	17	0.684	1.939	0.7770
UNHealthy	16	0.735	2.038	0.8350
Weikiva	15	0.873	2.365	0.8864

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Table 4-5. Mann-Whitney U-test results of summary statistics between reference and impaired LDI classes and between regional datasets.

	S (Richness)	E (Evenness)	H (Shannon Diversity)	D' (Simpson's Diversity)
Peninsular Dataset Impaired vs. Reference Z-statistic (*p*-value)	-0.301 (0.764)	1.418 (0.156)	0.847 (0.398)	0.971 (0.332)
Regional Dataset Regional Comparison Z-statistic (*p*-value)	1.998 (0.368)	2.111 (0.348)	2.901 (0.235)	1.913 (0.384)

North) for summary metrics (see Table 4-5). Two additional indices were also calculated from the macroinvertebrate data and compared between wetland condition classes: beta and gamma diversity (Ricklefs 1990, McCune and Grace 2002). Both were higher in reference sites than in impaired sites (reference sites average richness: 20.82, beta diversity: 7.06, gamma diversity: 147; impaired sites average richness: 21.03, beta diversity: 6.56, gamma diversity: 138).

Macroinvertebrate compositional similarities between wetland regions (*sensu* Lane 2000) were made using PSI and MRPP. In general, the South region and North region were most dissimilar, but the differences were slight. Comparisons using PSI indicated that South and Central regions shared 71% of the macroinvertebrate genera, while South and North regions only shared 56%. Central and North regions were 64% similar. MRPP results using the full dataset were equivocal as abundance measures and presence measures differed in interpretation. Global tests (South vs. Central vs. North) of genera abundance data indicated a significant compositional difference between wetland regions (A = 0.009, T = -2.41, p= 0.019). However, global test using genus presence, which is less subjected to temporal variation in data structure than abundance measures (McCune and Grace 2002), did not indicate a significant difference between wetland

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regions at $p<0.05$ (A = 0.006, T = - 1.674, p=0.060). Thus, while the abundance of macroinvertebrate genera differed between regions, the genera that comprised the macroinvertebrates assemblages identified did not. Iterative tests of both abundance and presence data indicated significant compositional differences between the South and North regions (Table 4-6), suggesting that South–North compositional variations were likely driving the differences identified in the global abundance MRPP tests.

Table 4-6. MRPP analyses of the macroinvertebrate compositional similarity between South, Central, and North wetland regions.

	Test	*A*	*T*	*p*-value
Abundance	S vs. C	0.003	-0.787	0.195
Iterative	S vs. N	0.017	-3.622	0.004
Tests	C vs. N	0.004	-1.017	0.015
Presence	S vs. C	-0.001	0.155	0.502
Iterative	S vs. N	0.015	-2.974	0.010
Tests	C vs. N	0.004	-1.001	0.155

Note: Abbreviations are S (South Region), C (Central Region), N (North Region). Iterative tests exclude one group (of three) during the MRPP procedure.

Simultaneous and iterative MRPP tests were also conducted using only the impaired (n = 37) or reference (n = 33) sites' data. The composition of reference sites was significantly different across regions when simultaneously tested (Table 4-7) with both abundance and presence data, and significant between all regions except Central and North when both abundance and presence data were iteratively tested. In stark contrast, there were no significant differences between the composition of impaired sites when either simultaneously or iteratively tested using either abundance or presence data (Table 4-8). Thus, while differences in the composition of reference sites were found through peninsular Florida, the composition of impaired sites was generally static.

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Table 4-7. MRPP analyses of the macroinvertebrate compositional similarity for reference sites between South, Central, and North wetland regions.

	Test	*A*	*T*	*p*-value
Abundance Global Test	S vs. C vs. N	0.016	-2.171	0.028
Presence Global Test	S vs. C vs. N	0.0177	-2.375	0.017
Abundance Iterative Tests	S vs. C	0.0192	-2.478	0.019
	S vs. N	0.0231	-2.693	0.013
	C vs. N	-0.0049	0.574	0.683
Presence Iterative Tests	S vs. C	0.0180	-2.671	0.022
	S vs. N	0.0260	-2.816	0.011
	C vs. N	-0.0030	0.041	0.636

Note: Abbreviations are S (South Region), C (Central Region), N (North Region). Global tests refer to simultaneous multivariate tests of the pooled macroinvertebrates from all three wetland regions. Iterative tests exclude one group (of three) during the MRPP procedure.

Community Composition and Environmental Gradients

Including the LDI, fourteen environmental variables were measured at each site. The measured water and soil parameters are given in the Appendix B. Significant differences between impaired and reference conditions, as measured by the Mann-Whitney U-test, were found for the following parameters: soil pH, soil TP, specific conductivity, water pH, ammonia, TKN, and water TP (Table 4-9). Water color was significant lower in reference conditions at $p<0.10$.

Colinearity among variables, which was identified as a VIF>5.0 and a tolerance of <0.20, was recognized for %TC, TKN, and water pH following regression analyses of the transformed data (SAS 1990). These variables were removed.

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Table 4-8. MRPP analyses of the macroinvertebrate compositional similarity for impaired sites between South, Central, and North wetland regions.

	Test	*A*	*T*	*p*-value
Abundance Global Test	S vs. C vs. N	0.0005	-0.064	0.431
Presence Global Test	S vs. C vs. N	0.0029	-0.410	0.319
Abundance Iterative Tests	S vs. C	-0.0037	0.583	0.687
	S vs. N	0.0044	-0.430	0.290
	C vs. N	0.0031	-0.434	0.293
Presence Iterative Tests	S vs. C	-0.0059	0.946	0.831
	S vs. N	0.0122	-1.134	0.130
	C vs. N	0.0064	-0.910	0.178

Note: Abbreviations are S (South Region), C (Central Region), N (North Region). Global tests refer to simultaneous multivariate tests of the pooled macroinvertebrates from all three wetland regions. Iterative tests exclude one group (of three) during the MRPP procedure.

Table 4-9. Comparison of the medians of water and soil variables between reference and impaired conditions with the Mann-Whitney U-test.

Water Values	Statistic (Z)	*p*-value
Color	-1.868	0.062
Specific Conductivity	-3.919	<0.001
Turbidity	-1.524	0.127
Water pH	-4.677	<0.001
Ammonia	-2.690	0.007
Nitrates/Nitrites	0.771	0.441
TKN	-2.932	0.003
TP	-4.836	<0.001

Soil Values	Statistic (Z)	*p*-value
Soil pH	-3.036	0.002
%TC	-0.818	0.414
%OM	-1.253	0.210
%TN	-1.147	0.251
%TP	-2.771	0.006

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The Mantel test was used to test the hypothesis of no relationship between the identified macroinvertebrates and the measured environmental parameters, including LDI score. Results indicated a moderate and significant relationship (Mantel's r =0.35, p=0.001), suggesting that the genus-level composition of peninsular herbaceous marshes was determined by the measured environmental driving factors.

An NMDS ordination was completed to examine the underlying structure of the sites in ordination space and to ascertain the environmental variables driving the composition of the sites sampled. Correlations for all measured variables are presented in Table 4-10, and a biplot of site ordination overlaid with vectors representing the environmental variables is presented in Figure 4-1. The stress, or "goodness of fit" of the ordination was 19.32 and indicated an adequate and representative decreased dimensionality of the dataset (Kruskal 1964, Clarke 1993). Approximately sixty-eight percent of the variance in the dataset was captured by the three-dimensional NMDS ordination (first axis: 12.9%, second axis: 25.3%, third axis: 30.3%). In NMDS, unlike other ordination methods (e.g., principal components analysis, detrended correspondence analysis), axes are not necessarily ordered based on their importance. The third axis contained the most data in the NMDS ordination.

As the second and third axes accounted for most of the variance in the dataset, the plot in Figure 4-1 is of sites along these two axes. The ordination scores for each site were linearly correlated (Pearson's r) with the measured environmental values, including LDI, latitude, and longitude. Correlations (Pearson's r^2) > 0.30 were found for LDI, specific conductivity, soil pH, water TP, and color, suggesting their importance in driving the structure of the wetland macroinvertebrate community.

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Table 4-10. Correlations of environmental variables and ordination scores for macroinvertebrates.

Parameter	Axis 1 (12.9%) r^2	Axis 1 (12.9%) tau	Axis 2 (25.3%) r^2	Axis 2 (25.3%) tau	Axis 3 (30.3%) r^2	Axis 3 (30.3%) tau
LDI Score	0.12	-0.24	0.06	-0.18	0.42	0.41
Soil pH	0.34	-0.39	0.11	0.21	0.19	0.36
% TN	<0.01	0.04	0.06	-0.22	<0.01	0.01
TP (mg/kg)	0.03	-0.15	0.09	-0.26	0.03	0.11
% OM	0.02	0.02	0.11	-0.26	<0.01	-0.03
Color (PCU)	<0.01	-0.03	0.30	-0.33	0.03	0.10
Spec. Cond. (umhos/cm)	0.30	-0.37	0.02	-0.14	0.36	0.45
Turbidity (NTU)	0.01	-0.03	0.10	-0.11	0.09	0.18
Ammonia (mg/L)	<0.01	-0.10	0.07	-0.17	0.08	0.31
Nitrate/Nitrite (mg/L)	0.01	<0.01	0.01	-0.12	<0.01	0.04
Water TP (mg/L)	0.07	-0.19	0.25	-0.38	0.33	0.43
Longitude	<0.01	<0.01	0.13	-0.24	<0.01	-0.07
Latitude	0.03	0.10	0.20	-0.32	0.01	-0.08

Note: Correlations marked with an asterisk were ≥ 0.30 and were considered strongly correlated with NMDS ordination scores. Kendal's tau, a non-parametric measure of association, is also given.

Metric Development

Compositional analyses between wetland regions equivocated on the ecological significance of the modeled wetland regions (Lane 2000) to macroinvertebrate distribution in isolated depressional marshes. Initial metric development utilized regional data sets – however, the small *n* of the regional data and the low to moderate correlation of macroinvertebrates to the disturbance gradient (C. Lane *unpublished research*, see also Tangen et al. 2003) limited development of regionally significant biological indicators of wetland condition. Perhaps due to the greater power of analysis, responsive patterns in the structure and composition of macroinvertebrate communities along the landscape modification gradient were discerned when the combined peninsular dataset (n=70) was examined. Thus, metrics to assess the condition of Florida's isolated herbaceous wetlands were identified utilizing the peninsular data set.

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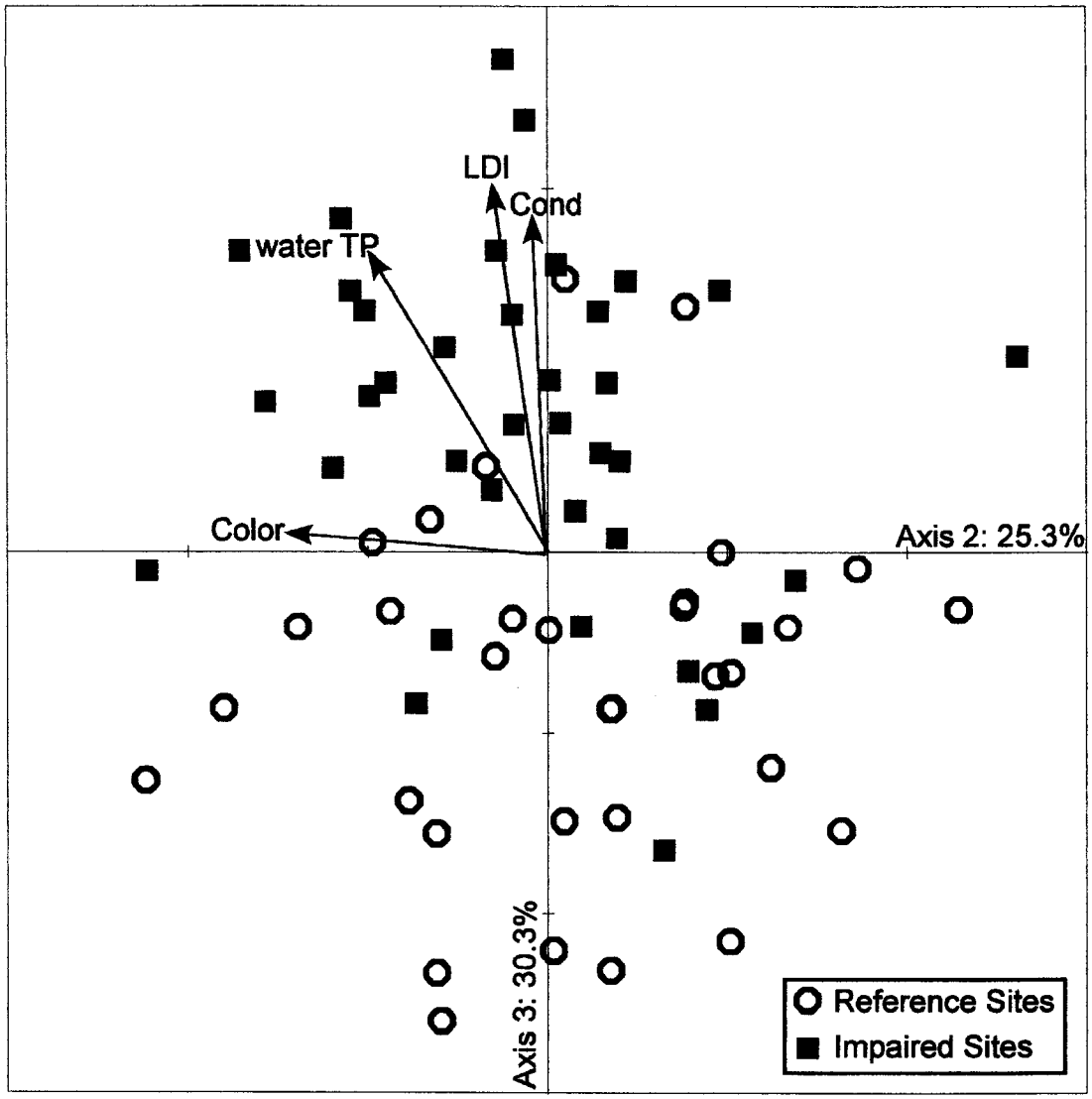

Figure 4-1. Biplot of macroinvertebrate NMDS ordination scores for each site and strongly correlated environmental variables. The vectors are shown at 150% of their original length for clarity. The length of the vectors represents the strength of the Pearson's correlation (all r^2 >0.30) and the angle represents the direction of maximum change. Four variables are shown: LDI (Landscape Development Intensity index), wTP (water total phosphorus), Spec (specific conductivity), and color. Soil pH was strongly correlated with the first axis, not shown.

