

The Florida Wetland Condition Index (FWCI):  
Developing Biological Indicators for Isolated Depressional Forested Wetlands

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By

Kelly Chinnere Reiss  
and  
Mark T. Brown

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Howard T. Odum Center for Wetlands  
University of Florida  
Gainesville, Florida 32611-6350

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## EXECUTIVE SUMMARY

### THE FLORIDA WETLAND CONDITION INDEX (FWCI): DEVELOPING BIOLOGICAL INDICATORS FOR ISOLATED DEPRESSIONAL FORESTED WETLANDS

Over 30 years ago, the federal Water Pollution and Control Act obliged states to protect and restore the chemical, physical, and biological integrity of waters, and charged states with establishing water-quality standards for all waters within state boundaries including wetlands. Criteria for defining water-quality could be narrative or numeric; and it could be addressed through chemical, physical, or biological standards. Initially, states used chemical and physical criteria (testing waters for chemical concentrations or physical conditions that exceeded criteria) and assuming losses in ecosystem integrity if the criteria were exceeded (Danielson 1998a). The United States Environmental Protection Agency (USEPA) recognized the potential of biological criteria to assess water-quality standards and in the late 1980s required states to use biological indicators to accomplish the goals of the Clean Water Act (USEPA 1990). In effect, biological assessment has evolved into one of the standard monitoring tools of water resource-protection agencies over the past 2 decades (Gerristen et al. 2000). Such biological criteria and monitoring programs have been created for lakes and streams throughout the United States (Barbour et al. 1996a; Karr and Chu 1999; Gerristen et al. 2000), and more recently efforts to assess wetland condition have been initiated (Mack 2001; USEPA 2002a). Within Florida, biological indices have been created based on macroinvertebrate community composition for streams (Barbour et al. 1996a; Fore 2003), lakes (Gerristen and White 1997), and isolated depressional freshwater herbaceous wetlands (Lane et al. 2003), and based on the community composition of the diatom and macroinvertebrate assemblages for freshwater herbaceous wetlands (Lane et al. 2003).

The primary objective of this research was to develop a Florida Wetland Condition Index (FWCI) for isolated depressional forested wetlands in Florida. Wetland study sites were sought in various landscape settings that included natural, agricultural, and urban land uses. Three independent measures of anthropogenic influence were calculated for each wetland including the Landscape Development Intensity index (LDI) (Lane et al. 2003; Brown and Vivas 2005), the Wetland Rapid Assessment Procedure (WRAP) (Miller and Boyd 1999), and the Minnesota disturbance index (Gernes and Helgen 1999). Strong correlations among LDI, WRAP, and MDI led us to adopt the LDI to represent the gradient of anthropogenic influence for further analyses. Compositional differences among the diatom, macrophyte, and macroinvertebrate assemblages were identified and related to the LDI measure of anthropogenic influence. Each assemblage (diatom, macrophyte, and macroinvertebrate) was used to construct an assemblage specific FWCI for isolated depressional forested wetlands in Florida.

While previous research has identified responses of wetland ecosystems to particular system impacts (such as increased nutrients or altered hydrology), few have combined multiple biotic components, environmental parameters, and landscape development intensity in an attempt to quantify ecological integrity. The contribution of this research to our understanding of changes in the community composition of isolated

depressional forested wetlands (based on the diatom, macrophyte, and macroinvertebrate assemblages) in relation to different development intensities in the surrounding landscape can be summarized in seven main points.

- Biological indicators along with physical and chemical parameters were useful in defining biological integrity;
- The variable turnover times and sensitivities of the three assemblages (diatoms, macrophytes, macroinvertebrates) suggest that a multi-metric multi-assemblage FWCI has more merit than a FWCI based on a single assemblage;
- Regionalization may strengthen the FWCI;
- A FWCI independent of wetland type may be feasible, given the strong likeness of the forested FWCI to the herbaceous FWCI (Lane et al. 2003);
- The Landscape Development Intensity (LDI) index was a useful tool correlating with the measured biological condition for isolated forested wetlands;
- Urban wetlands exhibit a different vector of change than do agricultural wetlands, and while the FWCI suggests low biological integrity of both agricultural and urban wetlands, these wetlands do provide services and do work in the environment.
- Richness, evenness, and diversity (for each assemblage) were not sensitive to different land uses or development intensities in the surrounding landscape.

The assemblage specific FWCIs provide a quantitative measure of the biological integrity of isolated depressional forested wetlands in Florida. The FWCI includes three separate measures of wetland biological integrity based on the community composition of three species assemblages. In total 19 metrics are used to address deviations from the wetland biological integrity potential based on the suite of reference wetlands, including seven diatom, six macrophyte, and six macroinvertebrate based metrics. Metrics were selected for inclusion in the FWCI based on the strength and significance (Spearman's correlation coefficient) of each metric in correlation with a quantitative gradient of LDI, the ability of a metric to visually distinguish correlations with LDI in scatter plots, and a significant difference between low and high LDI groups (Mann-Whitney U-test). The FWCI for each assemblage was composed of individual metrics specific to the assemblage, which were scaled and added together, creating the diatom FWCI (0-70 scale), macrophyte FWCI (0-60 scale), and the macroinvertebrate FWCI (0-60 scale), with the highest values reflecting the highest biological integrity whereas a score of 0 would reflect a lack of biological integrity or no similarity to the reference wetland condition.

The seven diatom metrics included in the FWCI, which were significant for both the Spearman's correlation coefficient ( $|r| > 0.45$ ,  $p < 0.01$ ) and the Mann-Whitney U-test between LDI groups ( $p < 0.10$ ), represented tolerance and autecological metrics. Tolerance metrics included tolerant and sensitive indicator species. The five autecological metrics included Pollution Class 1 (very tolerant to pollution), Nitrogen Uptake Metabolism Class 3 (need periodically elevated concentrations of organically bound nitrogen), Saprobity Class 4 (inhabit aquatic environments with an oxygen saturation between 10-25% and a biological oxygen demand of approximately 13-22 mg/L), pH Class 3 (circumneutral, mainly occurring at pH values around 7), and Dissolved Oxygen Class 1 (requiring continuously high dissolved oxygen concentrations

near 100%). Pollution Class was established by Bahls (1993), and Nitrogen Metabolism, Saprobity, pH, and Dissolved Oxygen Classes were defined by van Dam et al. (1994).

Six macrophyte metrics that were significant for both the Spearman's correlation coefficient ( $|r| > 0.39$ ,  $p < 0.001$ ) and the Mann-Whitney U-test between LDI groups ( $p < 0.001$ ) included in the FWCI were Tolerant Indicator Species, Sensitive Indicator species, Floristic Quality Assessment Index (FQAI), Exotic Species, Native Perennial Species, and Wetland Status Species. FQAI scores were obtained for species identified specific to this study.

The six macroinvertebrate metrics included in the FWCI, which were significant for both the Spearman's correlation coefficient ( $|r| > 0.3$ ,  $p < 0.05$ ) and the Mann-Whitney U-test between LDI groups ( $p < 0.05$ ), represented tolerance, community balance, and functional group metrics. Tolerance metrics included Tolerant Indicator Genera, Sensitive Indicator Genera, and Florida Index scores. Community balance metrics included Mollusca (phylum taxonomic level) and Noteridae (family taxonomic level). One functional groups metric was included, the percent of macroinvertebrates identified as scrapers.

Diatom FWCI Metrics	Macrophyte FWCI Metrics	Macroinvertebrate FWCI Metrics
% Tolerant species	% Tolerant species	% Tolerant species
% Sensitive species	% Sensitive species	% Sensitive species
% in Pollution Class 1	Modified FQI Score	FL Index Score
% in Nitrogen Class 3	% Exotic	% Mollusca
% in Saprobity Class 4	% Native Perennial	% Noterida
% in pH Class 3	% Wetland Status	% Scraper
% in Oxygen Class 1		

The variable turnover times and sensitivities of the three different assemblages (diatoms, macrophytes, macroinvertebrates) suggest that use of multiple assemblage specific FWCIs has more sagacity than a FWCI based on a single assemblage. The three species assemblage specific FWCIs can be used to infer influences in temporal and spatial changes to which a particular wetland has been exposed. While agreement in the ranking of the biological condition of study wetlands using the three species assemblage FWCIs was anticipated, discrepancies among the ranking from the different assemblages may provide great insight into wetland condition as different species assemblages respond to changes in driving energies over different time scales. While the *a priori* reference wetlands were generally differentiated from the agricultural and urban wetlands, differences between the agricultural and urban land uses were not as apparent. Ranges of FWCI scores for the *a priori* wetland categories (based on land use in the surrounding landscape) show the difficulties inherent in attempting to discern a specific mode of impact based on FWCI scores.

Though the FWCI suggested low biological integrity of both agricultural and urban wetlands, these wetlands provide services and do work in the environment. Therefore, a quantitative score of biological integrity established through the FWCI should not be used as a surrogate for wetland value, but rather as an objective, quantitative means of comparing changes in community composition along gradients of

landscape development intensity. In the future, an integrative multi-metric FWCI could be constructed for wetlands throughout the state, with lists of Indicator Species and metric scores dependent on Florida ecoregions and specific to wetland type.

While the FWCI can not be used to predict changes in the physical and chemical parameters of a wetland, its strength lies in providing an overview of biological integrity through the integration of changes in community composition from cumulative effects. Among *a priori* land use categories, differences in water and soil parameters were apparent (including dissolved oxygen, color, turbidity, water column pH, specific conductivity, water ammonia-nitrogen, water TKN, water TP, soil moisture, soil organic matter, and soil TP). When soil and water parameters were used to explain variation in the community composition for each assemblage based on Non-metric Multidimensional Scaling (NMDS) ordination techniques, water column pH was universally identified as a keystone parameter significantly correlating the community composition ordination. Additionally, total phosphorus concentrations correlated with the ordinations based on both the diatom and macrophyte assemblages. Perhaps preservation and restoration strategies could focus on limiting activities (in the surrounding landscape) that influence changes to water column pH and total phosphorus loading to wetlands in order to promote biological integrity.

## CHAPTER 1 INTRODUCTION AND OVERVIEW

With even casual observation, it is apparent that ecosystems change with increasing levels of human development, and that the extent of change is observably related to the magnitude of human activity. While previous research has identified the responses of forested wetlands to human induced changes such as increased nutrients (Nessel et al. 1982; Lemlich and Ewel 1984; Devall 1998) or altered hydrology (Marois and Ewel 1983; Lugo and Brown 1986; Young et al. 1995) few have studied the amalgamated response of ecosystems resultant from the combined effects of anthropogenic activities. This is particularly true for wetlands located within urban settings. Our study focused on understanding the effects of landscape development from anthropogenic activities on isolated depressional forested wetlands in Florida. More specifically, our goal was to define the condition of wetlands surrounded by different land use activities by describing the biological integrity of the ecosystem.

Determining wetland ecosystem integrity through the use of biological indicators requires an accepted definition of integrity. Karr and Dudley (1981) defined integrity 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.” This definition requires two things: a definition of the natural habitat (or reference standard condition) and appropriate regionalization. Reference standard wetlands have been defined as those wetlands surrounded by natural landscapes, with no apparent anthropogenic alterations. Ecosystem condition has been commonly defined by data collected at a suite of reference wetlands, which include a suite of reference standard wetlands as well as a suite of impaired wetland representing the full range of impaired conditions from minimal to severe impairment. Gerristen et al. (2000) concur that biological assessment relies on a characterization of the reference condition.