In an effort to discern potential macroinvertebrate metrics, more than 60 categorical and compositional metrics were examined for the strength of the correlation with the LDI. Five metrics were developed that were selected based on the strength of their

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correlations with the LDI. These metrics are described below: % Sensitive Taxa, % Tolerant Taxa, % Predators, % Odonata, and % Orthocladiinae.

Sensitive and tolerant taxa

Indicator species analysis (calculated using genus-level data) identified twenty-seven genera with specificity and fidelity to reference or impaired conditions. Fourteen genera were significantly associated with reference conditions (Table 4-11), and thirteen genera were significantly associated with impaired conditions (Table 4-12). The taxa, calculated indicator species value, and *p*-value of the Monte Carlo test are also given in Tables 4-11 and 4-12. Once the sensitive and tolerant taxa were identified, a metric was developed based on the abundance of the indicator taxa. The relationship between the abundance of sensitive and tolerant genera, respectively, and the LDI is presented in Figures 4-2 and 4-3. The abundance of sensitive and tolerant genera were correlated with the LDI: % Sensitive Spearman's $r = -0.65$, $p < 0.001$; % Tolerant Genera Spearman's $r = 0.71$, $p < 0.001$.

The distribution of the abundance of sensitive taxa appeared to be highly variable for any given LDI value, although a decreasing trend with increasing LDI scores was evident. The abundance of tolerant taxa did not appear to correlate with the LDI until a threshold of approximately 3.2 was reached. The abundance of tolerant taxa was also highly variable, although this was only evidenced after LDI values of 3.2 were obtained.

Percent Predators

The abundance of organisms classified in the predator functional feeding group was expected to decrease with increasing LDI scores (U.S. EPA 2002c). As demonstrated in Figure 4-4, these results support that hypothesis, as the abundance of predators decreased significantly (Spearman's $r = -0.32$, $p = 0.007$) along the increasing

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Table 4-11. Statewide indicators of reference conditions as determined from Indicator Species Analysis.

Indicator Genera (LDI <2.0)	Observed Ind. Value	*p*-value
Ablabesmyia	48.9	<0.001
Chaoborus	54.1	0.004
Clinotanypus	12.1	0.042
Corethrella	9.1	0.099
Hydroporus	9.1	0.097
Ischnura	16.0	0.088
Labrundinia	50.2	<0.001
Larsia	48.9	0.014
Lestes	12.1	0.046
Limnochares	9.1	0.097
Orthotrichia	9.1	0.097
Piona	12.1	0.045
Procladius	24.9	0.005
Sminthurinus	9.1	0.098

Table 4-12. Statewide indicators of impaired conditions as determined from Indicator Species Analysis.

Indicator Genera (LDI ≥2.0)	Observed Ind. Value	*p*-value
Atrichopogon	26.5	0.041
Beardius	27.7	0.071
Enochrus	25.4	0.060
Goeldichironomus	70.7	<0.001
Haliplus	13.5	0.055
Mansonia	21.6	0.006
Micromenetus	23.1	0.025
Monopelopia	62.5	<0.001
Odontomyia	27.7	0.004
Pachydrus	21.1	0.028
Physella	20.9	0.076
Ranatra	16.2	0.025
Zavreliella	16.3	0.079

LDI gradient. Additionally, predators were significantly more abundant in reference conditions than in impaired conditions (Mann-Whitney U-test $Z = 2.900$, $p = 0.004$). However, the data were highly variable with any given LDI value, indicating low precision as well as low accuracy, despite the significant correlation.

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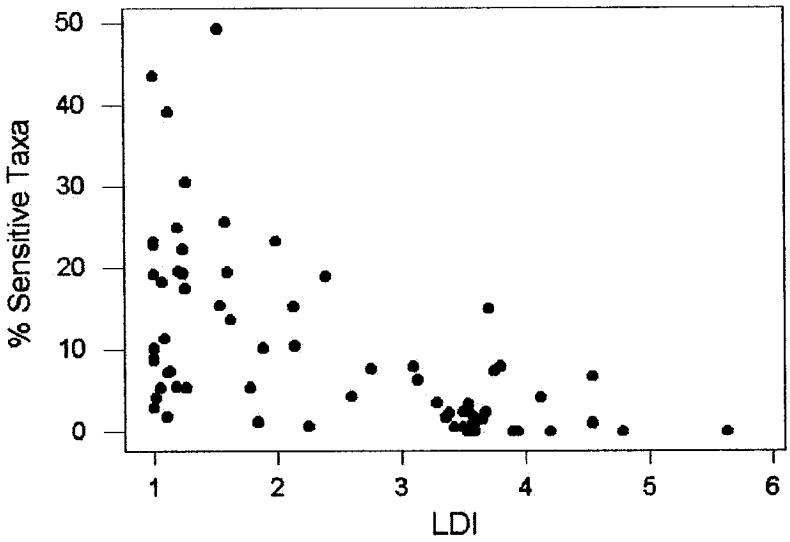

Figure 4-2. Abundance of sensitive taxa plotted against the LDI.

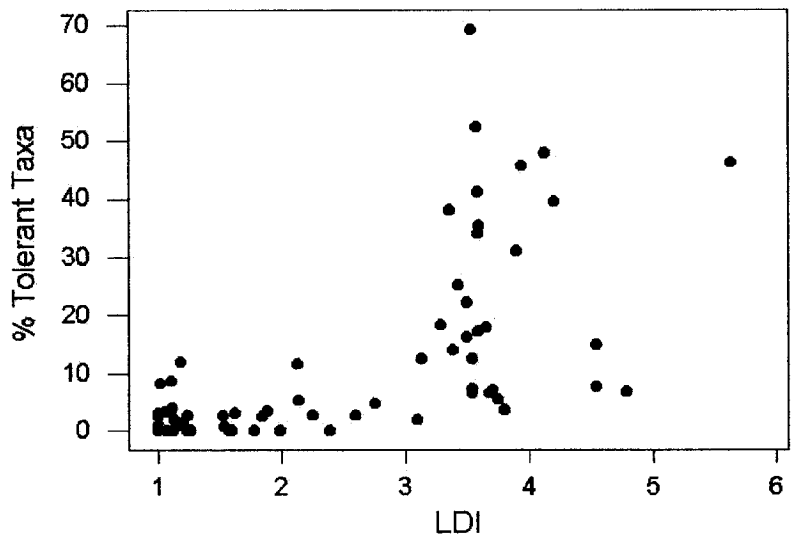

Figure 4-3. Abundance of tolerant taxa plotted against the LDI.

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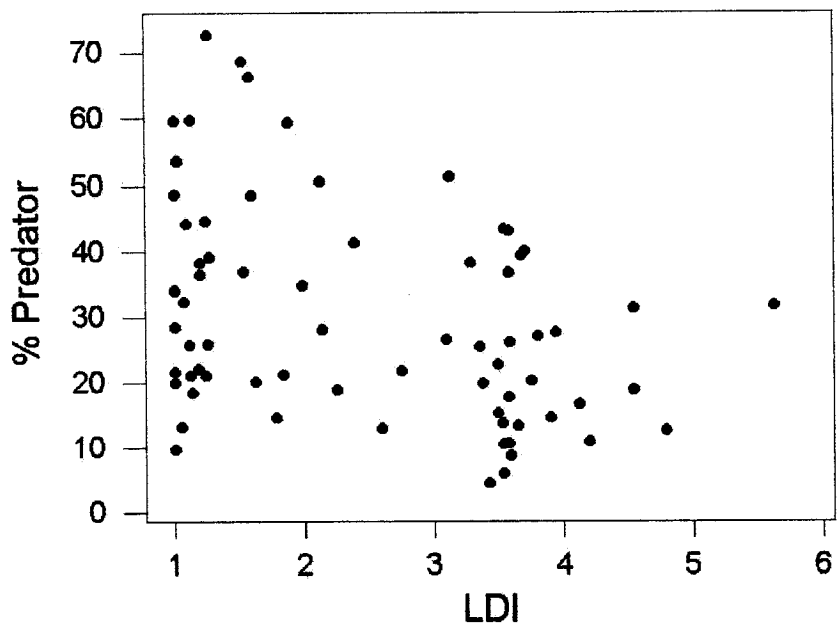

Figure 4-4. Predator abundance plotted against the LDI.

Percent Odonata

The abundance of members of the order Odonata (dragonflies and damselflies; family Insecta) was identified as an indicator of wetland condition in isolated depressional wetlands across peninsular Florida. With increasing LDI scores, the abundance of odonates decreased. A plot of the relationship between the abundance of odonates and the LDI is presented in Figure 4-5. Results of the Mann-Whitney U-test indicated a significant difference in Odonata abundance between reference and impaired wetlands ($Z = 2.334$, $p = 0.020$). The metric was significantly but moderately correlated (Spearman's $r = -0.33$, $p = 0.005$) with the disturbance gradient. The weak relationship between the LDI and the abundance of Odonata was also evidenced by highly variable values along the LDI gradient.

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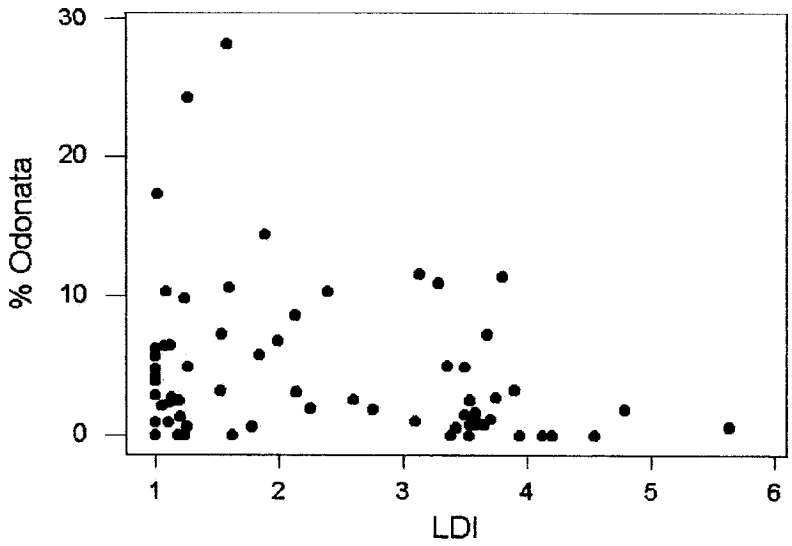

Figure 4-5. Odonata abundance plotted against the LDI

Percent Orthocladiinae

The abundance of the Orthocladinae tribe (midges, class Insecta, Chironomidae family) was correlated with disturbance gradient. Increases in LDI scores were generally reflected by increased abundance of Orthocladiinae midges (Figure 4-6), although the variance in the data was very high for any given LDI score. Orthocladinae were significantly more abundant at impaired than reference sites (Mann-Whitney U-test Z = 3.100, p = 0.002). The abundance of Orthocladiiae midges was moderately but significantly correlated with the LDI (Spearman's $r = 0.33$, $p = 0.006$).

Macroinvertebrate Index of Wetland Condition

The five final metrics were scaled, scored, and summed to create the Macroinvertebrate Index of Wetland Condition (MIWC). The 95th percentile and the quartile scores for each of the five metrics are presented in Table 4-13. The relationship between the MIWC and the LDI is presented in Figure 4-7. Scores for the MIWC range

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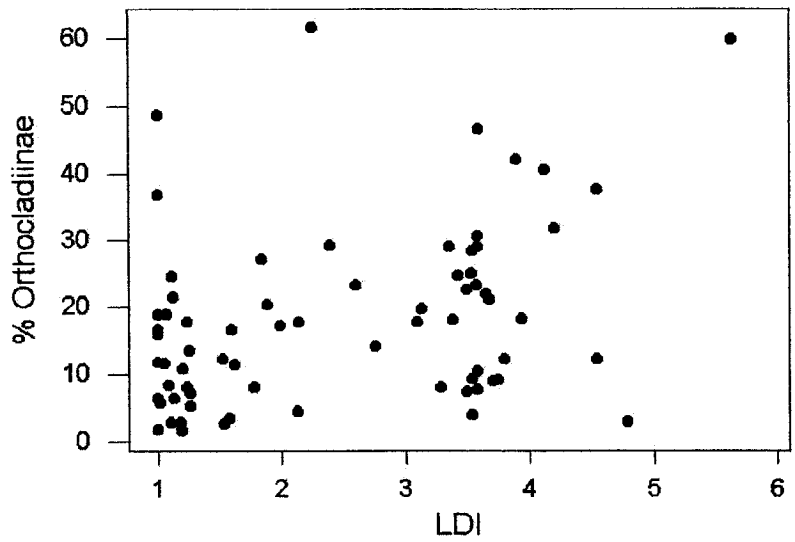

Figure 4-6. The abundance of Orthocladiinae plotted against the LDI.

from 0 (three sites) to 50 (one site) (Table 4-14). Two of the three low-scoring sites were located in cattle pastures, while the third was surrounded by a watermelon farm. The highest scoring site was located in Ocala National Forest. The MIWC was correlated with the LDI: (Spearman's $r = 0.79$, $p < 0.001$). Data distribution was highly variable, although a decreasing linear trend was apparent. A Mann-Whitney U-test of the difference in median MIWC scores between reference and impaired sites indicated a highly significant difference ($Z = 5.331$, $p < 0.001$).