### **Review of Isolated Depressional Forested Wetlands**

Throughout the world, wetlands have been categorized in many different ways (Mitsch and Gosselink 1993; Keddy 2000; Kent 2000). Probably the most widely recognized classification system in North America is that by Cowardin et al. (1979). Our study focuses on what Cowardin et al. (1979) categorized forested palustrine wetlands. In more general terms, the wetlands targeted in our study have been called pondcypress domes with reference to Vernon (1947), who was the first to name these systems after their characteristic silhouette in the landscape (Figure 1-1). Pondcypress domes range in size from less than 1 hectare to more than 10 hectares (Wharton et al. 1977), and host *Taxodium ascendens* (pondcypress) as the principal tree species (Devall 1998). Other tree species associated with *Taxodium ascendens* include: *Nyssa sylvatica* var. *biflora* *tupelo* (swamp tupelo), *Pinus* spp. (many southern pines), *Acer rubrum* (red maple), and *Magnolia virginiana* var. *australis* (sweetbay magnolia) (Wilhite and Toliver 1990; Devall 1998). Pondcypress trees characteristically dominate the center, with pondcypress



(A)



(B)



(C)

Figure 1-1. Characteristic silhouette shape of three isolated depressional wetlands sampled in this study. These pondcypress domes named by Vernon (1947) include: (A) CU2 in Orange County, (B) CR4 in Sumter County, and (C) PR2 in Santa Rosa County.



along the edge in competition with other species that are less tolerant of flooded conditions. There is a greater likelihood of fire and a larger number of seedlings in the drier edges (Odum 1978). While the target ecosystem type was the pondcypress dome, many of the study wetlands hosted co-dominant species or were dominated by canopy species other than pondcypress and may more appropriately be described as isolated depressional mixed-forested wetlands. The principal commonalities among the study wetlands include the isolated depressional nature of these wetlands and the occurrence of mature forest canopy.

The use of the word isolated to describe a wetland has been scrutinized because it has been interpreted to suggest separation of a wetland system from its landscape. However, the term isolated in this study simply refers to the lack of a regular or permanent surface water connection between the wetland and the surrounding landscape. However, in periods of high water and excessive rainfall, many of these isolated wetland systems receive surface run-in from nearby wetlands or discharge to other nearby wetlands or surface water features. Under typical conditions, inflows into isolated depressional wetlands are limited to sunlight, wind, water (rain, surface run-off, groundwater), and recruitment of plant and animal species. Water inflow comes almost entirely from rainwater, both as a direct input and as run-in from a relatively small watershed. As such these wetlands are often termed “isolated” due to their somewhat limited hydrologic connections.

Standing water is present in most isolated depressional forested wetlands much of the year (Odum 1978; Mitsch and Gosselink 1993). Some have deep central pools staying wet year round, while others go dry annually. There is also variation in maximum flooding depth and length of standing water between years, which reflects the larger scale climatic and physiogeographic influences to these wetland systems. Typically, the wettest period is summer and the driest spring and fall (Mitsch and Gosselink 1993).

Isolated depressional forested wetlands dominated with pondcypress are characterized by a tolerance of low nutrient levels and intermittent fire (Brandt and Ewel 1989), with major system inputs limited to rainfall and surface inflows (Mitsch and Gosselink 1993). These ecosystems are considered successional stable, but may be replaced by other ecosystems with changing environmental conditions, such as decreased water levels (Devall 1998). In drained pondcypress domes in northern Florida, Marois and Ewel (1983) found an increase in the densities of hardwood and shrub species. Furthermore, in south Florida, fire suppression has resulted in a shift in the canopy community composition to readily regenerating hardwood species over pondcypress dominance (Ewel 1990). Perhaps many of the depressional mixed-forested wetlands identified throughout the state were historically dominated by pondcypress, but have undergone a shift in canopy species composition due to drainage, fire suppression, timber harvesting, and other indeterminate anthropogenic activities.

Pondcypress and the most characteristic co-dominate swamp tupelo are deciduous, shedding their leaves from October to December. Ewel (1984) and Wharton et al. (1977) found that the perpetuation of pondcypress domes depends on fluctuating water levels, with a dry period without standing water necessary for pondcypress generation and higher water levels some time during the year necessary to prevent germination of more terrestrial faster-growing pines and hardwoods that are not tolerant

of standing water. Altering the typical hydroperiod of a pondcypress dome has a direct effect on species composition, resulting in encroachment of terrestrial species in drained pondcypress domes and a lack of regeneration of pondcypress in artificially flooded pondcypress domes. Anthropogenic activities in the surrounding up-slope landscape can create a wide array of changes to the inflows of these wetland systems. Some of the potential alterations to isolated depressional wetlands located within developed landscapes include changes in the seasonality and depth of flooding, increased nutrient inputs, increased toxin inputs, and physical impacts (canals, retaining walls, stormwater box culverts, etc.).

### **Changes in Hydrology**

There are two important mechanisms within the developed landscape leading to potential hydrologic alterations to an isolated depressional wetland ecosystem surrounded by developed land uses. First, increased run-off is considered a factor of the amount of increased impervious surface in the watershed supporting the wetland system and the amount of rainfall. This would be particularly apparent in an urban landscape, where previously vegetated lands are paved creating increased water flow during rain events, which might otherwise have been intercepted by the vegetation or permeated into the soil. A second mechanism of the developed landscape is an increased outflow of water storage from the wetland as a result of drainage.

In Florida, mature cypress trees (*Taxodium* spp.) are considered the most flood tolerant of all tree species (Harms et al. 1980; Ewel 1990). Past research shows that cypress trees can survive sustained deep flooding (Lugo and Brown 1986; Young et al. 1995), though the trees suffered decreased growth rates and no evidence of regeneration. This suggests that while mature cypress ecosystems may be able to withstand some threshold level of long-term flooding, regeneration may be impeded which is otherwise necessary to ensure the long-term continued existence of the ecosystem. Ultimately, removing the structure of the wetland will predictably alter other ecosystem components. For example, by removing the tree canopy, algae may flourish at the water surface where it would have otherwise been shaded out. This would in turn alter the food available for the fauna and decrease available sunlight at the soil surface. Ewel (1990) reported that increasing the length of flooding would also affect soil aeration and the ability of other plants to survive and reproduce.

Lugo and Brown (1986) looked at the response of floodplain tree species to sustained increases in water depth after the damming of the Ocklawaha River in Florida. They found that while the larger trees survived some depths of flooding, in the deepest areas, where mean water depth was 1 m, there was 100% tree mortality within 5 years of flooding. Whereas, in the control system, tree mortality was less than 1% per year. Additionally, flooded trees responded with a dieback of terminal branches, loss of leaves, reduction in leaf size, and loss of color brightness in leaves (Lugo and Brown 1986). Among the trees in the Ocklawaha River floodplain, 25-53 cm was the threshold flooding depth, beyond which tree mortality sharply increased. In a different study, Young et al. (1995) found that the annual radial growth of *Taxodium distichum* (baldcypress) significantly increased for 4 years after flooding, followed by declining growth in the subsequent 16 years. The researchers offered two potential explanations for the initial

growth increases: decreased competition due to the death of less flood tolerant species, or increased nutrient levels immediately following flooding.

Marois and Ewel (1983) studied the effects of ditches and berms on 15 pondcypress domes situated within an intensively managed slash pine plantation. In the pondcypress domes not ditched and bermed, the lengths of flooding and mean water depth were generally greater. Alternatively in the drier ditched and bermed pondcypress domes the density of hardwoods, shrubs, and vines increased. They concluded that while pondcypress tree growth increased in the years directly following the drying of the pondcypress domes, pondcypress regeneration may have been inhibited due to changes in vegetative species composition, soil chemistry, and hydrology.

Cypress seeds require soaking in water in order to germinate (Demaree 1932), so altering the seasonality and decreasing the depth of flooding in a pondcypress dome may inhibit or seriously diminish potential germination. The higher density of hardwoods, shrubs, and vines may also inhibit pondcypress regeneration by blocking sunlight from reaching the forest floor. Marois and Ewel (1983) found the highest percentage of light transmittance in unaltered pondcypress domes. The pondcypress domes with greater light also had an abundance of grasses and sedges. Ewel (1990) noted that drainage allows species with low flood tolerance to become established, resulting in an increased density of shrubs and hardwoods, poor pondcypress regeneration, increased fire potential, and a dramatic shift to arboreal wildlife species from aquatic and wading fauna (Marois and Ewel 1983; Harris and Vickers 1984). More specifically, Marois and Ewel (1983) found broadleaved predominantly evergreen mid-story plants (such as *Ilex cassine*, dahoon holly; *Lyonia lucida*, fetterbush; *Magnolia virginiana* var. *australis*, sweetbay magnolia; and *Persea palustris*, swamp bay), became more common in swamps when water levels were lowered. Harris and Vickers (1984) speculated that shifting species in the vegetation layer equates to altered structure and habitat for fauna which affects organisms in all other trophic levels.

Decreasing the mean water level could cause changes in the community composition of many species that rely on pondcypress domes for regeneration. Benthic invertebrates may not withstand increased dry periods, and reproduction may be difficult or unattainable. Often forming the base of swamp food chains (Ewel 1990), eliminating or decreasing the population of benthic macroinvertebrates could have repercussions throughout the food chain. Fish, amphibians, and reptiles may be eliminated from pondcypress domes with decreased hydroperiods due to reproduction difficulties or altered food availability (Means et al. 1998; Ewel 1990).

### **Increased Inflows of Nutrients and/or Toxins**

In undeveloped landscapes isolated depressional wetlands receive limited nutrient inputs from rainwater and surface water run-in (Wharton et al. 1977). An increase in the inflow of nutrients and toxins may come from both point (eg. wastewater additions) and non-point (ex. impervious surface run-off) sources. An increase in run-in from impervious surfaces in the surrounding landscape may increase the loading of nutrients and toxins to a wetland system (Harper 1994). Surface run-off carrying fertilizer used on agricultural crops or home lawns are examples of non-point source contributions. As nutrients flow into the wetland, the growth of living biomass increases, and nutrients

accumulate in the water and soil organic matter storages. Conversely, as toxins flow into and accumulate in the wetland, there is a deleterious effect on biomass.

Two nutrients of primary importance in pondcypress domes are phosphorus and nitrogen. Phosphorus, an element critical to plant growth, is mostly bound into forms unavailable to plants at pH levels below 5.7 (Brady and Weil 2004), higher than the average pH of pondcypress domes embedded in undeveloped landscapes (Coultas and Duever 1984). Dierberg and Brezonik (1984) found an annual average pH of 4.63 for pondcypress wetland with the primary source of water being rainfall, and an annual average pH of 5.4 for a pondcypress wetland with the primary source of water being shallow groundwater for wetlands in north-central Florida. Phosphorus is known to accumulate in the clay layers found beneath pondcypress domes, which makes pondcypress dome ecosystems dependent on a constant input of available phosphorus from rainfall. Raising the pH of a wetland increases the concentration of available phosphorus. In contrast, nitrogen does not accumulate in the clay layer or organic sediments at the bottom of pondcypress domes due to denitrification processes, and the rate of the nitrogen cycle seems dependent on the rate of decomposition of organic matter (Wharton et al. 1977).

Pondcypress trees respond to increased nutrient loading with increased tree growth rates (Nessel et al. 1982; Lemlich and Ewel 1984). Nessel et al. (1982) measured phosphorus concentrations in live cypress needles at a pondcypress dome embedded in silvicultural land use and a cypress strand receiving sewage for more than 40 years. Cypress needles in the silvicultural wetland had a lower average phosphorus concentration than cypress needles in the wetland receiving wastewater. Additionally, the top 20 cm of sediment in the wetland receiving wastewater had nearly 5.5 times as much phosphorus per square meter as in the silvicultural wetland. They concluded that the trees in the cypress strand were in fact responding to the increased nutrient inputs (from the sewage) with increased growth rates.

Traditionally pondcypress domes are oligotrophic systems (Mitsch and Gosselink 1993). Ewel (1984) and Harris and Vickers (1984) found that an increase in dissolved nutrients led to the development of thick mats of *Lemna* spp. (duckweed), *Spirodela* spp. (giant duckweed), and/or *Azolla caroliniana* (Carolina mosquito fern) on the water surface. Ewel (1984) also noted that a nutrient enriched pondcypress dome had similar understory species composition compared to a pondcypress dome not receiving wastewater, however the leaf area was significantly higher in the nutrient enriched pondcypress dome. Changes in the understory vegetation (from increased leaf area or covering of the water surface with a layer of vegetation) can have an effect on other trophic levels within the wetland ecosystem. In pondcypress domes receiving wastewater additions, Harris and Vickers (1984) reported an increase in the number of invertebrates and amphibians; however they also noted a shift in the invertebrate taxa and a high larval mortality of amphibians suggesting the fauna in the nutrient enriched pondcypress dome were different from the control wetland.

### **Physical Disturbance**

Examples of physical changes in depression forested wetlands include trampling and grazing by domestic cattle, rooting of feral pigs, construction of barriers such roads

and retention walls, and conduits such as stormwater culverts. Few publications were found that quantified the effects of physical modifications to wetlands. Findlay and Houlihan (1997) used existing biological surveys for 30 Ontario, Canada, wetlands, comparing species richness of plants, amphibians, birds, and reptiles with wetland areas, density of paved roads, and percent forest cover. They found a negative correlation between wetland species richness and density of paved roads on lands within 2 km of the wetland. They concluded that increasing the density of paved road surface or decreasing the forest cover by 20% within 2 km surrounding a wetland would pose significant risks to the biodiversity of the wetland and be as detrimental as losing 50% of the wetland itself, in terms of loss of species richness.

Another physical modification to isolated depressional forested wetlands is the removal of a portion or all of the canopy layer. Florida has a long history of timber harvesting, and Ewel (1990) suggested that nearly all of the pondcypress domes in north Florida have been logged since the late 1800s. Studies show that logged pondcypress domes maintain their defining characteristics after regeneration (Terwilliger and Ewel 1986; Ewel et al. 1989); however, during regeneration, there are shifts in the flora and fauna of logged wetlands. Physical modifications such as roads, canals, and stormwater culverts also act as direct conduits for the introduction of exotic species (Frappier and Eckert 2003).

### **Biological Indicators of Ecosystem Integrity**

Over 30 years ago, the Water Pollution and Control Act (later referred to as the Clean Water Act, 1972) required states to “restore and maintain the chemical, physical, and biological integrity of the Nation’s waters” (USEPA 1990). This legislation included establishing water-quality standards for all waters within state boundaries, including wetlands. Criteria for defining water-quality could be narrative or numeric; and it could be addressed through chemical, physical, or biological standards. Initially, states used chemical and physical criteria (testing waters for chemical concentrations or physical conditions that exceeded criteria) and assuming losses in ecosystem integrity if the criteria were exceeded (Danielson 1998a).

There are several shortcomings in deriving ecosystem integrity based on exceeding established limits for chemical and physical parameters. Such criteria have been considered rudimentary in their ability to reflect more than the temporal concentration of chemicals within a water body (Karr 1993). For instance, the use of toxicity parameters for determining ecosystem integrity may falsely indicate high ecosystem integrity simply because a single toxicity parameter went undetected. This same water body could have undesirable levels of other nontarget toxics or metals; or be physically altered so that it no longer resembles a fully functioning water body (Karr and Chu 1997). Furthermore, chemical and physical sampling may not occur during specific loading events and may therefore incompletely describe the biological and ecological condition of the system. Adams (2002) points out other environmental factors (such as sedimentation, alterations to habitat, varying temperature and oxygen levels, and changes in ecological aspects like food availability and predator-prey relationships) are not reflected with chemical criteria alone. James and Kleinow (1994) note that different organisms respond in different ways to the amount, persistence, and exposure of chemical

compounds otherwise foreign to an organism, and single-valued chemical and physical criteria of water quality may overlook important biological implications.

Alternatively, biological indicators integrate the spatial and temporal effects of the environment on resident organisms and are suitable for assessing the possible effects of multifaceted changes in aquatic ecosystems (Adams 2002). Adams (2002) and Karr and Chu (1997) note that biological indicators signal changes in the environment that might otherwise be overlooked or underestimated by methods that depend on chemical criteria alone. The underlying support for using biological indicators is that organisms have an intricate relationship with their environment, which reflects current and cumulative ecosystem conditions (Karr 1981). Biological indicators reflect chemical exposure and also integrate changes in the community composition of the ecosystem (from physical, chemical, and biological changes) (Adams 2002).

The United States Environmental Protection Agency (USEPA) recognized the potential of biological criteria to assess water-quality standards and in the late 1980s required states to use biological indicators to accomplish the goals of the Clean Water Act (USEPA 1990). In effect, biological assessment has evolved into one of the standard monitoring tools of water resource-protection agencies over the past 2 decades (Gerristen et al. 2000). Biological criteria and monitoring programs through the USEPA have been created for lakes and streams throughout the United States (Barbour et al. 1996a; Karr and Chu 1999; Gerristen et al. 2000), and more recently efforts to assess wetland condition have been initiated (USEPA 2002a).

Biological monitoring to assess ecosystem condition has been applied widely in ecological research. One trend in biological monitoring has led to the development of indices of biological integrity (often referred to as IBIs), for different species assemblages including diatoms (Fore and Grafe 2002); macrophytes (Galatowitsch et al. 1999a; Gernes and Helgen 1999; Mack 2001; Lane 2003); macroinvertebrates (Kerans and Karr 1994; Barbour et al. 1996b); fish (Schulz et al. 1999); and birds (O'Connell et al. 1998). Such indices have been applied to ecosystems throughout the world including in Europe (Kelly and Whitton 1998); widely throughout the United States (Karr 1981; Lenat 1993; Fore and Grafe 2002; Lane et al. 2003); and is beginning in Australia by J.E. Ling of the Royal Botanical Gardens, University of Western Sydney. The primary aim of biological monitoring is to detect changes in abundance, structure, and diversity of target species assemblages. Danielson (1998a) notes that biological signals are effective mainly because biological monitoring incorporates changes from various collective constant or pulsing sources.

Many studies have created multi-metric indices of biological condition, incorporating individual metrics into a quantitative value of community condition or ecosystem integrity. Karr and Chu (1997) defined metrics as biological attributes that have a consistent and predictable response to anthropogenic activities. Anthropogenic activities can alter the integrity of wetland ecosystems by causing one or more of the following conditions: eutrophication, contaminant toxicity, acidification, salinization, sedimentation, burial, thermal alteration, vegetation removal, turbidity, shading, dehydration or inundation, and/or habitat fragmentation (Danielson 1998a).

### *Diatoms as Biological Indicators*

Diatoms are unicellular or colonial algae with siliceous bodies. They are an important basis of wetland food webs and because they drive many wetland functions through their primary production, they are considered valuable in wetland biological assessment (Cronk and Fennessy 2001; Stevenson 2001). The USEPA (2002b) described six fundamental ecosystem functions of algae within water bodies: provide a food source for organisms at higher trophic levels, contribute to nutrient and biogeochemical cycling, oxygenate the water column, regulate water chemistry, create habitat for other organisms, and act as physical barriers to erosion. Because of their rapid turnover times, algae have a short response time to perturbations including nutrient and toxic contaminant inputs; and algae continue production throughout the winter, taking advantage of available nutrients when higher plants are dormant (Cronk and Fennessy 2001). While the standing stock of algae is typically lower than that of the macrophyte assemblage, algae can constitute a higher proportion of primary productivity within an aquatic community (Cronk and Fennessy 2001). These factors and others contribute to the utility of the algal assemblage for biological assessment. Among the main advantages of using algae for biological assessment include the high diversity within the algal community (particularly of diatom species) in aquatic environments (Stevenson 2001).

There is a depth of knowledge as to the sensitivity of many species to different environmental conditions based on their autecological characteristics, including two published tables of autecological relationships by Bahls (1993) and van Dam et al. (1994). Additionally, the rapid-response time of the algal community to changing environmental conditions is a major advantage to their use as biological indicators (Cronk and Fennessy 2001), as well as an overlap in the species present among different aquatic environments (van Dam et al. 1994; Fore and Grafe 2002). Diatoms in particular are considered easy to identify based on well-established taxonomic keys of their decay resistant siliceous structures (Stevenson et al. 1999), and there are well-tested protocols for sampling aquatic habitats (Goldsborough 2001).

Few significant disadvantages of using algae in biological assessment methodologies have been described. Among them is the necessity of a high-powered microscope for identification (Doherty et al. 2000), though identification is relatively easy, and good taxonomic keys have been established (Stevenson et al. 1999). However, taxonomic identification to lower taxonomic levels such as genus or species should be reserved for an expert taxonomist. Additionally, while most algae are not readily motile, wind and current translocation can complicate assessments based on scales of anthropogenic activity in the surrounding landscape. The third noted disadvantage includes natural seasonal variations in abundance and morphology (Vymazal and Richardson 1995).

Overall, algae are considered a valuable assemblage for assessing the biological condition of wetlands. In particular, diatoms are noted as a useful assemblage (Stevenson 2001; Doherty et al. 2000). Previous research has correlated the response of diatoms in streams, lakes, and wetlands, to changes in surrounding land use and to changes in water-column characteristics including nutrient loading (van Dam et al. 1994); pH (Pan and Stevenson 1996); heavy metal loading (Charles et al. 1996); and saprobity levels (Lange-Bertalot 1979). The USEPA (2002b) reported that diatoms are one of the most

commonly used assemblages in aquatic ecosystems for assessing biological, physical, and chemical conditions.

Research correlating changes in the diatom community composition to changes in their aquatic environment has been undertaken for isolated freshwater marshes in Florida (Lane 2003); large rivers in Idaho (Fore and Grafe 2002); streams (Barbour et al. 1999; Winter and Duthie 2000; Munn et al. 2002); depressional wetlands in Michigan (Pan and Stevenson 1996; Stevenson et al. 1999); prairie potholes (Adamus 1996); Mid-Atlantic streams (Pan et al. 1996); the Florida Everglades (Raschke 1993); and Florida lakes (Whitmore 1989). Most of the quantitative biological indices based on diatom community composition have been constructed for rivers and streams (Bahls 1993; Stevenson and Wang 2001).