Table 4-13. Quadrisect values and the 95th percentile data for each proposed biological indicator.

Metric	95th Percentile	0 Scores	3 Scores	7 Scores	10 Scores
% Sensitive Genera	28.31	< 1.27	1.27 – 5.28	> 5.28 – 13.06	> 13.06
% Tolerant Genera	46.00	> 12.58	> 3.45 – 12.58	0.85 – 3.45	< 0.85
% Predators	59.74	< 17.85	17.85 – 25.78	> 25.78 – 37.98	> 37.98
% Odonata	13.15	< 0.81	0.81 – 2.25	> 2.25 – 5.02	> 5.02
% Orthocladiinae	44.52	> 22.40	> 15.06 – 22.40	7.95 – 15.06	< 7.95

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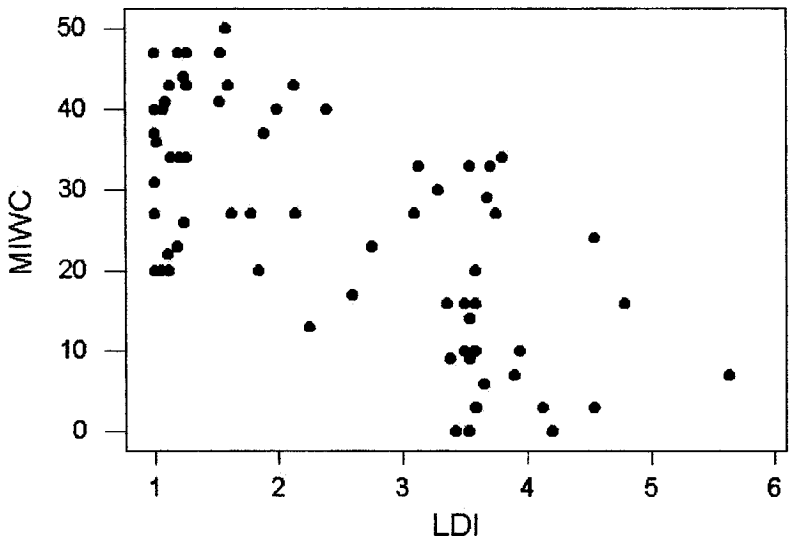

Figure 4-7. The Macroinvertebrate Index of Wetland Condition plotted against the LDI.

Discussion

Increases in land use intensity directly surrounding the study wetlands were manifested within the wetland through alterations in the composition of the macroinvertebrate community when compared with systems in reference, or low development intensity landscapes. Five metrics were discerned that permitted a measurement of the effect of increased landscape development on the macroinvertebrate community: the abundance of sensitive and tolerant genera, predators, odonates, and Orthocladiinae. The results of this study suggest that macroinvertebrate metrics, both compositional and categorical, were able to discern the relative condition of isolated depressional wetlands throughout peninsular Florida. Likewise, correlations between the LDI and the Macroinvertebrate Index of Wetland Condition, which synthesizes scores of

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Table 4-14. Macroinvertebrate Index of Community Condition scores for sampled sites.

Sites	MIWC	Sites	MIWC
Audubon	0	**Immokalee**	17
BearScat	23	**IRBlueCypress**	16
BigCow	3	**IRCanal**	3
BRSebastian	41	**IROJ**	29
CabPatch	9	**JD6**	26
Caravelle	33	**KellyPark**	6
Chuluota	37	**LCork**	20
CLBayard	40	**LLeeCounty**	36
CLCove	14	**LEGo**	31
CMWPast	27	**LESuwan**	43
CMWRef	27	**MALudy**	10
COBurgle	16	**MASpray**	34
COHole	22	**McArthur**	27
Crew	27	**MNElmer**	33
DEMelon	40	**MNOcala**	50
Deerfly	0	**MRPepper**	3
Garber	23	**Myakka**	34
GBarE	9	**OKCara**	16
GLDonut	10	**OKKiss**	47
GLPont	30	**OKPast**	20
Goethe	41	**PacificTom**	24
GreenSwamp	43	**PallMar**	27
HARare	10	**PBCorbett**	34
HagueI	3	**PBEnjay**	37
HagueII	7	**Penner**	43
HalfMoon	40	**POWales**	47
HEBad	16	**POWeowak**	47
HEL2	0	**RiceCreek**	37
HEOkay	20	**SANorthMya**	34
Highpast	33	**SAOscer**	20
HighRef	47	**SandhillCrane**	27
HillsRef	44	**Savannas**	43
HuntCamp	27	**STCow**	10
IFASI	13	**UNHealthy**	7
IFASII	20	**Weikiva**	40

each metric, supports the use of macroinvertebrates as indicators of freshwater wetland condition, although the relationships were not highly sensitive or precise.

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Environmental Correlates and Macroinvertebrate Composition

A premise behind the biological assessment of aquatic systems is the intimate relationship of organisms and the environment. Both exogenous and endogenous alterations to the environment, if sufficiently intense (or if synergistic relationships are sufficiently intense), are manifested in the community characteristics of various assemblages (Karr and Chu 1997). Allan and Johnson (1997) and Hynes (1975) theorized that aquatic communities (i.e., riverine and lacustrine systems) respond to environmental stimuli that operate at various spatial and temporal scales. In this study, the ordination of macroinvertebrate genera abundance data identified LDI score, specific conductivity, soil pH, water TP, and water color as significant variables determining macroinvertebrate community composition (see Table 4-10). Temporal and spatial changes in landscape-scale development intensity, as well as daily, weekly, and seasonal changes in localized variables such as water TP, soil pH, color, and specific conductivity affected community composition. Thus, the results of this study suggested that the notions proffered by Allen and Johnson (1997) and Hynes (1975) can also be applied to wetland systems.

As macroinvertebrates are responsive to a suite of anthropogenic alterations (this study; Adamus and Brant 1990, Adamus 1996, Danielson 1998, Anderson and Vondracek 1999, Euliss and Mushet 1999, Adamus et al. 2001, but see also Tangen et al. 2003), it is not surprising that a significant relationship with a general impairment indicator was identified. The use of a general, independent disturbance gradient to measure anthropogenic disturbance has enjoyed recent successes in biological assessment (Carpenter and Waite 2000, Cuffney et al. 2000, O'Conner et al. 2000, Munn et al. 2002, Fore and Grafe 2003). Landscape-scale assessment methods, such as the LDI, are

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generally adept at indirectly or directly capturing alterations not easily quantified –such as hydrologic modification through ditching, draining, and berm building, changes due to landscape variables such as fire frequency and intensity, and modifications to physical, chemical, and nutrient loading rates as landscapes change.

However, including smaller-scale (temporal and spatial) measurements such as soil and water chemistry are integral to understanding particular deterministic variables affecting aquatic communities and may assist in explaining high variance for given LDI values. As noted, in addition to LDI value, this study determined specific conductivity, (soil) pH, water TP, and water color to be important structuring components of macroinvertebrate communities in isolated herbaceous depressional marshes of peninsular Florida.

Elevated conductivity measures can generally affect aquatic organisms through osmotic regulatory alterations (Stevenson et al. 1999, Spieles and Mitsch 2000). Specific conductivity measures play an important part in macroinvertebrate community composition (Ormerod and Edwards 1987, Growns et al. 1992, Tate and Heiny 1995, Spieles and Mitsch 2000, Carlisle et al. 2003).

Studies by Malmqvist and Maki (1994) and Evans et al. (1999) have indicated the responsiveness of aquatic macroinvertebrates to alterations in pH. Danielson (1998), Doherty et al. (2000), and Adamus et al. (2001), reviewing macroinvertebrates as biological indicators, noted their utility in identifying pH-modified wetland systems It is well established that as a result of both the gradual translocation of materials available for cation exchange (Evans et al. 1999) and the extended anoxic conditions in inundated soils, wetlands in pine flatwoods are generally low pH systems characterized by

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organisms evolved to tolerate acidic conditions (Kushlan 1990, Evans et al. 1999). Conversely, elevated pH values are often found in agricultural settings due to application of agrochemicals composed of basic compounds, which can alter the pH of receiving water bodies and hence species composition (Fore and Grafe 2002).

Macroinvertebrate communities respond to alterations in phosphorous concentrations (Brightman 1984, Adamus and Brandt 1990, Malmqvist and Maki 1994, Tate and Heiny 1995, Adamus 1996, Danielson 1998, Doherty et al. 2000). Nutrients, such as TP, can increase productivity but also increase community respiration and thus affect dissolved oxygen levels and physical/chemical characteristics of the water body with subsequent effects on wetland communities (Dierberg and Brezonik 1984).

Few studies have implicated water color as directly affecting macroinvertebrate community composition (Malmqvist and Maki 1994, but see Crisman et al. 1998). It can be hypothesized, however, that increasing the opaqueness of the water may affect diatom productivity through decreases in the available light for photosynthetic processes. Possible decreases in macroinvertebrate assemblages consuming epiphytic and benthic algae (i.e., scrappers) could be expected, with potential interactions throughout the macroinvertebrate food web. In addition, increases in water color may affect visual predatory macroinvertebrates by decreasing the visibility of prey.

Regional Data Analyses

Data were initially analyzed at the regional level to account for differences between the macroinvertebrate composition of peninsular Florida wetlands that could be attributed to latitudinal, geophysical, or climatological vagaries. However, while significant differences were found between regions using the abundance dataset, no significant differences were identified using presence-absence data. While both presence

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and abundance may differ temporally, abundance data are much more dynamic and may be more a reflection of sampling period than of landscape characteristics affecting wetland communities. The lack of significant correlations between NMDS ordination scores and latitude and longitude (as analogs for wetland regions – see Lane 2000) similarly decreased the importance of regions for macroinvertebrate analyses in herbaceous wetlands. In addition, the increased power of analyses using a larger dataset (i.e., not incorporating the modeled wetland regions) made for stronger, more significant relationships between site composition and landscape characteristics. It was therefore determined that regionally unique metrics were not necessary, and the final metrics described in this chapter were developed utilizing the peninsular dataset (n=70). However, additional sampling and analyses within each wetland region may provide fruitful information germane to regionally unique macroinvertebrate metrics, and the development of additional metrics for macroinvertebrates may utilize regions as reports developed for stream and lake biological assessment in Florida (Barbour et al. 1996, Gerritsen et al. 2000) have concluded.

Final Metrics

Five final metrics were significantly correlated with increasing LDI scores: the abundance of sensitive and tolerant taxa, the abundance of predators, the abundance of Odonata, and the abundance of Orthocladiinae. Final metric correlations with the LDI were significant ($p< 0.05$) and Spearman's correlation coefficients were greater than |0.30|. While significant trends in the dataset were identified, lower correlation coefficients were obtained than anticipated and reflected the lack of sensitivity and precision inherent within the data that comprised the MIWC. The variance within each metric was large for any given LDI value, and only general trends were evidenced in the

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relationship between the LDI and the metrics. However, information germane to the development of biological indicators was nevertheless obtained, as suggested by the significance of the correlation between the LDI and the summed MIWC. The five final metrics are discussed below.

Sensitive and tolerant genera

To be able to statistically evaluate the specificity and fidelity of taxa to a particular class through a Monte Carlo randomization algorithm is an alluring characteristic of Indicator Species Analysis (ISA). Indeed, the use of ISA in ecological literature is increasing (McCune and Grace 2002), although a recent report suggested that ISA was conservative in dealing with rare taxa (Chytry et al. 2002). Nevertheless, use of ISA in this analysis allowed for the identification of twenty-seven taxa indicative of reference or impaired conditions.

However, the distribution of the data indicated that neither the abundance of sensitive taxa or tolerant taxa were particularly precise or accurate indicators of LDI values due to the highly variable nature of the relationship between the LDI and the metrics. A general trend, although highly variable, was evidenced by the abundance of sensitive taxa, while tolerant taxa did not appear to reflect greater than background values until LDI scores of approximately 3.3. The high variance in these metrics may be a result of the taxonomic level in the analyses.

Additional analyses to develop optima (i.e., U.S. EPA 2002e) for indicator species, or to further determine which environmental factors were most responsible for the distribution of the indicator taxa are suggested by these results and would assist in determining the ecological significance of the indicator taxa and the relationship of metrics to the LDI gradient.

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Categorical metrics–predators

A general decline was apparent in the abundance of predators identified within each sample with increasing LDI value, indicating the increasing importance of generalists and lower-structured food webs. However, the variance within the dataset was very large regardless of LDI value. This may be explained by several factors acting independently or in concert. For instance, Chovanec and Raab (1997) found that a physical disturbance in wetlands (such as vegetation removal by ruminates and subsequent lack of "perches") affected the abundance of predators. Altered timing, depth, and duration of water, which often occurs on managed landscapes, affected development of various instars of predatory macroinvertebrates (Anderson and Vondracek 1999). Agrochemical applications within a wetland watershed altered the trophic structure (i.e., increase the abundance of generalists) of wetlands in North Dakata (Euliss and Mushet 1999). A decrease in wetland water clarity from the constant re-suspension of flocculent materials in areas of high cattle densities (Newcombe and MacDonald 1991) negatively affected the abundance of predatory macroinvertebrates due to decreased success from visual predators.