In a study of isolated freshwater marshes in peninsular Florida, Lane et al. (2003) incorporated 14 metrics into the Diatom Index of Wetland Condition (DIWC). These included tolerant indicator species, sensitive indicator species, diatoms requiring low pH, diatoms requiring low salinity, diatoms tolerant of high salinity, diatoms tolerant of high pH, diatoms sensitive to high nitrogen, diatoms tolerant of high nitrogen, diatoms requiring elevated dissolved oxygen, diatoms tolerant of low dissolved oxygen, meso- and polysaprobious diatoms, diatoms characteristic of oligotrophic environments, diatoms characteristic of eutrophic environments, and pollution-tolerant diatoms. Environmental parameters correlating with diatom community composition included specific conductivity, water-column pH, water ammonia-nitrogen concentration, water total Kjeldahl nitrogen (TKN) concentration, water total phosphorus concentration (TP), soil pH, and soil TP (Lane 2003).

In a study of lotic (relating to moving water) systems in the Mid-Atlantic States, Pan et al. (1996) found the strongest correlation with diatom community composition and changes in water-column pH. Additional water-column parameters correlating with diatom community composition included turbidity, aluminum concentration, chlorine concentration, TP, total suspended solids, and dissolved organic-carbon concentration. Similarly, in a study of emergent permanently flooded floodplain wetlands in western Kentucky, Pan and Stevenson (1996) found significant correlations between diatom community composition and eight water variables, including alkalinity, conductivity, ammonia-nitrogen concentration, pH, silicon concentration, nitrate-nitrogen concentration, chlorine concentration, and TP. Another study of streams in Michigan also correlated the response of diatom community composition to different land use and water physical and chemical parameters (Stewart et al. 1999). These findings reiterate that usefulness of the algal assemblage in reflecting changes in the water environment.

### ***Macrophytes as Biological Indicators***

Wetland macrophytes are defined as aquatic emergent, submergent, or floating plants growing in or near water (USEPA 1998) and are described as distinguishing landscape features. The spatial distribution of macrophytes in the landscape occurs according to a multitude of factors, including hydroperiod, water chemistry, and substrate type, as well as other broader factors such as available seed source and climate. Fennessy et al. (2001) state that the community composition of wetland macrophytes typifies the physical, chemical, and biological wetland dynamics in time and space. Macrophytes



play a vital role in supporting the structure and function of wetlands by providing food and habitat for other assemblages including algae, macroinvertebrates, fish, amphibians, reptiles, birds, and mammals. As such, macrophyte populations can be used as diagnostic tools to assess other aspects of the wetland ecosystem. Crowder and Painter (1991) state that a lack of macrophytes where they are otherwise expected to grow suggests reduced wildlife populations from lack of food or cover and/or water quality concerns such as toxic chemical constituents, increased turbidity, or increased salinity. In contrast, an overgrowth of particular macrophytes may signify increased nutrient loading (USEPA 1998).

Many advantages of studying macrophytes as indicators of wetland condition have been noted, including their large, obvious size; ease of identification, to at least some useful taxonomic level; known response to toxicity tests; and general lack of ability to move to avoid unfavorable conditions (Danielson 1998a; Cronk and Fennessy 2001). Additionally, macrophytes readily respond to changes in nutrient, light, toxic contaminant, metal, herbicide, turbidity, water, and salinity levels. They can also be sampled in the field with transects, or from the office with aerial photography, and well-established field methods of sampling macrophytes exist (USEPA 2003). Furthermore, the USEPA (2003) states that macrophytes do not require laboratory analysis, can easily be used for calculating simple abundance metrics, and are superb integrators of environmental condition. In general, macrophytes represent a useful assemblage for describing wetland condition (Mack 2001). Schindler (1987) alleged that macrophytes can provide a more integrated picture of wetland function than static measures such as nutrient cycling, productivity, decomposition, or chemical and physical composition.

There are however some noted shortcomings of using macrophytes as biological indicators. These include the potential delay in response time for perennial vegetation, difficulty identifying taxa to the species level in certain seasons and for some genera, different herbivory patterns, and varied pest-management practices (Cronk and Fennessy 2001). Despite these limitations, macrophytes have provided strong signals of anthropogenic influence (USEPA 2003). In fact, many states have begun using macrophytes in their wetland biological assessment programs, including Minnesota (Galatowitsch et al. 1999a; Gernes and Helgen 1999), Montana (Apfelbeck 2000), North Dakota (Mushet et al. 2002), and Ohio (Mack 2001).

Previous biological assessment studies have included unique and varied macrophyte metrics dependent on wetland type and bioregion. Lane et al. (2003) calculated five metrics based on the macrophyte community composition for inclusion in the marsh Vegetative Index of Wetland Condition (VIWC). The five core metrics of the VIWC included tolerant indicator species, sensitive indicator species, exotic species, annual to perennial ratio, and average coefficient of conservatism score (Lane 2003). In Minnesota, Vegetative Indices of Biotic Integrity (V-IBIs) have been created for eight wetland types (Galatowitsch et al. 1999a), and included 15 metrics for high-order river floodplain wetlands, 12 for low-order river floodplain wetlands, eight for mid-order river floodplain wetlands, seven for calcareous littoral wetlands, six for noncalcareous littoral wetlands, seven for wet prairie-sedge meadows, four for forest glacial marshes, and a single metric for prairie glacial marshes. Another comprehensive biological assessment used to construct multi-metric indices of biotic integrity for Ohio wetlands was designed by Mack et al. (2000). Separate biological multi-metric indices were developed for

emergent, forested, and shrub wetlands. Twelve metrics were incorporated, including *Carex* species, dicot species, shrub species, hydrophyte species, Rosaceae species, Floristic Quality Assessment Index (FQAI), tolerant species, intolerant species, invasive graminoids, shrub density, small-tree density, and maximum importance value.

The FQAI has been included in many of the multi-metric indices created for the macrophyte assemblage. The concept of FQAI was developed by Wilhelm and Ladd (1988) for vegetation around Chicago, Illinois. This method of scoring plant species based on expert botanist opinion has been used in Michigan (Herman et al. 1997), Ohio (Andreas and Lichvar 1995; Fennessy et al. 1998; Mack 2001), Ontario (Francis et al. 2000), North Dakota (Mushet et al. 2002), and Florida (Lane et al. 2003; Cohen et al. 2004). The FQAI provides a quantitative means of assessing the fidelity of a plant to a particular environment through the Delphi technique (Kent 2000), where individual botanists assign coefficients to each species, and then reevaluate their scores based on the group mean scores. This technique assumes that the collective decision by a group of expert botanists is more accurate than the professional judgment of one individual (Kent 2000).

### ***Macroinvertebrates as Biological Indicators***

Biological assessment based on the macroinvertebrate assemblage has been widely applied for indications of environmental quality, and often more specifically water quality (Lenat 1993; Cummins and Merritt 2001). Invertebrates participate in many fundamental ecological processes, including the breakdown of organic matter and recycling of nutrients. Additionally, invertebrates are a vital component of the food web, making up a large portion of the diets of other organisms (such as fish, amphibians, and birds) (Cummins and Merritt 2001; Helgen 2001). In fact, Voshell (2002) recognized that freshwater invertebrates have been used more often than any other group of organisms for assessing freshwater ecosystems.

A great deal of research has been completed on the specific ecology of lotic macroinvertebrates (of, relating to, or living in flowing waters), though less is known about lentic macroinvertebrates (of, relating to, or living in still waters). Williams and Feltmate (1992) noted that, while not well studied, the communities of aquatic insects in wetlands include species from most of the major aquatic groups. The community composition of wetland macroinvertebrates differs from that of flowing waters, because of differences in substrate, dissolved-oxygen level in the water column, hydroperiod, and annual water fluctuations. Macroinvertebrates have been useful indicators of environmental condition in streams; and Karr and Chu (1997) speculate macroinvertebrates also may be appropriate indicators of environmental integrity in wetlands.

Since 1997, the use of the macroinvertebrate assemblage for biological assessments has been initiated in 48 states for lakes and streams (Karr and Chu 1999). Macroinvertebrate-based wetland biological assessment methodologies have been initiated in many states, including Florida (Lane et al. 2003), Minnesota, Montana, North Dakota, and Ohio (Danielson 1998b). Within the state of Florida and throughout the southeastern Coastal Plain, ecological research on the macroinvertebrate community has included many ecosystem types from isolated marshes of peninsular Florida (Kushlan

1990; Lane 2003); isolated wetlands in south Florida (Stansly et al. 1997); southeastern wetlands (Pickard and Benke 1996); nontidal wetlands (Batzer and Wissinger 1996); sloughs of the northern Everglades (Rader and Richardson 1992; Rader and Richardson 1994); floating islands in Orange Lake in north central Florida (Haag et al. 1987); and bottomland hardwood swamps (Wharton et al. 1982).

Doherty et al. (2000) conclude that the structure and function of the macroinvertebrate community accurately reflects the biological condition of a wetland, and that the macroinvertebrate community composition changes in predictable ways with increased human influence. Because wetland macroinvertebrates complete part or all of their lives in the wetland, they are directly exposed to conditions in the wetland water and soils (Merritt and Cummins 1996; Helgen 2001). Also, because of the short length of their life cycles (compared to most macrophytes and vertebrates), Stansly et al. (1997) noted that macroinvertebrates respond quickly to changes in the physical, chemical, or biological parameters of their host environment. Their quick response time, reliance on water (both for the water quality and duration of inundation), and ease of collection make macroinvertebrates a favorable assemblage for use as biological indicators. Noted disadvantages to using macroinvertebrates include the amount of time and knowledge necessary for identification to lower taxonomic levels (Cummins and Merritt 2001).

The Florida Department of Environmental Protection (FDEP) has initiated the development of biological indices based on the macroinvertebrate assemblage for freshwater bodies in Florida. Macroinvertebrate-based biological indices have been created for isolated marshes through the Macroinvertebrate Index of Wetland Condition (MIWC; Lane et al. 2003), for Florida streams through the Stream Condition Index (SCI; Barbour et al. 1996a; Fore 2003), for surface waters in south Florida canals using SCI protocol (Snyder et al. 1998), for the evaluation of restoration in the Kissimmee River Basin (Merritt et al. 1996), and in freshwater lakes through the Lake Condition Index (LCI; Gerristen and White 1997). Different core metrics comprise each multi-metric biological index.

Lane (2003) incorporated five core metrics as biological indicators of wetland condition for isolated marshes in the MIWC, including sensitive taxa, tolerant taxa, predators, Odonata, and Orthocladinae. The SCI was developed with ten core metrics within six categories of biological organization including were taxonomic richness (total number of taxa, number of Trichoptera taxa, and number of Ephemeroptera taxa); feeding group (percentage filterer individuals); voltinism (long-lived taxa richness); habit (clinger taxa richness); community structure (percentage dominance of the most abundant taxon and percentage Tanytarsini midges); and sensitivity and tolerance (sensitive taxa richness and percentage very tolerant individuals) (Fore 2004). The LCI incorporated seven metrics, including taxa richness, Shannon diversity, Hulbert index, ETO taxa (Ephemeroptera, Trichoptera, and Odonata), percent dominance, filter feeders, and gatherers (Gerristen and White 1997).