Categorical metrics–Odonates

Larval odonates comprise an important part of many aquatic system food webs, including fish, amphibians, birds, and other organisms (Merrit and Cummins 1996). As predators, members of the order Odonata would be expected to decrease with increasing LDI scores, as occurred in this study, due to similar reasons described above (see Categorical Metrics – Predators). However, the relationship between the LDI and the abundance of Odonates was highly variable for any given LDI value, decreasing the information content of the relationship, as well as the sensitivity and accuracy. Odonates

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tend to equivocate on their response to disturbances, which may explain some of the variance in the dataset. Odonates are tolerant to many disturbances – such as altered pH, and low to moderate nutrient loading (Chovanec and Raab 1997), which would tend to decrease their utility as biological indicators. However, they have been identified as indicators of, "the ecological integrity of ecosystem structures, the ecological quality of the land-water interface, and the connectivity of aquatic ecosystems to other landscape units" (Chovanec and Raab 1997, p. 383). These abilities may be due in part to the long life cycle of odonates, as they would be expected to integrate long term and short, episodic perturbations to the wetland. Conrad et al. (1999) also reported the importance of landscape-level connectivity and structure to odonate population dynamics. Often, marsh systems are drained and filled in the course of agricultural operations–decreasing the abundance of these systems on a landscape scale when compared to marsh systems located in reference landscapes (Pearlstine et al. 1997).

Categorical metrics–Orthocladiinae

That midges (Diptera: Chironomidae) comprise a metric for the assessment of wetland condition was not surprising, given that they are often the most numerous insects present in freshwater environments (Cranston 1995, Coffman and Ferrington 1996). Midges have been so abundant in the benthos of both lentic and lotic aquatic systems as to be used in classifying streams and lakes (Lindegaard 1995), and were used as some of the earliest indicator organisms of anthropogenic eutrophication as the Chironomidae family contains genera extremely sensitive to disturbances (such as nutrient enrichment, Rosenberg and Resh 1993). The Chironomidae also contain genera that are extremely tolerant of such conditions, which implies identification to at least sub-family, as suggested by Lindegaard (1995).

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The majority of biological assessment research on midges (and macroinvertebrates in general) has been conducted for assessment of streams and lakes, and not wetlands (Rosenberg and Resh 1993, but see Driver 1977, Delettre 1989, Streever et al. 1995, Leeper and Taylor 1998, Gernes and Helgen 1999, Kashian and Burton 2000, King and Richardson 2002, U.S. EPA 2002c). Thus, many of the tolerance values or responses to disturbance present in the literature for cosmopolitan midges of those habitats (i.e., Beck 1954, Lenat 1993) may not be viable for the assessment of wetland environments due to the different driving forces acting on them.

Despite apparent benefits in the assessment of herbaceous wetland systems, use of the Chironomidae in biotic assessment should include caveats: first, many chironomids may only be identified to species after being mounted on microscope slides and examined under high-magnification, with complimentary increases in sample processing time and cost. To wit, the time spent in-lab washing, sorting, subsampling, and identifying 100 to 150 organisms, including the Chironomidae, averaged over 20 hours per site (R. Frydenborg, FDEP, *personal communication*). Second, the variance within the dataset indicated that the relationship between the LDI and the Chironomidae was highly variable for any given LDI score, which would decrease their relative utility in wetland assessment at a fine scale. It may be important in future analyses to target the Chironomidae and thus decrease the laboratory sampling time spent on other families, or to examine larval Chironomidae further for developmental abnormalities as deformations have been correlated to exogenous perturbations (Johnson et al. 1993).

Macroinvertebrate Index of Wetland Condition

The macroinvertebrate metrics were significantly correlated with the LDI, although the data were generally neither sensitive nor precise at that scale, as reflected by

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high variance for any given LDI value. While many agencies and researchers have touted use of macroinvertebrates to assess stream and lake systems (Barbour et al. 1996, Gerritsen and White 1997), as well as wetland systems (U.S. EPA 2002c), recent literature has indicated that the macroinvertebrate community may not be particularly responsive to agricultural perturbations (Tangen et al. 2003). These results support the use of macroinvertebrates as biological indicators of wetland condition in agricultural settings at the scale provided, but also suggest exploration of macroinvertebrate composition and structure along additional scales to increase the strength of the relationship.

Field Sampling and Macroinvertebrate Identification

Field sampling

The very nature of wetlands predisposes macroinvertebrates to wide fluctuations in hydrology, as well as associated fluctuations in temperature, salinity, and dissolved oxygen levels (Sharitz and Batzer 1999, Leslie et al. 1999, Evans et al. 1999). Florida is generally wettest during summer and driest during late winter and early spring (Chen and Gerber 1990). However, an expansive drought subjected many peninsular Florida wetlands to depressed or non-existent hydroperiods during the sample period of 1999 and 2000 (Lawrimore et al. 2001). As such, the study wetlands were sampled late in the summer growing season to ensure that the wetlands were sufficiently hydrated (mean sampling date in 1999 was September 5, and September 29 in 2000). This late sampling date, and the range in dates for each region and year (Table 4-15), may have had ramifications on the observed macroinvertebrates, since the late summer to fall sampling

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Table 4-15. Sampling dates for each region.

Region	Earliest Date 1999	Earliest Date 2000	Latest Date 1999	Latest Date 2000
South	August 3	August 29	November 4	October 25
Central	July 23	September 9	August 17	October 26
North	July 28	October 16	November 11	November 1

window may have excluded some species that complete their life cycle early in the growing season (Anderson and Vondracek 1999). Additionally, the range in sampling dates from July and August to early November could affect the results, and thus comparative

site scoring as some sites might have been sampled early in the summer while others in the same region were sampled in the fall, yet they were compared in the analyses to each other. In essence, some organisms might have been collected early in the season but were not found later in the season not as a function of the land use around the wetland but as a function of the date sampled. Thus, the presence or abundance of an organism at a site may be related to the sampling date instead of the character of the landscape surrounding the wetland.

Sites were selected for inclusion based on the providence of supportive landowners and managers and the prevalent land use around each wetland. The sites sampled were generally identified in early spring to mid summer, and subsequently sampled in late summer to early fall. Most sites identified in spring were dry, yet all sites studied (for macroinvertebrate analyses) were hydrated when sampled. However, no gauges were placed on-site to discern the time span between wetland hydration and the sampling date. Some sites sampled may have only recently been hydrated and thus may have supported only a paltry abundance of organisms or a particular composition not due to landscape

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factors but due to the short window of opportunity for emergence for many macroinvertebrates (Wiggins et al. 1980, Wallace and Anderson 1996).

Many authors have noted seasonal differences in the emergence of macroinvertebrates (Anderson and Vondracek 1999, Stewart and Loar 1994, Wallace and Anderson 1996) and the existence of delay between hydration and emergence of macroinvertebrates (Sharitz and Batzer 1999, Wallace and Anderson 1996, Battle and Golladay 2001, Euliss and Mushet 1999). Current research (C. Lane, *unpublished research*), including resampling studied wetlands across years and various seasons, may assist in understanding these relationships and their effect on biological assessment.

Macroinvertebrate identification and enumeration

Taxonomic resolution. Macroinvertebrates were identified to the lowest possible taxon, with 82% being identified to genus. Incomplete specimens, early instars, or lack of diagnostic features hampered identification to species level. With only 48% of the organisms encountered identified to species, a substantial amount of information was lost from the inability to identify the organism to a lower phylogenic level. For instance, Bailey et al. (2001) reported that there was substantial variation in the response of organisms at the family, genus, and species level to perturbations. Resh and Unzicker (1975) reported that assessment analyses conducted at the species level differed from results obtained when the data were analyzed at the genus level. In the only analysis of taxonomic resolution in wetland macroinvertebrate data to date, King and Richardson (2002) found that metrics consisting of family-level indicators were inferior in their environmental relationship to genus and species level indicators. However, multivariate analyses completed by Bowman and Bailey (1998) and Furse et al. (1984) indicated that the difference between lentic study sites in ordinate space was very similar between

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genus and order levels, indicating that they both reflect similar site information. Many state wetland bioassessment programs incorporate analyses at the family level (Montana, Ohio, Minnesota, cited in U.S. EPA 2002c) or order level (Maine, Minnesota, Montana, Ohio, cited in U.S. EPA 2002c). Often, these programs are trying to categorize aquatic systems as impaired or unimpaired, or as good, fair, or poor, and not attempting to delve into the specific impairments. Using a coarse taxonomic resolution appears to permit such statements but may prevent elucidation of a more detailed assessment regarding the types of perturbations affecting a target aquatic system (Bailey et al. 2001).

Fixed count methodology. The Florida Department of Environmental Protection mandates fixed counts of approximately 100 organisms for both the Stream Condition Index (Barbour et al. 1996) and Lake Condition Index (Gerritsen et al. 2000). The organisms colleted in this study were identified by the FDEP Central Lab utilizing the same 100-count protocols as these indices. There has been substantial dialogue in the literature regarding the efficacy of fixed-count subsampling to 100 organisms in rapid assessment programs. Doberstein et al. (2000) agreed with Courtemanch (1996), Sovell and Vondracek (1999), and Karr and Chu (1999) that subsampling using a 100-organism fixed count results in substantial information loss in analyses of stream biota. In a study of wetlands of the Everglades, King and Richardson (2002) found that fixed counts of 200 did substantially better at determining impairment than counts of 100 organisms, but increases above 200 organisms resulted in no change in impairment detection ability. Doberstein et al. (2000) also commented that counting 100 organisms introduces within-site variability with subsampling replicates, and that the variance decreases with increasing subsample size.

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The results of this study suggest that fixed count sampling to approximately 100 organisms provides adequate information to characterize the fauna and to discern the relative quality of the sampled isolated depressional herbaceous wetlands. As the metrics proposed are abundance metrics, they are less affected by the increases in sampling effort due to expected isometric responses (Barbour and Gerritsen 1996). However, the sites sampled in this study were generally polarized along the disturbance gradient. Classification into two (or three) groups (such as good, fair, or poor) is generally reliable under such circumstances (Karr and Chu 1999, but see Doberstein et al. 2000, King and Richardson 2002). It may be more difficult to discern differences in mid-quality wetland assessments using fixed counts of 100 organisms (Doberstein et al. 2000), and more organisms or additional search methods (i.e., select for large, rare macroinvertebrates) may be warranted under such circumstances (Barbour et al. 1999).

Another criticism of fixed-count subsampling is that rare species are often overlooked (Sovell and Vondracek 1999, Cao et al. 2001, Bailey et al. 2001). This study did not analyze species-level information, which decreases the likelihood of rare taxa. Including species-level information may provide additional data from which metrics may be identified, however, additional costs and time are involved. The relationship between rare species and wetland characterization warrants additional attention.

Conclusions and Recommendations

Five attributes of macroinvertebrate community composition or structure were identified for use as biological indicators. The summed value of these five metrics, creating the Macroinvertebrate Index of Wetland Condition (MIWC), was correlated with the LDI gradient >0.75, although the precision and sensitivity of the metrics that comprise the MIWC was generally low. The macroinvertebrate composition of isolated

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systems in Florida was driven by variation in Landscape Development Intensity, soil pH, specific conductivity, and water TP. Specific actions to decrease the influence of these deterministic environmental variables in isolated wetland systems may result in wetland systems with community structure and function more similar to reference sites.

Additional information on the relationship of the macroinvertebrate community along a disturbance gradient may be gleaned from a more detailed taxonomic examination of the composite samples. Stronger correlations, including the development of optima may be ascertained from species-level studies, although generalizations could be made using genus-level (or perhaps higher, e.g., Hilsenhoff 1988). However, as previously mentioned, laboratory identification to species of certain families of macroinvertebrates can be laborious and difficult. Also, as diagnostic features may be temporally available, or development seasonally dependent, species identification may be fortuitous at best.

It is suggested, therefore, that additional sites be sampled and the temporal study window expanded to both increase the power of the analyses and to expand the applicability of the results. As these metrics were developed with genus-level data, increasing the sample size will necessarily increase the power of the analyses and also decrease variation in the data due to the relatively coarse level of identification. For instance, some taxa have species that are sensitive to, and species that are tolerant of, measured changes such as decreased dissolved oxygen (Barbour et al. 1999). Increasing sample size may assist in further elucidating trends between environmental variables of interest and macroinvertebrate community response. In addition, increasing sample size will likewise increase the likelihood of improved taxonomic resolution as laboratories

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become more familiar with taxa of isolated depressional wetlands and increase their reference collections. Increase in sample size may also permit the development of additional metrics or the exploration of different disturbances (e.g., urban systems).

Finally, as wetland macroinvertebrate community composition was also related to environmental gradients (such as TP, specific conductivity, pH, etc.), additional laboratory studies of the relationship between particular gradients (i.e., increases in pH) may be fruitful. Controlling for particular effects may further the knowledge of the response of wetland macroinvertebrates to anthropogenic disturbances and assist in identifying optima and other discursive and useful information on wetland macroinvertebrates autecology.

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CHAPTER 5
SUMMARY AND SYNTHESIS

Metric Summary

Twenty-four metrics were developed for the three assemblages sampled from isolated depressional herbaceous wetlands located throughout peninsular Florida: fourteen algal metrics, five macrophyte metrics, and five macroinvertebrate metrics. The metrics identified were comprised of compositional, trophic, and autecological attributes that varied with respect to changes in the development intensity within a 100m buffer immediately surrounding the study wetlands. Correlations between the three indices (Diatom Index of Wetland Condition, Vegetative Index of Wetland Condition, and Macroinvertebrate Index of Wetland Condition) and the measured Landscape Development Intensity index (LDI) were each greater than |0.65| and were highly significant ($p<0.001$), although lacking in sensitivity and precision.

Landscape Assessment and Index Development

An assumption inherent in this study is the direct relationship between the land use within the wetland basin and the composition and function of the wetland itself. This assumption was predicated on the use of landscape-scale characteristics obtained from the literature to quantify the level of disturbance within a system (Cuffney et al. 2000, Galatowitsch et al. 1999a). Landscape-scale measurements of human disturbance, such as the Landscape Development Intensity index (LDI), provided an independent assessment of possible deleterious inputs (e.g., phosphorous loading from agricultural operations) and alterations to the proper functioning of the wetland (e.g., changes in fire

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or hydrologic regime due to land use change). Landscape-scale measurements, when coupled with GIS analyses, also provided repeatable measurements when compared with subjective on-site procedures (e.g., Wetland Rapid Assessment Procedure, Miller and Gunsalus 1997).

The scale of the disturbance assessment may have had ramifications on the correlation coefficient of the measured ecological condition of the wetland sampled. For instance, the LDI does not incorporate slope, elevation, or direction of flow into the assessment. Thus, a system with a potentially disruptive land use (e.g., a dairy farm) within the 100m buffer but down hill would have the same score as a site with a dairy farm within the 100m buffer but uphill – despite the obvious advantages to being located uphill of such a potential perturbation. This landscape assessment artifact may be reflected in the high variance, low precision, and low sensitivity of the metric values for any given LDI score (i.e., Figure 2-6). Improvements to the LDI, such as incorporating the existence of buffers to flow (i.e., berms) or access (i.e., *Serenoa repens*) may improve the relationship between organismal response and landscape perturbation.

The Landscape Development Intensity index, as an agglomerative measure, incorporates and assigns disturbance coefficients to multiple land uses based on non-renewable fuel use (Brown and Vivas *submitted*). However, ecological responses to increases in non-renewable fuel use may not follow the rank order inherent within the LDI. For instance, agricultural practices generally use less non-renewable fuel per hectare than single family residential land use (Table 1-1). The pesticides and fertilizers used in agricultural practices, however, have a direct effect on the composition and functioning of wetland systems (Euliss and Mushet 1999). Single family residential land

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use may use pesticides and fertilizers, but likely at a much lower per hectare dosage – in other words the direct effect on ecosystems may not be as intense. Thus higher LDI scores may not be directly analogous to increased stressors on wetland assemblages. This may led to highly variable responses from metrics for any given LDI score (Figure 2-6).

The LDI was developed as an agglomerative index of energy intensity, but based on the lack of sensitivity and precision in these analyses, it appears that additional information germane to development intensity might improve the relationship between the biological data and the landscape data. For instance, improvements to the development index could come from separating out the effect of altered hydrology from other values due to its relative importance to wetlands (Mitsch and Gosselink 2000). In the current LDI, hydrological alterations are impossible to identify due to the methodology inherent within the LDI (Brown and Vivas *submitted*).

Additional improvements to the fit of the biological data could be made through multiple regression. Combining land use data with compositional data could decrease the variance within the dataset through parsimonious selection of variables that necessarily improve the fit (Zar 1999).

With two exceptions (mean Coefficient of Conservatism and Annual to Perennial ratio), metrics identified in this analysis are based on the abundance of various compositional, functional, or trophic assemblages. Data are constrained (Aitchison 1986) due to the abundance values necessarily ranging from 0% - 100%. The use of constrained data in developing the three indices of wetland condition may be implicated in the highly variable empirical scores for metrics along increasing LDI values. Membership in one group (e.g., % Orthocladiinae) may be inversely related to

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membership in another group (e.g., % Tolerant). For instance, a high abundance of Orthocladiinae in any given sample necessitates a lower abundance of tolerant organisms, as the non-Orthocladiinae portion of the sample would be small (i.e., 100% - Orthocladiinae % = remaining sample for categorizing). Thus, some metric values may be lower than anticipated for any given LDI score not as a function of the surrounding land use but due to the effects of constrained data.

In summary, as a general, landscape level development intensity index, the LDI was significantly correlated with each of the metric developed, although the sensitivity and precision were low. However, the LDI remains a useful relative indicator of wetland condition despite the relatively low resolution as conclusions may be drawn regarding the expected landscape relationship to down-slope wetlands. In addition, the LDI, compared to field work, provides a relatively simple assessment for a minor expenditure of effort and money. In addition, the use and acceptability of GIS in landscape assessment is increasing as the number of available coverages and GIS applications continues to increase (Cuffney et al. 2000).

Environmental Variables Driving Wetland Composition Across Assemblages

Soil and water samples from wetlands in reference and developed landscapes were shown to be significantly different for the following non-collinear environmental variables: soil pH, soil TP, specific conductivity, ammonia, and water TP. Dimension-reducing ordination with non-metric multidimensional scaling (NMDS) and subsequent correlations of environmental variables with ordination scores indicated that for algal, macrophyte, and macroinvertebrate assemblages, LDI score, soil pH, specific conductivity, and water TP were important components driving wetland composition.

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Latitude and water color were also correlated with ordination scores for macrophytes and macroinvertebrates, respectively.

While the relationship of each assemblage with the four (or five, in the case of macrophytes and macroinvertebrates) environmental variables was discussed, the strong correlation of the composition of these three very different assemblages with the variables LDI, soil pH, specific conductivity, and water TP warrants additional attention. The four driving environmental variables were measures that acted on various scales. LDI was a coarse measurement of human landscape modification. Specific conductivity and water TP were finer-scale measurements that typically emanated from the interaction of precipitation and constituent mobilization and runoff into the wetland system. These two variables may also have been modified by human landscape alterations such that irrigation, rather than natural rainfall, may have played a role in either mobilizing the constituents to the wetland or in increasing the specific conductivity of the receiving water body (Fore and Grafe 2002). Soil pH, another environmental variable operating on a finer scale, would be expected to be less reactive to landscape modifications since direct "pH loading" generally does not occur. However, agrochemicals are typically more basic, thus runoff and transportation of agrochemicals into receiving water bodies such as wetlands may affect not only the nutrient levels in the receiving waters, but also the pH of the system (Fore and Grafe 2002).

Since the LDI was a general landscape modification measurement, it was expected that wetland species composition would be correlated with changes in the LDI across each assemblage. The results suggested that the LDI was able to couple the disparate effects of human landscape modification, such as altered hydrology (strongly

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affecting diatoms and macroinvertebrates) or trampling/selective herbivory (strongly affecting macrophytes), into a single value, which was assumed to be manifested by changes in assemblage composition.

Phosphorous in the system would be expected to increase metabolic rates throughout the aquatic system. Thus, diatoms and macrophytes would increase production due to increased availability of a typically limiting nutrient, and macroinvertebrates would likewise increase in abundance as additional resources would be made available from plant production and subsequent decay. Increases in nutrient levels, however, can have deleterious impacts on wetland systems that can cascade through the various assemblages. For instance, increased nutrient loading can affect dissolved oxygen levels with the water body as chemical and biological oxygen demand may surpass available oxygen levels. This would drastically affect the three assemblages sampled (as well as most other organisms within the system). Chemical reactions to anoxia, such as the release of bound phosphorous from the soil, could further alter community composition.

Specific conductivity and soil pH directly affect diatom community composition, generally through decreases in bio-available constituents (Pan and Stevenson 1996, U.S. EPA 2002e). Compositional changes related to either variable were generally less pronounced for macroinvertebrates (Spieles and Mitsch 2000) and macrophytes (Adamus et al. 2001). As diatoms are an important component of macroinvertebrate food webs, alterations to the macroinvertebrate food base may be manifested through compositional changes of the macroinvertebrate assemblage. Macrophytes are affected minimally by alterations in specific conductivity and pH (Danielson 1998, Adamus et al. 2001),

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although changes in these constituents may often indicate increased nutrient loading or groundwater irrigation (and associated landscape changes).

Environmental Variables and Management Concerns

Four environmental variables (LDI, soil pH, specific conductivity, and water TP) were strongly correlated with the community composition of diatoms, macrophytes, and macroinvertebrates of isolated herbaceous depressional wetlands of peninsular Florida. As the assemblages sampled represent primary, secondary, and tertiary trophic classes, structural components of the wetland itself, and a portion of the landscape food web (i.e., food source for passerines and wading birds, reptiles and amphibians, small mammals, etc.), changes in wetland management to decrease the effect of the controlling environmental variables would be advantageous to meeting the goals of the Clean Water Act. To ameliorate the impact of human landscape modification on wetland systems, it is hypothesized that creating buffer zones around wetlands would improve the community composition (i.e., the system would become more similar to wetlands in reference conditions) and improve biotic integrity. Buffer zones emanating from the wetland/upland boundary would likely improve wetland water quality through particle retention and nutrient uptake by upland/facultative plants of the buffer zone. Preventing domestic herbivores from grazing in the wetland through fencing or shrubbery (e.g., *Serenoa repens*–saw palmetto) would decrease damage from selective herbivory, as well as decrease nutrient loading from cattle wastes. Specific conductivity may decrease as a result of constituent uptake by buffer vegetation and immobilization of micro-constituents of the overland or subsurface flow. Soil pH in the wetland, considered a function of agrochemical loading, may also decrease as a result of wetland buffers as

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agrochemicals of the overland flow could be assimilated by buffer vegetation, and agrochemicals adsorbed onto soil particles would be sequestered in buffer vegetation.

Conclusions and Recommendations

Isolated, depressional wetlands provide many functions to the landscape and to the State of Florida. However, their spatial location, generally smaller size, and irregular or patchy distribution on the landscape have contributed to their filling or destruction such that they have been greatly reduced in number (Kirkman et al. 1999). Those that remain, if in developed landscapes, often have decreased functionality and integrity (*sensu* Karr 1981) due to deleterious anthropogenic inputs or landscape activities. To attempt to meet the goals of the Clean Water Act, which include maintaining the chemical, physical, and biological integrity of wetland systems, methods to assess the relative condition of these systems had to be developed. Once the relative condition was known, efforts to restore wetland systems could be undertaken.

In this study, methods were developed to assess the relative condition of isolated herbaceous wetland systems in peninsular Florida using endemic wetland flora and fauna. Seventy-five isolated wetlands were sampled throughout peninsular Florida, and twenty-four metrics were developed from the algal, macrophyte, and macroinvertebrate assemblages identified. Individual metrics were summed for each of the assemblages and three indices developed: the Diatom Index of Wetland Condition, the Macrophyte Index of Wetland Condition, and the Macroinvertebrate Index of Wetland Condition. Correlations of these three indices with the Landscape Development Intensity index, an independent, GIS analysis of human landscape modification were significant and $>|0.65|$ (Spearman's r).

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In addition to developing biometrics for the three assemblages, environmental variables responsible for algal, macrophyte, and macroinvertebrate community composition were discerned. The parameters responsible, identified through site ordination, were of both coarse (LDI scores, and latitude for macrophytes) and finer scales (soil pH, water TP, and specific conductivity, as well as water color for macroinvertebrates). Efforts to decrease the impact of these inputs on wetland community composition through the development of wetland buffers were proffered.

Due to the highly variable and low sensitivity of the LDI vis-à-vis the twenty-four metrics identified, efforts to improve the goodness of fit between the LDI and the biological data are warranted. Significant correlations were identified, however improvements in the correlation may be obtained through modifying the LDI (e.g., revising calculations) or changing the scale of the landscape analyses to include hydrologic alterations (currently not an itemized perturbation). An additional method to improve the fit of the data would through multiple regression analyses of sampled data and land use (before combined into the LDI).

Recommendations for future work following these analyses are of two veins: validation (expanding the current *n* to compare and contrast how additional sites relate to the sites used to develop these indices) and calibration (amending the metrics developed from these analyses based on the results of the validation and the larger *n*), and expansion. Revisiting sites, increasing the dataset through the addition of new sites, and reevaluating the metrics developed based on the additional sampling effort are the hallmarks of a thorough validation and calibration program. An *n* of 75, while statistically satisfying, should be increased to capture the breadth and depth of the

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wetland systems of peninsular Florida more thoroughly. An expansion of this research into other impairments and marshes of varying size and location (i.e., the Florida panhandle) is also recommended. Impairments of different intensity, such as industrial or urban development, may be manifested by markedly different attributes in isolated wetlands located in those landscape matrices. Finally, including additional assemblages, such as reptiles/amphibians, soil microbes, or birds, could increase the information obtained from the wetland systems and perhaps permit a deeper understanding of the relationship between stressors, sources of stress, and the manifestation of exogenous perturbations on the flora and fauna, and ultimately the integrity itself, of isolated depressional wetlands throughout Florida.

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APPENDIX A
SITE DESCRIPTIONS

Seventy-five sites were sampled throughout peninsular Florida. Access to many sites was obtained through cooperation with landowners and was often contingent on site anonymity. Coordinates for sites sampled, exclusive of the anonymous sites, are given below (Table A-1), as are Landscape Development Intensity (LDI) scores. Additional information on LDI scores may be found in Brown and Vivas (*submitted*).

Table A-1. Site coordinates and LDI score.

Site	LDI	Latitude	Longitude
ALPaynes	1.38	29.60210	-82.23563
Audobon	3.54	28.11827	-82.11629
Bear Scat	1.18	26.18502	-81.25442
Big Cow	3.58	26.23075	-81.26520
BRSebastian	1.09	27.83263	-80.58833
CabPatch	3.54	29.43420	-81.34030
Caravelle	3.54	29.51767	-81.72119
Chuluota	1.00	28.61731	-81.05781
CLBayard	1.07	29.96117	-81.61504
CLCove	3.54	29.95187	-81.66341
CMWPast	2.14	26.85486	-81.78479
CMWRef	1.62	26.94567	-81.84494
COBurgle	3.36	n/a	n/a
COHole	1.11	26.02437	-81.26467
CREW	1.78	26.48973	-81.53834
Deerfly	2.39	29.17017	-81.62333
DEMelon	4.20	n/a	n/a
GarberRanch	2.75	27.21615	-82.15127
GBarE	3.38	n/a	n/a
GLDonut	3.59	n/a	n/a
GLPont	3.29	n/a	n/a
Goethe	1.52	29.16072	-82.59969
GreenSwamp	1.60	28.35458	-82.01761
HagueI	4.12	29.78267	-82.41186
HagueII	5.63	29.78350	-82.41133
Half Moon	1.99	28.91872	-82.25719
HARare	3.58	n/a	n/a
HEBad	3.49	26.30340	-81.24560
HEL2	3.43	n/a	n/a
HEOkay	1.05	26.59035	-81.32581
HighPast	3.71	27.42338	-81.56088
HighRef	1	27.48100	-81.55022
HillsRef	1.23	28.14610	-82.23110
Hunt Camp	1	29.32275	-81.74680
IFASI	2.25	29.72395	-82.40680
IFASII	1.84	29.72678	-82.40619
Immokalee	2.6	26.46316	-81.44605
IRBlueCypress	3.58	n/a	n/a
IRCanal	3.59	n/a	n/a
IROJ	3.68	n/a	n/a
JD6	1.24	27.00147	-80.14629
Kelly Park	3.65	28.79637	-81.43891
LCork	1	26.47915	-81.54871
Lego	1	29.13782	-82.63217
LESuwan	2.12	29.31693	-83.04337
LLeecounty	1.02	26.70824	-81.65356
MALudy	3.5	n/a	n/a
MASpray	3.8	n/a	n/a
McArthur	3.75	27.16536	-81.19631
MNElmer	3.13	n/a	n/a
MNErik*	3.06	n/a	n/a
MNOcala	1.58	29.10181	-81.89731

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Table A-1. Continued.