Numerous studies have documented the response of the benthic macroinvertebrate community to anthropogenic activities. Two primary areas of research include changes in trophic state, and additions of stormwater and wastewater. Gerristen and White (1997) and Cairns and Pratt (1993) found that the benthic macroinvertebrate community composition responded to changes in trophic status. In the northern Everglades, Rader and Richardson (1994) found that macroinvertebrates responded to nutrient enrichment

with a greater number of Coleopteran species present in nutrient-enriched and intermediate areas (especially those in the families Hydrophilidae and Dysticidae) than in non-enriched areas. With shifts in trophic status, the structure of other assemblages also changed, affecting the benthic macroinvertebrate community composition. For example, Adamus and Brandt (1990) found that shading from dense stands of emergent vegetation altered the distribution among functional feeding groups by limiting the production of benthic algae (thus favoring detritivores over grazers). De Szalay and Resh (1996) similarly found that increased shading caused fine particulate organic matter to settle out, making rich detritus accessible to support a large population of benthic macroinvertebrate detritivores.

While adding stormwater and wastewater alters the natural hydrology of an isolated wetland, it also increases the inflow of nutrients, sediments, and toxic metals. Harris and Vickers (1984) found that adding wastewater to pondcypress domes shifted the macroinvertebrate community toward a less-complex trophic structure. Similarly, when wastewater was directed into Florida pondcypress domes, *Lemna* spp. (duckweed) mats covered the water surface, blocking sunlight from the water column, and creating anoxic conditions (Dierberg and Brezonik 1984). This reduced the diversity and biomass of benthic invertebrates, leaving only a few pollution-tolerant organisms (Brightman 1984). In Florida streams, Barbour et al. (1996b) found that the occurrence of tubificid oligochaetes increased with organic enrichment.

Other studies have focused on the effects of adding stormwater to freshwater wetlands. Freshwater marshes in Savannas Preserve State Park, Florida, receiving stormwater additions showed increased phosphorus levels, lowered oxygen levels, increased water-column pH and hardness, and a change in the macroinvertebrate community toward pollution-tolerant species and those intolerant of the typical acidic and oligotrophic environment (Graves et al. 1998). Barbour et al. (1996b) reported that some chironomids of the family Orthocladiinae, including those in the genus *Cricotopus*, were found to be tolerant of metal pollution; while other Orthocladiinae, including *Rheocricotopus* spp. and *Corynoneura* spp., were thought to be sensitive to metal pollution in Florida streams.

Aside from the nutrient, sediments, and toxics loading, wastewater or stormwater additions also alter the water level and hydroperiod of a wetland system, which affects the macroinvertebrate community composition. Toth (1993) reported a response of the macroinvertebrate community to water-level manipulation in a Kissimmee River demonstration project. Hydroperiod had a distinct influence on community composition, as some macroinvertebrates either temporarily relocated or coped with behavioral and biological adaptations to changing water conditions. Macroinvertebrates with adaptations to wetland hydroperiods demonstrate both behavioral and physiological adaptations for draw-down conditions. For example, in south Florida hydric flatwoods, Gore et al. (1998) found that *Crangonyx* spp. and several other aquatic insects burrowed into moist sediments to avoid desiccation.

Some macroinvertebrates are thought to be indicative of water level and seasonality, with *Caenis* spp., *Anax* spp., *Libellula* spp., and *Pantala* spp. indicative of persistent water; some Chironomus, some Tanytarsus, *Beardius* spp., and *Zavreliella marmorata*, indicative of permanent standing water; and *Ablabesmyia rhamphe* grp., *Krenopelopia* spp., and *Tanytarsus* sp. g. indicative of ephemeral wetlands (Doherty et al.

2000). Stansly et al. (1997) concluded that in isolated wetlands of south Florida, the presence of macroinvertebrates with long life cycles or predatory behavior may indicate hydroperiod stability. Snyder et al. (1998) found that macroinvertebrates with comparatively short life cycles that are capable of rapid colonization were typical of canals surrounded by urban land uses, whereas the occurrence of macroinvertebrates with longer life cycles were more common in canals surrounded by more natural landscapes.

### **Quantifying Anthropogenic Influence**

Wetlands occupy a large portion of the Florida landscape. An estimate from the 1780s reported 8,225,000 ha of wetlands in Florida (Dahl 2000). By the mid-1980s, the National Wetlands Inventory estimated Florida had 4,467,000 ha of wetlands remaining, translating into a loss in Florida of 46% of the pre-1780s wetland area (Mitsch and Gosselink 1993; Dahl 2000). Throughout the continental United States, similar trends were apparent, with a drastic decline in the surface area of wetlands. Dahl (2000) reported that 98% of all wetland losses throughout the continental United States from 1986 to 1997 were losses to freshwater wetlands. Of the remaining freshwater wetlands, 40% of those wetlands sampled were adjacent to agricultural lands and therefore potentially affected by land use practices such as herbicide and pesticide application, irrigation, livestock watering and wastes, soil erosion, and deposition. An additional 17% of the remaining wetlands were adjacent to urban or rural development. Freshwater non-tidal wetlands experienced the greatest development pressure just inland from coastlines as the demand for housing, transportation infrastructure, and commercial and recreational facilities increased (Dahl 2000). These changes in land use are proportionally more widespread in Florida than much of the continental United States due to the remarkable length of coastline along both the Atlantic Ocean and Gulf of Mexico coasts of Florida. Spanning the populated coasts from Jacksonville to Miami on the east coast and from Naples to Tampa along the west coast, most coastal counties in Florida are reported to have high wetland loss of non-tidal freshwater wetlands from 1986 to 1997 (Dahl 2000). Dahl (2000) suggested that many of these wetlands were harvested and succeeded into shrub wetlands.

Anthropogenic activities can influence an array of changes to the physical, chemical, and biological characteristics in surrounding ecosystems. There have been numerous attempts at quantifying anthropogenic influence based on varying scales. Three primary indices of anthropogenic influences were incorporated throughout our study to compare wetland condition, including the Landscape Development Intensity (LDI) index (Brown and Vivas 2005), the Wetland Rapid Assessment Procedure (WRAP; Miller and Boyd 1999), and the Minnesota disturbance index (Gernes and Helgen 1999).

#### ***Landscape Development Intensity Index***

The LDI index has been used as a gauge of human activity based on a development intensity measure derived from nonrenewable energy use in the surrounding landscape. The underlying concept behind calculating the LDI (quantifying the nonrenewable energy use per unit area in the surrounding landscape) stems from earlier

works by Odum (1995), who pioneered emergy analysis for environmental accounting. [Emergy is an environmental accounting term referring to expressing energy use in solar equivalents (Odum 1995).] Brown and Ulgiati (2005) suggest that landscape condition, or ecosystem health, is strongly related to the surrounding intensity of human activity, and that ecological communities are affected by the direct, secondary, and cumulative impacts of activities in the surrounding landscape. Healthy ecosystems are defined as those with integrity and sustainability, which correlate to limited development in the surrounding landscape and the maintenance of ecosystem structure and function, even when stressors (e.g. flooding, drought, etc.) are present (Brown and Ulgiati 2005).

The LDI does not account for any individual causal agent directly, but instead, may represent the combined effects of air and water pollutants, physical damage, changes in the suite of environmental conditions (ex. groundwater levels, increased flooding), or a combination of such factors, all of which enter the natural ecological system from the surrounding developed landscape (Brown and Vivas 2005). Wetlands surrounded by more intense activities such as highways and multi-family residential land uses receive higher LDI index values. The highest LDI coefficient of 10.0 is assigned to the urban land use category of Central Business District. Undeveloped land uses such as wetlands, lakes, and upland forests, are assigned an LDI coefficient of 1.0, the lowest possible value, based on no use of nonrenewable energy in these ecosystems.

### ***Wetland Rapid Assessment Procedure***

The Wetland Rapid Assessment Procedure (WRAP) was designed to provide accurate and consistent evaluations of wetland sites, and relies on an evaluator with an adequate understanding of the functions of and species found throughout Florida ecosystems (Miller and Gunsalus 1999). WRAP consists of a qualitative score describing the functional capacity of a wetland. Scores range from 0.0 to 3.0, in 0.5 increments. The primary scoring categories for WRAP include: 1) Wildlife utilization; 2) Overstory/shrub canopy; 3) Vegetative ground cover; 4) Adjacent upland support/buffer; 5) Field indicators of wetland hydrology; and 6) Water quality input and treatment. A score of 3.0 indicates an “intact” wetland, whereas a score of 0.0 indicates a wetland with a reduced functional capacity (Miller and Gunsalus 1999).

### ***Minnesota Disturbance Index***

The Minnesota disturbance index is considered a gradient of human disturbance, or a measure of land use disturbance based on investigator knowledge, observations, and best professional judgment about the degree of influence to the ecosystem. Gernes and Helgen (1999) used the Minnesota disturbance index as a baseline for creating an index of vegetative biotic integrity for depressional wetlands. There are two primary categories and three secondary categories used to calculate the Minnesota disturbance index score. The primary categories include stormwater and agricultural influence, and are weighted twice as high as the secondary categories. Wetlands receive scores assigned according to significantly affected (S = 8), moderately affected (M = 4), least affected (L = 2), and not applicable (NA = 0) depending on the scorers opinion as to the degree of influence. Wetlands only receive a score in one of the primary categories, and reference standard

wetlands receive a score of zero in both primary categories. The three secondary categories include hydrologic/miscellaneous influence, historical influence, and buffer, receiving scores of significantly affected (S = 3), moderately affected (M = 2), least affected (L = 1), and not applicable (NA = 0). Wetlands can receive scores in all of the secondary categories, with possible scores ranging from 0 to 17, with a score of 0 representing the reference standard condition.

### **Project Overview**

Physical and chemical environmental parameters and the community composition of the diatom, macrophyte, and macroinvertebrate assemblages were sampled to develop the Florida Wetland Condition Index (FWCI) for isolated depressional forested wetlands for Florida. Wetland study sites were sought in various landscape settings that included undeveloped, agricultural, and urban land uses. Compositional differences in the diatom, macrophyte, and macroinvertebrate assemblages among wetlands were identified and related to the Landscape Development Intensity (LDI) index, a quantitative calculation of anthropogenic activity in the landscape surrounding a wetland which is based on a development intensity measure derived from nonrenewable energy use in the surrounding landscape. Each assemblage was used to construct the three assemblage specific Florida Wetland Condition Indices (FWCIs) for isolated depressional forested wetlands in Florida.

## CHAPTER 2 METHODS

Biological, physical, and chemical parameters were sampled in 118 forested wetlands less than 2 ha in size. This chapter describes site selection, calculations of the gradient of anthropogenic activity including the Landscape Development Intensity (LDI) index, field-data collection, and laboratory analyses. Statistical analyses are described for each assemblage and for the creation of the assemblage specific Florida Wetland Condition Indices (FWCIs).

### Site Selection

Field research spanned two growing seasons with 72 wetlands sampled between May-September in 2001 and an additional 46 wetlands sampled between May-October in 2002. Figure 2-1 shows the location of the 118 sample wetlands indicated by generalized *a priori* land use categories (reference, agricultural, urban). Hereafter wetlands surrounded by greater than 50% undeveloped landscapes were called reference (short for reference standard), wetlands surrounded by greater than 50% agricultural land uses were called agricultural and wetlands surrounded by greater than 50% urban land uses were called urban.