Site	LDI	Latitude	Longitude
MRPepper	4.54	n/a	n/a
Myakka	1.25	27.23684	-82.33043
OKCara	4.79	n/a	n/a
OKKiss	1.20	27.55004	-81.00910
OKPast	3.58	27.59890	-81.03172
PacificTom	4.54	26.52025	-81.67369
PallMar	1.00	26.94969	-80.31057
PBCorbett	1.13	26.94206	-80.36113
PBEnjay	1.00	26.95468	-80.18508
Penner	1.27	29.49614	-81.82489
POWales	1.26	27.66359	-81.42379
POWeowak	1.53	27.77768	-81.44651
PUPond*	1.42	29.71649	-81.96575
RiceCreek	1.88	29.68086	-81.74208
Sandhillcrane	3.10	26.86677	-80.21878
SANorthmya	1.20	27.27050	-82.25933
SAOscer	1.12	27.19536	-82.45383
Savannas	1.13	27.30214	-80.27348
STCow	3.94	n/a	n/a
SUVaca*	3.17	28.91047	-82.25994
SUWarhol*	3.66	n/a	n/a
UNHealthy	3.9	30.04499	-82.17451
Weikiva	1.00	28.73703	-81.48396

Note: Latitude and longitude positions marked with "n/a" were accessed through agreements with anonymous landowners.

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APPENDIX B
SOIL AND WATER SAMPLING METHODS AND RESULTS

To relate the distribution of sampled communities to environmental parameters, soil and water physical and chemical constituents were measured (Tables B-1 and B-2, respectively). Soil chemistry samples were obtained at each site and the following parameters determined in the lab: total phosphorous (soil TP, mg/kg), percent total nitrogen (%TN), percent total carbon (%TC), percent organic matter (%OM), and pH. For soil samples the study wetland was separated in to dominant vegetation zones and four soil cores were proportionally distributed within the dominant vegetation zones (e.g., if 75% of the wetland was *Panicum hemitomon* and 25% *Pontederia cordata*, 3 samples would be taken in the *Panicum* zone and 1 sample in the *Pontederia* zone). The cores were taken with an approximately 8cm inner-diameter beveled PVC pipe. At each sample location, the duff layer was removed and the PVC pipe outline traced with a 15cm knife to reduce sample compaction. The PVC pipe was pounded into the wetland soil to an approximate depth of 10cm and extracted using a trowel. The surficial water was gently poured from the top, and the 10cm sample extruded. Samples from the same zone were mixed and a composite sample of approximately one liter scooped into a labeled container. All samples were placed on ice in the field and stored in a laboratory refrigerator until analyzed by the University of Florida Soil Science Department. In the lab, pH readings were taken and subsamples were dried to a constant weight and finely ground (<0.2 mm). Percent C and N were obtained from the ground samples utilizing a Carlo-Erba NA-1500 CNS Analyzer (Haak-Buchler Instruments, Saddlebrook, NJ).

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Total P analysis was performed by ashing additional subsamples at 550°C for 4 hours, dissolving the dried sample in 6M HCl (Anderson 1976), and analyzing the digestate for P following U.S. EPA method 365.4 (U.S. EPA 1983). Percent organic matter was obtained during the analysis of the total P. For subsequent site-specific analyses, a weighted average for each soil constituent was obtained by utilizing the percent extent of each wetland vegetation zone sampled.

Samples for analysis of water chemistry parameters were collected at each site. Water samples for nutrient analysis were taken from the center of the wetland at an approximate depth of 10cm with a 0.5L pre-rinsed Polypropylene bottle and preserved with 2mL 1:1 H_2SO_4. The same method was used for chemical analysis (color, specific conductivity, turbidity, and pH) samples, with the exception that no preservative was added. Both bottles were placed on ice and delivered overnight to the FDEP Central Laboratories for processing. The following parameters were measured by FDEP following FDEP protocols (SOP #TA-06.04-5): color (PCU), specific conductivity (umhos/cm), turbidity (NTU), pH, ammonia (mg/L), nitrate/nitrite (mg/L), total Kjeldahl Nitrogen (TKN, mg/L), and total Phosphorus (water TP, mg/L). In the event that the constituent measured was below detection levels, the value entered for analysis was half of the lowest detectable level.

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Table B-1. Soil characteristics measured at each study site.

Sites	pH	% TC	% OM	% TN	TP (mg/kg)
ALPaynes	3.82	42.73	3.00	787.40	83.91
Audubon	4.25	9.11	16.48	0.72	659.83
BearScat	7.12	5.75	10.76	0.53	81.16
BigCow	6.87	4.09	6.35	0.39	67.82
BRSebastian	4.12	9.71	19.58	0.50	99.78
CabPatch	5.66	7.46	15.71	0.52	321.64
Caravelle	4.75	11.98	24.32	0.64	251.89
Chuluota	3.90	19.34	36.63	1.17	223.45
CLBayard	3.65	19.07	34.94	0.88	16.36
CLCove	5.52	10.66	22.87	0.78	591.44
CMWPast	5.91	1.93	4.53	0.12	46.94
CMWRef	5.33	2.10	3.97	0.12	53.65
COBurgle	6.29	17.95	29.32	1.38	6.48
COHole	7.35	11.90	14.66	0.72	137.28
Crew	5.28	3.91	9.75	0.24	59.30
DEMelon	5.66	6.47	11.67	0.46	202.29
Deerfly	4.79	46.55	83.95	2.68	340.87
Garber	5.13	16.52	31.34	0.94	394.21
GBarE	3.98	48.07	3.19	371.79	91.88
GLDonut	4.90	9.43	19.39	0.68	73.36
GLPont	5.22	17.01	33.33	1.14	292.14
Goethe	4.09	14.42	26.73	0.88	200.91
GreenSwamp	4.60	13.83	25.09	0.87	372.60
HaRare	6.18	11.43	20.66	0.79	144.37
HagueI	5.51	7.22	98.80	0.49	969.13
HagueII	6.28	3.47	7.46	0.23	803.59
HalfMoon	4.11	15.91	29.18	1.07	95.75
HEBad	6.42	10.53	17.63	0.82	470.63
HEL2	6.53	22.57	49.44	1.81	418.91
HEOkay	5.10	21.02	5.03	1.43	37.16
Highpast	5.01	3.31	6.13	0.23	105.21
HighRef	4.26	8.81	18.83	0.56	325.15
HillsRef	5.13	10.21	20.73	0.57	185.93
HuntCamp	4.55	34.83	57.02	2.53	271.52
IFASI	4.39	49.08	96.15	3.42	649.48
IFASII	4.60	37.62	72.61	2.75	1011.63
Immokalee	4.47	8.53	14.94	0.50	145.91
IRBlueCypress	5.67	17.56	22.43	1.34	322.85

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Table B-1. Continued.

Sites	pH	% TC	% OM	% TN	TP (mg/kg)
IRCanal	5.95	15.63	29.15	1.03	521.02
IROJ	6.09	8.46	15.85	0.68	317.99
JD6	4.92	3.05	7.77	0.18	25.93
KellyPark	3.91	49.45	89.95	3.33	460.89
LCork	6.35	0.77	4.35	0.06	53.64
LLeeCounty	5.30	7.55	12.54	0.57	138.68
LEGo	4.23	9.87	11.03	0.67	110.37
LESuwan	5.02	6.73	11.17	0.41	77.94
MALudy	5.53	5.42	9.18	0.34	209.99
MASpray	4.15	25.72	11.58	1.41	45.99
McArthur	4.71	16.10	28.67	1.07	300.08
MNElmer	4.23	26.59	54.12	1.37	790.86
MNErik	3.98	48.07	3.19	371.79	91.88
MNOcala	4.52	11.86	22.51	0.81	166.61
MRPepper	4.83	48.02	93.75	2.72	492.98
Myakka	4.56	7.62	13.15	0.42	42.16
OKCara	5.76	4.18	7.69	0.30	58.68
OKKiss	4.85	3.10	9.21	0.19	77.80
OKPast	6.10	2.46	5.77	0.17	67.25
PacificTom	5.98	0.98	4.83	0.06	32.54
PallMar	5.50	6.37	11.36	0.37	40.47
PBCorbett	5.82	5.42	13.24	0.25	262.93
PBEnjay	4.78	2.81	5.37	0.20	42.87
Penner	4.75	8.30	9.88	0.46	91.55
POWales	4.22	5.02	7.96	0.32	73.44
POWeowak	5.06	11.73	21.32	0.87	312.90
PUPond	3.82	42.73	3.00	787.40	83.91
RiceCreek	4.31	14.12	27.89	0.84	478.98
SANorthMya	4.88	3.53	4.34	0.26	53.16
SAOscer	4.89	3.31	4.94	0.29	55.46
SandhillCrane	4.80	3.05	8.67	0.20	61.06
Savannas	4.66	7.33	13.08	0.37	42.67
STCow	5.55	13.77	25.17	1.01	393.92
SUVaca	3.98	48.07	3.19	371.79	91.88
SUWarhol	3.98	48.07	3.19	371.79	91.88
UNHealthy	6.63	3.14	8.74	0.21	261.10
Weikiva	3.38	45.02	85.24	2.16	518.94

Note: Average values of impaired site soil parameters were given to site GBarE as access for soil sampling was not available.

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Table B-2. Physical/Chemical water parameters.

Site	Color (PCU)	Specific Conductivity (umhos/cm)	Turbidity (NTU)	pH	Ammonia (mg/L)	Nitrates/ Nitrites (mg/L)	TKN (mg/L)	TP (mg/L)
ALPaynes†	281.5	76.8	2.08	4.92	0.112	0.007	1.73	0.0434
Audubon	500	64.0	12.00	5.41	0.170	0.007	3.90	0.3500
BearScat	40	350.0	1.40	7.66	0.013	0.002*	0.41	0.0190
BigCow	100	180.0	2.10	7.22	0.021	0.002*	1.00	0.0380
BRSebastian	140	45.0	0.40	4.25	0.015	0.002*	0.99	0.0075*
CabPatch	800	210.0	0.70	6.46	0.027	0.008	3.10	0.5500
Caravelle	3000	61.0	320.00	5.09	1.400	0.002*	6.50	1.1000
Chuluota	250	39.0	1.10	4.36	0.017	0.008	1.20	0.0500
CLBayard	400	67.0	10.00	4.12	0.082	0.002	2.80	0.1000
CLCove	500	100.0	16.00	6.10	0.093	0.002*	7.30	0.5400
CMWPast	100	130.0	2.00	7.33	0.023	0.002*	1.50	0.0190
CMWRef	400	22.0	6.60	5.47	0.028	0.011	1.30	0.0380
COBurgle	250	100.0	1.80	6.89	0.026	0.002*	2.20	0.1650
COHole	30	410.0	0.55	7.72	0.013	0.002*	0.48	0.0075*
Crew	100	17.0	0.75	5.40	0.013	0.002*	0.76	0.0230
DEMelon	200	230.0	1.80	6.69	0.031	0.002*	2.00	0.0910
Deerfly	150	46.0	1.30	4.75	0.024	0.002*	1.10	0.0250
Garber	500	42.0	2.10	5.91	0.027	0.002*	2.40	0.0450
GLDonut	200	170.0	2.90	6.30	0.070	0.002*	1.70	0.1600
GLPont	200	270.0	1.60	7.05	0.036	0.012	1.50	0.0580
Goethe	200	31.0	3.80	4.57	0.018	0.002*	1.90	0.0800
GreenSwamp	400	40.0	1.40	4.47	0.013	0.002*	1.70	0.0240
HaRare	300	300.0	1.90	6.68	0.041	0.002*	3.00	2.9000
HagueI	300	310.0	2.70	6.55	0.050	0.006	2.60	2.4000
HagueII	8000	1100.0	5600.00	7.09	48.000	0.011	110.00	45.0000
HalfMoon	120	46.0	0.45	5.01	0.024	0.002*	1.40	0.0250
HEBad	400	350.0	0.75	7.80	0.017	0.002*	1.20	1.6000
HEL2	300	155.0	1.95	6.16	0.046	0.002*	4.20	0.2600
HEOkay	200	96.0	0.95	5.83	0.019	0.002*	2.20	0.0230
Highpast	300	22.0	2.40	5.56	0.036	0.002*	2.50	0.1400
HighRef	600	53.0	2.00	4.31	0.014	0.002*	2.10	0.0730
HillsRef	300	34.0	2.40	4.90	0.016	0.015	1.70	0.0270
HuntCamp	400	47.0	2.70	5.32	2.600	0.002*	5.90	0.0390
IFASI	500	68.0	1.50	4.62	2.200	0.002*	6.00	0.0730
IFASII	300	40.0	1.40	4.95	0.033	0.002*	2.50	0.0690
Immokalee	150	18.0	2.30	5.17	0.014	0.002*	0.79	0.0280
IRBlueCypress	200	170.0	0.95	5.87	0.005*	0.002*	1.40	0.0640
IRCanal	150	1400.0	1.00	7.20	0.017	0.002*	1.50	0.0400
IROJ	150	230.0	3.40	7.75	0.021	0.002*	1.70	0.0350
JD6	150	32.0	0.70	4.69	0.018	0.002*	0.95	0.0075*
KellyPark	600	99.0	2.40	5.68	0.150	0.008	4.40	0.2200

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Table B-2. Continued.

Site	Color (PCU)	Specific Conductivity (umhos/cm)	Turbidity (NTU)	pH	Ammonia (mg/L)	Nitrates/Nitrites (mg/L)	TKN (mg/L)	TP (mg/L)
LCork	100	120.0	3.90	6.57	0.081	0.002*	1.50	0.0370
LLeeCounty	500	99.0	1.00	6.00	0.023	0.002*	2.90	0.0320
LeGo	200	30.0	0.80	4.31	0.018	0.002*	2.20	0.0450
LeSuwan	300	92.0	0.40	5.90	0.027	0.002*	3.30	0.0270
MaLudy	300	370.0	0.85	6.75	0.042	0.002*	1.70	1.0000
MaSpray	300	35.0	0.65	4.13	0.019	0.002*	1.50	0.0280
McArthur	600	170.0	6.00	5.55	0.022	0.002*	3.90	2.8000
MnElmer	700	58.0	1.10	5.58	0.080	0.002*	1.80	0.0880
MnErik†	610.8	225.3	162.86	6.17	1.437	0.003	5.57	1.6819
MnOcala	300	77.0	4.40	4.88	0.083	0.002*	3.30	0.0430
MrPepper	250	180.0	1.20	5.12	0.023	0.002*	2.20	0.1700
Myakka	200	79.0	2.00	4.57	0.020	0.002*	1.20	0.0430
OKCara	500	240.0	2.00	6.03	0.025	0.002*	2.60	0.4900
OKKiss	500	55.0	0.50	4.75	0.330	0.002*	2.30	0.0075*
OKPast	600	140.0	16.00	6.54	0.220	0.002*	5.40	0.4200
PacificTom	200	69.0	2.50	6.18	0.012	0.002*	1.70	0.1800
PallMar	200	64.0	3.70	4.18	0.041	0.091	1.20	0.0270
PBCorbett	60	110.0	0.55	7.05	0.038	0.002*	2.30	0.0075*
PbEnjay	50	21.0	1.60	4.58	0.015	0.002*	0.83	0.0075*
Penner	150	29.0	2.30	4.36	0.020	0.002*	0.96	0.0330
PoWales	200	40.0	0.75	4.21	0.016	0.002*	1.50	0.0260
PoWeowak	100	58.0	0.65	5.35	0.005*	0.009	0.67	0.0075*
PuPond†	281.5	76.8	2.08	4.92	0.112	0.007	1.73	0.0434
RiceCreek	300	99.0	0.90	4.10	0.015	0.010	1.60	0.3000
SaNorthMya	200	34.0	1.40	4.50	0.021	0.008	1.30	0.0270
SaOscer	500	46.0	0.75	4.76	0.027	0.002*	1.90	0.0480
SandhillCrane	100	31.0	0.40	5.29	0.015	0.002*	0.73	0.0075*
Savannas	500	13.0	4.60	4.69	0.005*	0.002*	1.00	0.0075*
STCow	400	590.0	6.90	6.60	0.043	0.002*	3.40	0.4400
SuVaca†	610.8	225.3	162.86	6.17	1.437	0.003	5.57	1.6819
SuWarhol†	610.8	225.3	162.86	6.17	1.437	0.003	5.57	1.6819
UnHealthy	200	470.0	1.30	7.14	0.062	0.006	2.80	0.5300
Weikiva	1200	190.0	2.10	3.43	0.005*	0.010	2.20	0.1200

Notes: Values for sites marked with an asterisk (*) were below detection limits and the value given is half the detection limit. Sites marked with (†)were sampled while dry and average values of reference wetlands were given to AlPaynes and PuPond, while average values for impaired wetlands were given to MnErik, SuVaca, and SuWarhol. Average pH for reference sites was given for site Savannas due to a measurement error.

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APPENDIX C
DIATOM AUTECOLOGICAL VALUES

Autecological values (Bahls 1993, Van Dam et al. 1994) for the epiphytic diatoms are given below. For metric development, the values were recoded as given in Table 2-19.

Table C-1. Diatom Autecological Values.

Site	pH	Salin.	Nitro.	D.O.	Sapro,	Troph.	Poll.
Achnanthes exilis	5	2	1	1	1	2	3
Achnanthes hungarica	4	2	2	4	3	6	3
Achnanthes minutissima	3	2	2	1	2	7	3
Achnanthes minutissima scotica	3	2					
Amphora veneta	5	3	2	3	4	5	2
Anomoeneis serians	1	1	1	2	1	1	
Anomoeneis vitrea	4	2	1	2	1	2	
Asterionella formosa	4	2	2	2	2	4	3
Aulacoseira alpigena	2	1	1	1	1	1	3
Aulacoseira crenulata	3	1	1	1	1	1	
Aulacoseira granulata	4	2	2	3	2	5	3
Caloneis bacillum	4	2	1	2	2	4	2
Cocconeis placentula lineata	4	2	2	3	2	5	3
Cyclotella meneghiniana	4	3	3	5	4	5	2
Cyclotella pseudostelligera	3	2	2	3	3	5	2
Cymbella cesatii	3	1	1	1	1	1	3
Cymbella gracilis	2	1	1	1	1	2	3
Cymbella microcephala	4	2	1	1	1	4	2
Cymbella minuta	3	2					2
Diploneis parma							
Eunotia bilunaris	6	2	2	2	2	7	
Eunotia bilunaris mucophila	2	2	2	2	1	2	
Eunotia circumborealis		1					
Eunotia faba	2	1	1	1	1	2	
Eunotia fallax	2	1	1	1	1	1	
Eunotia flexuosa	2	1	1	1	1	2	3
Eunotia formica	2	2	1	1	1	3	
Eunotia glacialis	2	1	1	1	1	2	3
Eunotia implicata	2	1					
Eunotia incisa	2	1	1	1	1	1	3

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Table C-1. Continued.

Site	pH	Salin.	Nitro.	D.O.	Sapro,	Troph.	Poll.
Eunotia monodon	2	1	1	1	1	1	3
Eunotia monodon bidens	2	1	1	1	1	1	
Eunotia naegelii	2	1	1	1	1	1	
Eunotia paludosa	1	1	1	1	1	1	
Eunotia paludosa trinacria	1	1	1	1	1	1	
Eunotia pectinalis	2	1	2	1	2	3	3
Eunotia pectinalis undulata	2	1	2	1	2	1	3
Eunotia pirla							
Eunotia praerupta	2	1	1	1	1	2	3
Eunotia rhomboidea	2	1	1	1	1	1	
Eunotia soleirolii	3	1	2	1	2	1	
Eunotia veneris	2	1	1	1	1	2	
Fragilaria vaucheriae	4	2	2	3	3	5	2
Frustulia rhomboides	2	1	1	1	1	1	3
Frustulia rhomboides amphipleuroides	2	1	1	1	1	1	3
Frustulia rhomboides crassinerva	2	1	1	1	1	1	
Frustulia rhomboides saxonica	1	1	1	1	1	1	3
Gomphonema affine	4	2	1	1	2	3	3
Gomphonema angustatum							2
Gomphonema augur turris							
Gomphonema clavatum	3	1	1	1	1	4	2
Gomphonema gracile	3	2	1	1	1	3	2
Gomphonema parvulum	3	2	3	4	4	5	1
Gomphonema pseudotenellum							
Hantzschia amphioxys	3	2	2	2	3	7	2
Mastogloia smithii	4	4			2		2
Navicula accommoda	4	2	4	5	5	6	
Navicula arvensis	4	2	3	4	4	5	2
Navicula bicephala							3
Navicula clementis	4	3	2	1	2	4	2
Navicula cohnii	4	3	2	1	2	5	2
Navicula confervacea	3	3	3	3	3	5	2
Navicula cryptocephala	3	2	2	3	3	7	3
Navicula cryptotenella	4	2			2	7	2
Navicula detenta							3
Navicula erifuga	4	3				5	2
Navicula evanida	3	2	1	1	1	3	
Navicula festiva	1	1	1	1	1	1	2
Navicula hambergi	2			1			
Navicula kotschyi	4	2		1	1		2
Navicula kriegerii							
Navicula laevissima	3	1	1	1	1	3	3
Navicula mediocris	2	1	1	1	1	1	
Navicula minima	4	2	3	4	4	5	1
Navicula molestiformis	4	2	3	4	4	5	1

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Table C-1. Continued.

Site	pH	Salin.	Nitro.	D.O.	Sapro,	Troph.	Poll.
Navicula mutica	3	3	2	1	3	5	2
Navicula notha	2	1	1	1	1	2	2
Navicula pseudoventralis	4	2	1	1	1	2	2
Navicula pupula	3	2	2	3	3	4	2
Navicula pupula elliptica	4	2				4	2
Navicula pupula mutata	3	2			2	2	2
Navicula pupula rectangularis	3	2	2	3	3	4	2
Navicula pygmaea	5	3	3	3	3	5	2
Navicula radiosa	3	2	2	2	2	4	3
Navicula schadei	3	2	1	1	1	3	
Navicula seminulum	3	2	3	4	4	5	1
Navicula subtilissima	1	1	1	1	1	1	2
Navicula tenelloides	4	2	1	1	1	5	1
Navicula trivialis	4	3	2	3	3	5	2
Navicula viridula rostellata	4	2	2	2	2	5	2
Neidium alpinum	2	1			1	1	3
Neidium bisulcatum	3	1	1	1	1	1	
Neidium hercynicum	2	2					3
Neidium javanicum							
Nitzschia acidoclinata	3	1	1	1	2	3	3
Nitzschia agnita		4					1
Nitzschia amphibia	4	2	3	3	3	5	2
Nitzschia brevissima	3	3		3	2	5	2
Nitzschia capitellata	4	4			5	6	2
Nitzschia debilis	4	2	2	1	3		
Nitzschia dissipata	4	2	2	2	2	4	3
Nitzschia frustulum	4	3	4	3	2	5	2
Nitzschia gracilis	3	1		2	2	3	2
Nitzschia intermedia	3	2			2	5	3
Nitzschia microcephala	4	2	4	3	3	5	1
Nitzschia nana	3	2		1	2	3	
Nitzschia palea	3	2	4	4	5	6	1
Nitzschia palea debilis	3	1			1	1	
Nitzschia palea tenuirostris	3	2					1
Nitzschia paleaformis	1	2	2	2	2	7	
Nitzschia perminuta	4	2	1	1	1	2	3
Nitzschia pura		2			1		2
Nitzschia pusilla	3	2	2	2	2	7	1
Nitzschia solita		3				5	
Nitzschia subacicularis	4	2	1	1	2	7	2
Nitzschia terrestris	3	2		1			
Pinnularia braunii	1	1			1	1	
Pinnularia gibba	3	2	2	3	3	7	3
Pinnularia gibba mesogongyla	3	1					
Pinnularia interrupta	3	1	1	1	1	2	

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Table C-1. Continued.

Site	pH	Salin.	Nitro.	D.O.	Sapro,	Troph.	Poll.
Pinnularia maior	3	2	2	2	2	4	3
Pinnularia microstauron	3	2	2	3	2	7	2
Pinnularia obscura	3	2	1	1	1		3
Pinnularia subcapitata	2	2	2	3	2	2	3
Pinnularia subrostrata							3
Pinnularia viridis	3	2	2	3	2	7	3
Rhopalodia gibberula							3
Stauroneis anceps	3	2	2	2	2	4	3
Stauroneis kriegeri	3	2	2	2	2	4	3
Stauroneis phoenicenteron	3	2	2	3	2	4	2
Stauroneis thermicola	3	2	2	1	2	7	3
Stenopterobia curvula	2	1	1	1	1	1	
Stenopterobia delicatissima	2	1	1	1	1	1	
Synedra tenera	2	1	1	1	1	2	3

Note: Abbreviations are as follows: Salin. (Salinity); Nitro (Nitrogen); D.O. (Dissolved Oxygen); Sapro. (Saprobity); Troph. (Trophic); Poll. (Pollution Tolerance).

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APPENDIX D
COEFFICIENT OF CONSERVATISM

Each macrophyte taxon identified was given a Coefficient of Conservatism (CC) score (Wilhelm and Ladd 1988) as detailed in Cohen et al. (*submitted*). The table below provides a list of the average confidence-weighted Coefficient of Conservatism for each species identified.

Table D-1. Coefficient of Conservatism scores for sampled macrophytes.