Random site selection was not feasible given the necessity of obtaining permission to access private lands and the non-random pattern of land development in Florida. Site selection for agricultural wetlands was accomplished with the aide of the Natural Resources Conservation Service under the United States Department of Agriculture and University of Florida Institute of Food and Agricultural Sciences extension agents. Sample wetlands were targeted spatially throughout Florida, so that a nearly equal distribution of wetlands was sampled within each of the four Florida wetland region. Boundaries of the Florida wetland regions were determined with a hydrologic model by Lane (2000). Florida freshwater palustrine wetlands were classified using a hierarchical classification technique, and physical (surficial geology, soils, digital elevation model, slope) and climatic (precipitation, potential evapotranspiration, runoff, annual days of freezing) variables were tested for correlation with wetland clusters. Final wetland region boundaries were based on a spatial water balance model.

The number of wetlands sampled per *a priori* land use category per region varied, with 28 wetlands in the south (n = 9 reference, n = 9 agricultural, n = 10 urban), 31 in the central (n = 11 reference, n = 9 agricultural, n = 11 urban), 31 in the north (n = 9 reference, n = 12 agricultural, n = 10 urban), and 28 in the panhandle (n = 8 reference, n = 10 agricultural, n = 10 urban) wetland regions. All 72 wetlands sampled in 2001 and 39 (of the 46) wetlands sampled in 2002 hosted *Taxodium ascendens* (poncycypress) as a dominant or co-dominant canopy species. The remaining seven wetlands sampled in 2002 had a canopy layer comprised of a mixture of species that was not dominated or co-dominated by *Taxodium ascendens*. These wetlands exhibited the characteristic depressional shape in the landscape and from GIS based digital orthophotography. Wetlands surrounded by natural landscapes were generally located on conservation lands



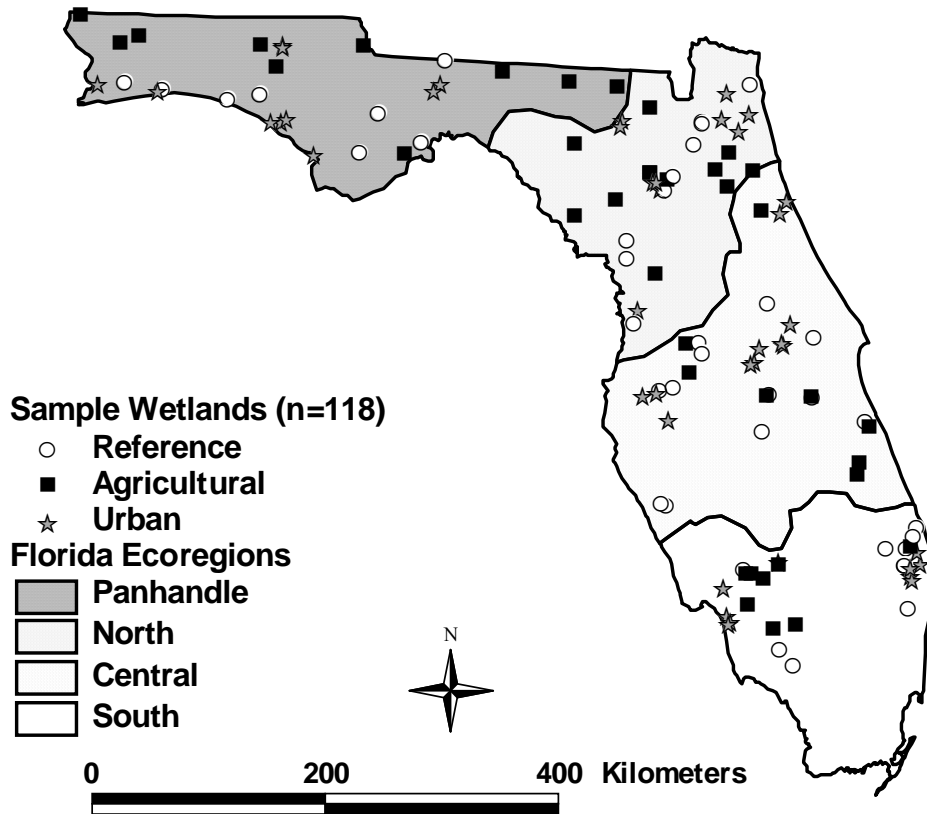


Figure 2-1. Study site location of 118 isolated depressional forested wetlands in Florida. The state of Florida was separated into four ecoregions (Lane 2000). Sample wetlands were designated by *a priori* surrounding land use categories: ○ reference, ■ agricultural, or ★ urban.

including state and national parks and forests, county and city lands, and private conservation tracts. Wetlands currently surrounded by cattle pasture, row crops, citrus, and silvicultural land uses were included in the agricultural *a priori* land use category. Urban wetlands located in an urban land use matrix for the longest period of time were given priority for sampling. However, due to the widespread historic loss of wetlands throughout Florida (FDNR 1988) and early incentives to drain swamp lands, few isolated depressional forested wetlands were found in the oldest urban areas. Many of the urban wetlands sampled were suspected to previously been embedded in agricultural land uses.

Table 2-1 provides some general information about each sample wetland, including sample date, surrounding land use, and land ownership. The sample date provided is the earliest sample date, and correlates to macrophyte sampling. A minimum water level of 10 cm was standardized to ensure sampling did not occur immediately following a small rain event or too soon after initial hydration for the growing season, which would not allow the biological assemblages dependent on inundation time to respond. Wetlands sampled without sufficient standing water were revisited later in the field season once the wetlands held at least 10 cm of water. Table 2-2 identifies data collected at each wetland. Site codes reflect the wetland region (S = south; C = central;

Table 2-1. Surrounding land use, land ownership, and sample date for 118 study wetlands in Florida.

Site Code*	Sample Date	Surrounding Land Use^	Land Owner	Site Code*	Sample Date	Surrounding Land Use^	Land Owner
SA1	6/5/01	Cattle & Crops	Public	CR3	6/20/01	State Park	Public
SA2	6/6/01	Citrus	Private	CR4	8/10/01	WMD	Public
SA3	6/27/01	Cattle	Public	CR5	8/13/01	State Park	Public
SA4	7/30/01	Crops	Private	CR6	8/15/01	State Forest	Public
SA5	7/31/01	Cattle & Crops	Public	CR7	5/30/02	City Owned	Public
SA6	9/5/01	Cattle	Private	CR8	7/2/02	State Forest	Public
SA7	7/31/02	Woodland	Public	CR9	7/11/02	State Preserve	Public
SA8	7/31/02	County Park	Public	CR10	10/9/02	State Park	Public
SA9	8/1/02	Cattle	Private	CR11	10/9/02	State Park	Public
SR1	6/28/01	County Park	Public	CU1	5/31/01	Univ. Campus	Public
SR2	7/3/01	State Park	Public	CU2	6/15/01	Residential	Private
SR3	7/24/01	State Reserve	Public	CU3	7/16/01	Commercial	Private
SR4	8/1/01	National Park	Public	CU4	8/14/01	Road Side	Private
SR5	8/21/01	State Preserve	Public	CU5	9/11/01	Road Side	Private
SR6	9/18/01	NWR	Public	CU6	9/12/01	Golf Course	Private
SR7	7/15/02	County Park	Public	CU7	5/30/02	City Owned	Public
SR8	7/17/02	County Airport	Public	CU8	7/1/02	Industrial	Private
SR9	7/24/02	County Park	Public	CU9	7/8/02	Commercial	Private
SU1	6/6/01	Resid. & Golf	Private	CU10	8/7/02	Park	Public
SU2	6/29/01	School Campus	Public	CU11	8/8/02	Park	Public
SU3	7/4/01	Residential	Public	NA1	5/21/01	Cattle	Public
SU4	8/22/01	Residential	Private	NA2	6/4/01	Cattle	Private
SU5	8/23/01	Industrial	Private	NA3	6/19/01	Silviculture	Public
SU6	9/30/01	Industrial	Private	NA4	7/20/01	Crops	Private
SU7	7/16/02	Commercial	Private	NA5	7/27/01	Cattle	Private
SU8	7/16/02	Comm. & Res.	Private	NA6	7/31/01	Silv., Cat.,Crops	Private
SU9	7/23/02	Residential	Private	NA7	5/22/02	Crops	Public
SU10	7/30/02	Roads & Canals	Public	NA8	5/21/02	Silviculture	Private
CA1	5/23/01	Crops	Private	NA9	6/10/02	Silviculture	Ease.
CA2	5/30/01	Cattle	Private	NA10	7/12/02	Silviculture	Ease.
CA3	6/7/01	Pullet Farm	Private	NA11	7/24/02	Cattle	Public
CA4	6/21/01	Cattle	Public	NA12	7/26/02	Cattle & Crops	Public
CA5	7/10/01	Cattle	Private	NR1	5/26/01	University Land	Public
CA6	7/23/01	Citrus	Private	NR2	6/18/01	City Park	Public
CA7	7/3/02	Silv. & Cattle	Public	NR3	7/10/01	State Forest	Public
CA8	7/19/02	Dairy Farm	Public	NR4	7/11/01	WMD	Public
CA9	7/24/02	Citrus	Private	NR5	8/6/01	Military	Private
CR1	5/30/01	Conserv. Tract	Private	NR6	8/21/01	State Park	Public
CR2	6/14/01	Conserv. Tract	Private	NR7	5/28/02	State Park	Public

Table 2-1. Continued.

Site Code*	Sample Date	Surrounding Land Use <sup>^</sup>	Land Owner	Site Code*	Sample Date	Surrounding Land Use <sup>^</sup>	Land Owner
NR8	8/5/02	State Park	Public	PA9	8/13/02	Row Crops	Public
NR9	8/29/02	State Forest	Public	PA10	8/14/02	Silviculture	Public
NU1	5/22/01	Road Side	Private	PR1	6/15/01	National Forest	Public
NU2	6/11/01	Resid. & Golf	Private	PR2	7/3/01	WMD	Public
NU3	6/26/01	Residential	Private	PR3	7/4/01	Military	Public
NU4	6/27/01	Residential	Private	PR4	8/9/01	State Forest	Public
NU5	6/28/01	Residential	Private	PR5	8/10/01	State Forest	Public
NU6	8/1/01	Resid. & Inst.	Private	PR6	8/18/01	National Forest	Public
NU7	5/15/02	Comm. & Res.	Private	PR7	6/4/02	Conserv. Tract	Private
NU8	6/3/02	Res. & Golf	Private	PR8	8/7/02	NWR	Public
NU9	6/12/02	Industrial	Private	PU1	6/14/01	Residential	Private
NU10	7/29/02	Res. & Instit.	Private	PU2	7/5/01	Residential	Private
PA1	5/24/01	Cattle	Private	PU3	8/17/01	Res. & Comm.	Private
PA2	5/29/01	Cattle	Private	PU4	8/17/01	Res. & Park	Private
PA3	7/3/01	Crops/Turf Grass	Public	PU5	9/28/01	Comm. & Silv.	Private
PA4	7/2/01	Crops	Private	PU6	9/29/01	Commercial	Private
PA5	8/8/01	Cattle	Private	PU7	6/18/02	Res. & Orchard	Private
PA6	8/9/01	Cattle	Private	PU8	6/19/02	Ind. & Silv.	Private
PA7	6/5/02	Cattle	Private	PU9	6/20/02	Residential	Private
PA8	8/8/02	Silviculture	Public	PU10	7/25/02	Institutional	Private

\*Site Codes correspond to the region, land use category, and sample order: S = south, C = central, N = north and P = panhandle; R = reference, A = agricultural, and U = urban.