Site Name	CC	Site Name	CC
Acalypha gracilens	3.29	*Axonopus furcatus*	2.12
Acer rubrum	4.65	*Azolla caroliniana*	1.81
Aeschynomene indica	0.49	*Baccharis glomeruliflora*	6.12
Agalinis filifolia	6.69	*Baccharis halimifolia*	2.53
Agalinis linifolia	7.04	*Bacopa caroliniana*	5.31
Agalinis obtusifolia	6.50	*Bacopa inominata*	7.48
Alternanthera philoxeroides	0.00	*Bacopa monnieri*	4.49
Alternanthera sessilis	0.11	*Bidens laevis*	7.19
Alternanthera tenella	0.00	*Bidens mitis*	6.31
Amaranthus blitum	0.00	*Bigelowia nudata*	7.59
Amaranthus spinosus	0.04	*Blechnum serrulatum*	7.15
Ambrosia artemisiifolia	0.95	*Boehmeria cylindrica*	5.91
Ammannia latifolia	4.55	*Boltonia diffusa*	4.96
Ampelopsis arborea	3.25	*Brasenia schreberi*	8.79
Amphicarpum muhlenbergianum	5.70	*Burmannia capitata*	8.13
Andropogon glomeratus	3.90	*Canna flaccida*	6.75
Andropogon virginicus	3.44	*Caperonia castaneifolia*	4.94
Aristida affinis	8.23	*Caperonia palustris*	0.52
Aristida lanosa	8.73	*Cardamine pensylvanica*	4.42
Aristida palustris	8.84	*Carex alata*	4.27
Aristida patula	4.85	*Carex albolutescens*	3.47
Aristida purpurascens	5.58	*Carex fissa*	3.90
Aristida stricta	8.67	*Carex stipata*	4.46
Asclepias lanceolata	6.73	*Carex verrucosa*	7.97
Aster dumosus	2.53	*Carphephorus odoratissimus*	6.93
Aster elliottii	6.76	*Cassytha filiformis*	3.34
Aster subulatus	5.74	*Celtis laevigata*	5.08
Axonopus affinis	1.89	*Centella asiatica*	1.92

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Table D-1. Continued.

Site Name	CC
Cephalanthus occidentalis	7.27
Chenopodium album	0.78
Chenopodium ambrosioides	0.59
Chrysobalanus icaco	5.63
Cirsium nuttallii	3.08
Cladium jamaicense	9.04
Coelorachis rugosa	8.91
Coelorachis tuberculosa	10.00
Commelina diffusa	2.02
Conoclinium coelestinum	4.37
Conyza canadensis	1.01
Crinum americanum	8.67
Cuphea carthagenensis	1.92
Cynodon dactylon	0.29
Cyperus articulatus	6.64
Cyperus compressus	2.74
Cyperus croceus	1.30
Cyperus distinctus	5.00
Cyperus haspan	5.68
Cyperus lanceolatus	2.04
Cyperus odoratus	4.25
Cyperus polystachyos	1.56
Cyperus retrorsus	1.79
Cyperus strigosus	4.49
Cyperus surinamensis	2.03
Cyperus virens	5.70
Decodon verticillatus	7.80
Desmodium triflorum	0.43
Dichromena colorata	6.18
Dichromena latifolia	6.62
Digitaria bicornis	0.00
Digitaria ciliaris	1.30
Digitaria serotina	1.39
Diodia virginiana	4.96
Diospyros virginiana	5.76
Drosera brevifolia	8.21
Drosera capillaris	7.09
Drosera intermedia	8.23
Drymaria cordata	2.72
Dulichium arundinaceum	7.31
Echinochloa colona	0.24
Echinochloa crusgalli	0.22
Echinochloa muricata	6.01
Echinochloa walteri	3.36

Site Name	CC
Eclipta prostrata	3.21
Eichhornia crassipes	0.00
Eleocharis atropurpurea	5.69
Eleocharis baldwinii	2.82
Eleocharis cellulosa	7.80
Eleocharis elongata	6.97
Eleocharis equisetoides	9.10
Eleocharis interstincta	7.80
Eleocharis microcarpa	5.78
Eleocharis vivipara	3.81
Elephantopus elatus	2.72
Eleusine indica	0.00
Eragrostis atrovirens	1.58
Eragrostis elliottii	4.14
Eragrostis spectabilis	3.44
Erechtites hieracifolia	1.37
Erianthus giganteus	6.34
Erigeron quercifolius	3.31
Eriocaulon compressum	7.50
Eriocaulon decangulare	7.50
Eupatorium capillifolium	0.83
Eupatorium compositifolium	2.72
Eupatorium leptophyllum	4.94
Eupatorium mohrii	6.87
Eupatorium perfoliatum	5.85
Euthamia carolinana	3.25
Ficus aurea	3.38
Fimbristylis dichotoma	3.55
Fimbristylis miliacea	1.95
Fuirena breviseta	7.60
Fuirena pumila	5.92
Fuirena scirpoidea	6.50
Galactia elliottii	5.54
Galium pilosum	4.77
Galium uniflorum	5.80
Gaylussacia dumosa	5.44
Gomphrena serrata	0.87
Gordonia lasianthus	9.03
Gratiola pilosa	6.63
Gratiola ramosa	6.87
Habenaria repens	4.58
Hedyotis corymbosa	2.31
Hedyotis uniflora	4.04
Helianthus floridanus	5.85

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Table D-1. Continued.

Site Name	CC
Hibiscus grandiflorus	6.86
Hydrochloa caroliniensis	4.79
Hydrocotyle umbellata	1.92
Hydrolea corymbosa	5.85
Hymenachne amplexicaulis	0.00
Hypericum brachyphylum	7.55
Hypericum cistifolium	6.32
Hypericum fasciculatum	7.27
Hypericum hypericoides	5.44
Hypericum mutilum	4.04
Hypericum myrtifolium	6.56
Hyptis alata	4.58
Ilex cassine	7.66
Ilex glabra	5.85
Ipomoea quamoclit	0.26
Ipomoea sagittata	6.42
Iris hexagona	6.97
Itea virginica	7.09
Iva microcephala	4.68
Juncus coriaceus	8.51
Juncus effusus	3.25
Juncus marginatus	3.65
Juncus megacephalus	5.70
Juncus polycephalus	4.96
Juncus repens	6.91
Juncus scirpoides	4.33
Justicia angusta	8.56
Justicia ovata	8.88
Kosteletzkya virginica	7.49
Kyllinga brevifolia	1.42
Kyllinga pumila	5.53
Lachnanthes caroliniana	3.76
Lachnocaulon anceps	7.15
Lachnocaulon beyrichianum	9.18
Lachnocaulon minus	7.97
Lechea cernua	8.67
Leersia hexandra	5.61
Lemna minor	3.77
Leucothoe racemosa	9.44
Limnobium spongia	4.79
Lindernia grandiflora	3.60
Liquidambar styraciflua	5.56
Lobelia glandulosa	6.03
Lobelia paludosa	8.08

Site Name	CC
Ludwigia alata	5.85
Ludwigia alternifolia	6.24
Ludwigia arcuata	5.32
Ludwigia decurrens	6.76
Ludwigia lanceolata	6.15
Ludwigia linearis	5.72
Ludwigia linifolia	7.04
Ludwigia maritima	5.85
Ludwigia microcarpa	4.81
Ludwigia octovalvis	4.09
Ludwigia palustris	4.77
Ludwigia peruviana	0.62
Ludwigia repens	5.20
Ludwigia suffruticosa	6.23
Ludwigia virgata	6.73
Lycopodiella alopecuroides	7.61
Lycopodium appressum	8.01
Lycopus rubellus	6.75
Lygodium microphyllum	0.78
Lyonia ferruginea	8.39
Lyonia lucida	7.06
Lythrum alatum	3.55
Macroptilium lathyroides	0.41
Magnolia virginiana	9.44
Mayaca fluviatilis	8.45
Melaleuca quinquenervia	0.00
Melochia corchorifolia	2.24
Melothria pendula	3.31
Micranthemum umbrosum	5.66
Micromeria brownei	6.34
Mikania scandens	1.95
Mollugo verticillata	1.30
Murdannia keisak	2.34
Murdannia nudiflora	1.42
Myrica cerifera	3.82
Myriophyllum laxum	5.85
Nuphar luteum	6.09
Nymphaea odorata	7.18
Nymphoides aquatica	6.09
Nyssa biflora	9.04
Osmunda cinnamomea	6.44
Osmunda regalis	8.04
Oxypolis filiformis	8.69
Panicum abscissum	9.22

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Table D-1. Continued.

Site Name	CC
Panicum aciculare	6.01
Panicum chamaelonche	7.69
Panicum ciliatum	7.15
Panicum commutatum	7.57
Panicum dichotomiflorum	4.96
Panicum dichotomum	5.61
Panicum ensifolium	6.50
Panicum erectifolium	7.39
Panicum hemitomon	5.82
Panicum hians	6.63
Panicum maximum	0.78
Panicum repens	0.41
Panicum rigidulum	5.47
Panicum spretum	6.63
Panicum tenerum	8.67
Panicum tenue	5.85
Panicum verrucosum	6.83
Parthenocissus quinquefolia	3.43
Paspalum acuminatum	1.06
Paspalum conjugatum	3.84
Paspalum distichum	5.54
Paspalum laeve	5.79
Paspalum monostachyum	9.80
Paspalum notatum	0.14
Paspalum praecox	6.50
Paspalum repens	6.69
Paspalum setaceum	3.44
Paspalum urvillei	0.00
Persea borbonia	8.02
Persea palustris	8.31
Phyla nodiflora	1.92
Phyllanthus urinaria	0.22
Phytolacca americana	2.09
Pinus elliottii	4.21
Pinus palustris	4.77
Pinus taeda	5.34
Pluchea foetida	6.65
Pluchea longifolia	5.85
Pluchea odorata	4.96
Pluchea rosea	5.45
Polygala cymosa	7.67
Polygala rugelii	8.17
Polygonum densiflorum	5.32
Polygonum hirsutum	8.17

Site Name	CC
Polygonum hydropiperoides	4.02
Polygonum lapathifolium	1.95
Polygonum punctatum	4.02
Polypremum procumbens	1.71
Pontederia cordata	5.38
Portulaca amilis	0.87
Proserpinaca palustris	5.85
Proserpinaca pectinata	5.50
Pteridium aquilinum	3.90
Quercus laurifolia	5.14
Quercus nigra	4.14
Quercus virginiana	4.85
Rhexia cubensis	7.22
Rhexia mariana	5.50
Rhexia nashii	7.80
Rhexia nuttallii	7.93
Rhexia petiolata	7.90
Rhus copallinum	3.65
Rhynchospora cephalantha	6.19
Rhynchospora debilis	7.80
Rhynchospora divergens	5.53
Rhynchospora fascicularis	5.92
Rhynchospora fernaldii	4.77
Rhynchospora filifolia	8.13
Rhynchospora inundata	7.25
Rhynchospora microcarpa	5.29
Rhynchospora microcephala	6.50
Rhynchospora nitens	5.20
Rhynchospora perplexa	5.20
Rhynchospora pusilla	7.54
Rhynchospora rariflora	8.63
Rhynchospora tracyi	9.03
Rhynchospora wrightiana	7.80
Ricciocarpus natans	4.55
Richardia scabra	0.00
Rosa palustris	6.01
Rubus argutus	3.56
Rubus cuneifolius	3.90
Rubus trivialis	2.60
Sabatia grandiflora	7.09
Sacciolepis indica	0.92
Sacciolepis striata	5.35
Sagittaria graminea	5.53
Sagittaria lancifolia	4.96

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Table D-1. Continued.

Site Name	CC
Sagittaria latifolia	6.50
Salix caroliniana	2.95
Salvinia minima	2.03
Sambucus canadensis	1.48
Sarcostemma clausum	3.81
Saururus cernuus	7.33
Schinus terebinthifolius	0.00
Schizachyrium scoparium	5.44
Schoenolirion albiflorum	9.10
Scleria baldwinii	8.67
Scleria georgiana	8.78
Scleria reticularis	6.79
Scleria triglomerata	6.74
Scleria vaginata	0.00
Scoparia dulcis	2.36
Senna occidentalis	0.00
Serenoa repens	7.03
Sesbania herbacea	1.49
Sesbania vesicaria	1.44
Setaria parviflora	3.40
Sida rhombifolia	1.65
Smilax auriculata	3.96
Smilax bona-nox	3.78
Smilax pumila	6.01
Solanum americanum	1.16
Solanum carolinense	2.13
Solanum viarum	0.00
Solidago fistulosa	4.49
Solidago latissimifolia	7.51
Solidago stricta	5.49
Solidago tortifolia	6.96
Sorghastrum secundum	7.73
Spartina bakeri	5.98
Spermacoce assurgens	3.09
Spirodela polyrhiza	2.95
Sporobolus domingensis	2.47
Sporobolus indicus	0.99
Stillingia aquatica	8.32
Stillingia sylvatica	7.30
Syngonanthus flavidulus	6.93
Taxodium ascendens	7.21
Taxodium distichum	7.21
Teucrium canadense	6.44
Thalia geniculata	7.12
Thelypteris interrupta	6.74
Toxicodendron radicans	1.44
Triadenum virginicum	8.16
Triadenum walteri	7.92
Tripsacum dactyloides	6.03
Typha domingensis	0.59
Typha latifolia	1.60
Ulmus americana	7.68
Urena lobata	0.00
Utricularia cornuta	7.46
Utricularia foliosa	6.44
Utricularia purpurea	6.50
Utricularia radiata	6.01
Utricularia subulata	7.23
Vaccinium corymbosum	5.63
Vaccinium darrowii	7.15
Verbena bonariensis	0.56
Viola lanceolata	5.32
Viola primulifolia	6.11
Vitis rotundifolia	1.18
Woodwardia areolata	7.68
Woodwardia virginica	6.50
Xyris ambigua	6.43
Xyris baldwiniana	6.97
Xyris brevifolia	7.20
Xyris caroliniana	6.14
Xyris difformis	7.50
Xyris elliottii	6.69
Xyris fimbriata	7.08
Xyris flabelliformis	7.43
Xyris jupicai	3.51
Xyris platylepis	5.32
Xyris smalliana	7.80

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BIOGRAPHICAL SKETCH

Charles Raymond Lane was born on August 2, 1972. He was raised in Ho-Ho-Kus, New Jersey, and attended Lloyd Public School and Midland Park High School, graduating with Honors in 1991. Charles completed his bachelor's degree in economics in 1995 from the University of North Carolina at Chapel Hill. He began his graduate research with Dr. Mark T. Brown in 1997 and received his Master of Science degree from the University of Florida in 2000.

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I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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Associate Professor of Environmental Engineering Sciences

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Thomas L. Crisman
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