<sup>^</sup>Surrounding Land Use abbreviations: NWR = National Wildlife Refuge; WMD = Water Management District; Res. = Residential; Cat. = Cattle; Comm. = Commercial; Inst. = Institutional; Ind. = Industrial; Crops = Row Crops; Silv. = Silviculture; Ease. = Easement.

N = north; P = panhandle), land use category, and order of sampling. Site codes were assigned to preserve the anonymity of land owners.

### Gradients of Anthropogenic Activity

Three independent indices of anthropogenic activity were calculated including the Landscape Development Intensity (LDI) index, the Wetland Rapid Assessment Procedure (WRAP), and the Minnesota disturbance index (DI). LDI scores were calculated prior to site visits using 1999 digital orthophoto imagery available from Labins, The Land Boundary Information System from the Florida Department of Environmental Protection (FDEP) (available at <http://www.labins.org/2003/index.cfm>). Sample wetlands were delineated from aerial images, and a 100 m buffer was constructed around the edge of each wetland in ArcView GIS 3.2 (Environmental Systems Research Institute, Inc. 1999). Land uses within the 100 m wetland buffer were delineated based on digital orthophoto

Table 2-2. Field-data collected at 118 sample wetlands.

Site	Soil	Water	Diatoms	Macrophytes <sup>^</sup>	Macro-invertebrates	Site	Soil	Water	Diatoms	Macrophytes <sup>^</sup>	Macro-invertebrates	Site	Soil	Water	Diatoms	Macrophytes <sup>^</sup>	Macro-invertebrates
SA1	✓			◇		CR4	✓	✓	✓	●	✓	NU1	✓			◇	
SA2	✓	✓	✓	●	✓	CR5	✓	✓	✓	●	✓	NU2	✓	✓	✓	◇	✓
SA3	✓	✓	✓	◇	✓	CR6	✓	✓	✓	●	✓	NU3	✓			◇	
SA4	✓	✓	✓	●	✓	CR7	✓			◇		NU4	✓	✓	✓	◇	✓
SA5	✓	✓	✓	●	✓	CR8	✓	✓		●	✓	NU5	✓	✓	✓	◇	✓
SA6	✓	✓	✓	●	✓	CR9	✓	✓		●	✓	NU6	✓	✓	✓	◇	✓
SA7	✓	✓		●	✓	CR10	✓	✓		●	✓	NU7	✓			◇	
SA8	✓	✓		●	✓	CR11	✓	✓		●	✓	NU8	✓			◇	
SA9	✓	✓		●	✓	CU1	✓	✓	✓	◇	✓	NU9	✓			◇	
SR1	✓	✓	✓	●	✓	CU2	✓			◇		NU10	✓	✓		●	✓
SR2	✓	✓	✓	●	✓	CU3	✓	✓	✓	●	✓	PA1	✓			◇	
SR3	✓	✓	✓	●	✓	CU4	✓			◇		PA2	✓	✓	✓	◇	✓
SR4	✓	✓	✓	●	✓	CU5	✓	✓	✓	●	✓	PA3	✓	✓	✓	●	✓
SR5	✓	✓	✓	●	✓	CU6	✓	✓	✓	●	✓	PA4	✓			◇	✓
SR6	✓		✓	●	✓	CU7	✓	✓		◇	✓	PA5	✓	✓	✓	●	✓
SR7	✓	✓		●	✓	CU8	✓	✓		●	✓	PA6	✓	✓	✓	●	✓
SR8	✓	✓		●	✓	CU9	✓	✓		●	✓	PA7	✓			◇	
SR9	✓	✓		●	✓	CU10	✓	✓		●	✓	PA8	✓			◇	
SU1	✓	✓	✓	◇	✓	CU11	✓			◇		PA9	✓			◇	
SU2	✓	✓	✓	◇	✓	NA1	✓			◇		PA10	✓			◇	
SU3	✓	✓	✓	●	✓	NA2	✓			◇		PR1	✓		✓	●	✓
SU4	✓	✓	✓	●	✓	NA3	✓			◇		PR2	✓			◇	
SU5	✓	✓	✓	●	✓	NA4	✓	✓		●	✓	PR3	✓			◇	
SU6	✓	✓	✓	●	✓	NA5	✓			◇		PR4	✓	✓	✓	●	✓
SU7	✓	✓		●	✓	NA6	✓	✓	✓	●	✓	PR5	✓	✓	✓	●	✓
SU8	✓	✓		●	✓	NA7	✓			◇		PR6	✓	✓	✓	●	✓
SU9	✓			●	✓	NA8	✓			◇		PR7	✓	✓		●	✓
SU10	✓			◇		NA9	✓			◇		PR8	✓	✓		●	✓
CA1	✓			◇		NA10	✓	✓		●	✓	PU1	✓	✓		◇	
CA2	✓	✓	✓	◇	✓	NA11	✓	✓		●	✓	PU2	✓			◇	
CA3	✓	✓	✓	◇	✓	NA12	✓			◇		PU3	✓	✓	✓	●	✓
CA4	✓	✓	✓	◇	✓	NR1	✓			◇		PU4	✓	✓	✓	●	✓
CA5	✓	✓	✓	●	✓	NR2	✓	✓	✓	◇	✓	PU5	✓			◇	
CA6	✓		✓	●	✓	NR3	✓	✓	✓	◇	✓	PU6	✓	✓		◇	
CA7	✓	✓		●	✓	NR4	✓	✓	✓	●	✓	PU7	✓			◇	
CA8	✓	✓		◇	✓	NR5	✓			◇		PU8	✓			◇	
CA9	✓			●	✓	NR6	✓	✓	✓	●	✓	PU9	✓			◇	
CR1	✓	✓		◇		NR7	✓			◇		PU10	✓	✓		●	✓
CR2	✓			◇		NR8	✓	✓		●	✓						
CR3	✓	✓	✓	◇	✓	NR9	✓	✓		●	✓						

✓ = Data collected

<sup>^</sup> ●-sampled with >10 cm standing water; ◇-sampled with < 10 cm standing water

imagery, and were updated during the site visit to reflect changes in land use since the 1999 images were recorded. The following equation was used to calculate LDI:

$$LDI_{\text{Total}} = \sum \% LU_i * LDI_i \quad (2-1)$$

where %LU<sub>i</sub> is the percent of a land use within wetland buffer and LDI<sub>i</sub> is the LDI coefficient for a particular land use based on the amount of nonrenewable energy use per unit area in the surrounding landscape (Table 2-3). The LDI coefficient values and LDI equation are based on work by Brown and Vivas (2005). Potential LDI scores ranged from a minimum of 1.0 (Natural Land/Open Space) to a maximum of 10.0 (Central Business District).

WRAP was scored during the initial 30 minutes at each study wetland according to descriptions from Miller and Gunsalus (1999). The DI was scored in the office after the field visit by the field crew leader using information obtained from ArcView GIS 3.2 (Environmental Systems Research Institute, Inc. 1999) and field notes according to categories established by Gernes and Helgen (1999). The three measures of anthropogenic activity were compared using the Pearson correlation coefficient.

### **Field-data Collection**

A concise summary of field-data collection procedures follows. Appendix A provides more detailed descriptions of field-data collection techniques in the format of Standard Operating Procedures (SOPs) for field use. Field methods are described as transect establishment followed by water, soil, diatom, macrophyte, and macroinvertebrate sampling techniques.

Figure 2-2 shows the positioning of the four transects established at each wetland that were situated as perpendicular crossing axes running through the center of each wetland. Transect axes always corresponded to the cardinal directions (north, east, south, west). The upland/wetland boundary was determined using the Florida Unified Wetland Delineation Methodology (Chapter 62-340, F.A.C.), using a combination of wetland plant presence according to wetland plant status (e.g. obligate, facultative wetland, facultative, or upland) and wetland hydrologic indicators (e.g. lichen lines, moss collars).

### ***Water Samples***

A grab style water sample was taken in the deepest pool of each wetland when a minimum of 10 cm of standing water was present throughout at least 50% of the wetland surface area. The area with the deepest pool often coincided with the center of each wetland as depicted in Figure 2-2. Water samples were collected at 75 wetlands, including 14 in the panhandle wetland region (reference n = 5; agricultural n = 4; urban n = 5), 14 in the north wetland region (reference n = 6; agricultural n = 3; urban n = 5), 23 in the central wetland region (reference n = 9; agricultural n = 6; urban n = 8), and 24 in the south wetland region (reference n = 8; agricultural n = 8; urban n = 8).

Dissolved oxygen and water temperature were taken on-site using an YSI-55 Dissolved Oxygen hand meter. Grab water samples were sent to the FDEP Central Chemistry Laboratory, Tallahassee, Florida. Analysis included color (EPA 110.2), turbidity (EPA 180.1), pH (150.1), specific conductance (EPA 120.1), ammonia- nitrogen

Table 2-3. Landscape Development Intensity coefficients (LDI<sub>i</sub>) used in the calculation of the Landscape Development Intensity (LDI) index.

Land Use (i)	Nonrenewable Energy Use (E14 solar equivalent joules/ha/yr)	LDI <sub>i</sub>
Natural Land / Open Water	1.0	1.0
Pine Plantation	5.1	2.0
Low Intensity Open Space / Recreational	6.7	2.1
Unimproved Pastureland (with livestock)	8.3	2.6
Improved Pasture (no livestock)	19.5	3.7
Low Intensity Pasture (with livestock)	36.9	4.5
Medium Intensity Open Space / Recreational	51.5	4.8
High Intensity Pasture (with livestock)	65.4	4.9
Citrus	67.3	5.2
Row crops	117.1	5.9
High Intensity Agriculture (dairy farm)	201.0	6.6
Recreational / Open Space (High-intensity)	1077.0	6.9
Single Family Residential (Low-density)	1230.0	7.6
Single Family Residential (Med-density)	2461.5	7.7
Single Family Residential (High-density)	3080.0	8.0
Low Intensity commercial (Comm Strip)	3729.5	8.0
Institutional	3758.0	8.1
Highway (2 lane)	4042.2	8.3
Industrial	5020.0	8.3
Multi-family residential (Low rise)	5210.6	8.7
Highway (4 lane)	7391.5	8.9
High intensity commercial (Mall)	12661.0	9.2
Multi-family residential (High rise)	12825.0	9.2
Central Business District (Avg 2 stories)	16150.3	9.4
Central Business District (Avg 4 stories)	29401.3	10.0

(EPA 350.1), nitrate/nitrite-nitrogen (EPA 353.2), total Kjeldahl nitrogen (EPA 351.2), and total phosphorus (EPA 365.4).

### ***Soil Samples***

A composite soil sample was collected at all 118 sample wetlands. Cores were taken using a 7.6 cm diameter PVC pipe driven 10 cm into the soil. One soil core was collected in the approximate middle of each transect (Figure 2-2), and soil cores were homogenized into a composite sample per site. Soil moisture (Gardner 1986), organic matter, total Kjeldahl nitrogen (USEPA 1993), and total phosphorus (USEPA 1979) were analyzed. Nitrogen and phosphorus samples were processed through the Institute of

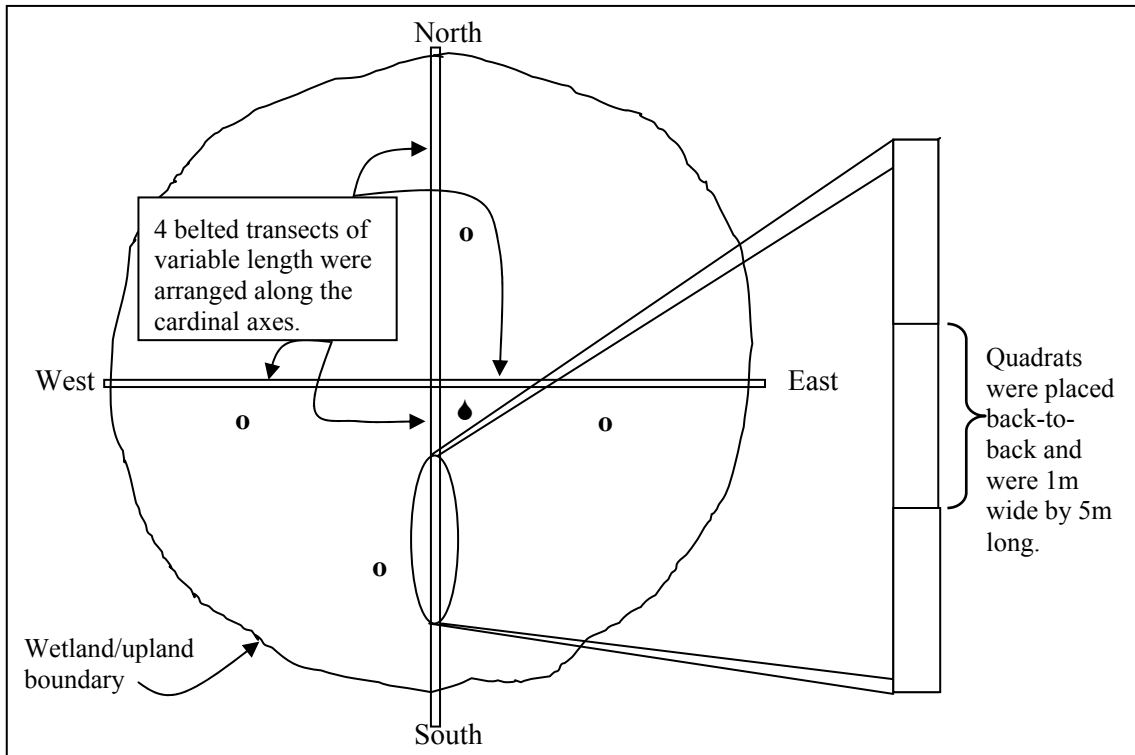


Figure 2-2. Belted transect layout for macrophyte sampling and the location of the water and soil samples. One soil core (○) was taken along each transect and compiled for each wetland. One water sample (●) was taken in the approximate center of each wetland.

Food and Agricultural Sciences (IFAS) Analytical Research Laboratory, Gainesville, Florida.

### ***Sampling Design***

#### ***Benthic diatoms***

Benthic diatom samples were collected at 50 isolated depressional forested wetlands throughout Florida between May-September 2001, as listed in Table 2-2. Figure 2-3 shows the spatial location of the wetlands with benthic diatom samples in Florida. Sites were sampled in the panhandle (n=10), north (n=10), central (n=13), and south (n=17) wetland regions. Sample wetlands were situated in three *a priori* described land use categories (reference n=18; agricultural n=16; urban n=16).

A minimum of 10 cm of standing water was necessary for benthic diatom collection. A hollow cylinder was placed on the soil surface to isolate an area of substrate with a surface area of 28 cm<sup>2</sup>. A bulb pipette was used to loosen the top 0.5 cm layer at the soil surface-water interface, and a 10 mL sample was extracted. This was repeated 10 times throughout the flooded portion of the wetland, resulting in a final sample volume of 100 mL. For preservation, 5 mL of M<sup>3</sup>, a standard preservative for

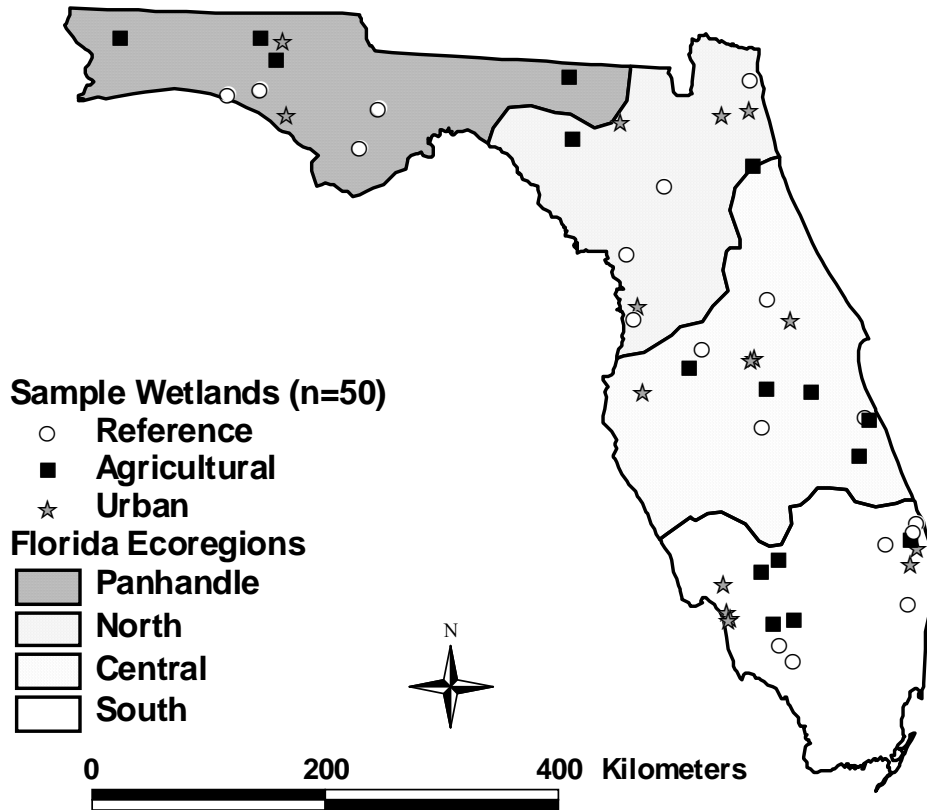


Figure 2-3. Benthic diatom samples were collected at 50 isolated depressional forested wetlands. The state of Florida was separated into four wetland regions (Lane 2000). Sample wetlands were designated by surrounding land use: o reference, ■ agricultural, or ★ urban.

algae samples (APHA 1995), was added to the 100 mL algae sample. Appendix A provides the recipe for creating a batch of  $M^3$  preservative.

Benthic diatom samples were shipped to Michigan State University for identification and enumeration under the supervision of R. J. Stevenson. Samples were homogenized prior to sub-sampling for laboratory identification. Sub-samples were digested following Hasle and Fryxell (1970), which removed the organic matter from the diatom frustules to aide in identification. Following rinsing with distilled water, the digested sub-samples were mounted on microscope slides using Naphrax (Northern Biological Supplies Limited, Ipswich, England). Five hundred valves were counted along microscope transects and identified to the lowest possible taxonomic level, preferably species (following FDEP SOP#AB-03.1 <http://www.dep.state.fl.us/labs/sop>).

### *Macrophytes*

Macrophyte vegetation was sampled at all 118 wetlands throughout Florida (Figure 2-1). Sampling was conducted along four transects situated as perpendicular crossing axes running through the center of each wetland (Figure 2-2). Along each



transect, a series of 1 m wide by 5 m long quadrats was established back to back. Living macrophytes rooted within each quadrat were identified to the lowest taxonomic level possible.

*Supplementary data.* Taxonomic information including species, genus, and family were compiled for all of the macrophytes identified. Additional characteristics were collected for use in developing potential metrics, including category (annual or perennial, evergreen or deciduous, indigenous or exotic) and growth form (aquatic, fern, grass, herb, sedge, shrub, tree, or vine). References specific to Florida were consulted first (Tobe et al. 1998; Wunderlin and Hansen 2003). Additional information was supplemented from other sources (in the following order: Godfrey and Wooten 1981, Wunderlin 1998, and USDA NRCS 2002. When information was still unavailable in published literature, Florida botanists (who also participated in the Floristic Quality Assessment Index) were consulted.

*Floristic Quality Assessment Index.* A Floristic Quality Assessment Index (FQAI) has been included in many of the multi-metric biotic indices created for the macrophyte assemblage. The concept of FQAI was developed by Wilhelm and Ladd (1988) for vegetation around Chicago, Illinois. This method of scoring plant species based on expert botanist opinion has been used in Michigan (Herman et al. 1997), Ohio (Andreas and Lichvar 1995; Fennessy et al. 1998; Mack 2001), Ontario (Francis et al. 2000), North Dakota (Mushet et al. 2002), and Florida (Lane et al. 2003; Cohen et al. 2004). The FQAI provides a quantitative means of assessing the fidelity of a plant to a particular environment through the Delphi technique (Kent 2000), where individual botanists assigned coefficients (termed coefficients of conservatism) to individual species. This technique assumes that the collective decision by a group of expert botanists is more accurate than the professional judgment of one individual (Kent 2000).

The Florida FQAI enlisted regional expert botanists to provide quantitative scores for vegetation in the form of a coefficient of conservatism assigned individually to each species. The FQAI score for an individual wetland was calculated as:

$$FQAI = [ \sum (C_1 + C_2 + \dots C_n) ] / N \quad (2-2)$$

where C = species specific coefficient of conservatism score (CC), and N = species richness. This equation was considered a modified FQAI because previous studies used the square root of native species richness as the denominator. Theories on the importance of species richness suggest that higher species richness signifies a more valuable ecosystem, which can be quantified by using the square root function (Fennessy et al 1998). However, a recent study by Cohen et al. (2004) found a stronger relationship along a disturbance gradient for the FQAI of Eq. 2-2 than with the traditional FQAI equation (using the square root of native species richness in the denominator). They also reported that using total species richness (i.e. including exotic species in the calculation of species richness) improved the relationship of mean CC with the human disturbance gradient (measured with LDI). The sum of the species CC scores was divided by total species richness (Eq. 2-2) in this study to account for potential differences in species richness due to differences in wetland regions (Lane 2000), bioregions (Griffith et al. 1994), *a priori* land use categories, or other unspecified differences.

CC scores were obtained from the Florida botanists surveyed. Each botanist was sent a complete list of species identified in the isolated forested wetlands in the 2001 field season (n = 482 species), and was asked to score each species based on its faithfulness to Florida isolated forested wetlands. After the 2002 field season, one botanist scored the additional 79 taxa not previously encountered, raising the number of taxa with CC scores to 561 species. Potential CC scores ranged from 0 to 10:

- 0 - Exotic and native species that act as opportunistic invaders, included species that commonly occur in disturbed ecosystems
- 1-3 - Species that were widely distributed and occurred in disturbed ecosystems
- 4-6 - Species with a faithfulness to a particular ecosystem, but also tolerant of moderate levels of disturbance
- 7-8 - Species typical of well-established ecosystems that sustain only minor disturbances
- 9-10 - Species occurring within a narrow set of ecological conditions

Species with low CC scores were considered tolerant of many disturbances, whereas species with high CC scores were considered to occur within a narrow set of stable ecological condition. Appendix B lists the CC scores for the macrophyte species identified in this study.

#### *Macroinvertebrates*

Macroinvertebrates were collected at 79 isolated depression forested wetlands throughout Florida (Figure 2-4). Field research spanned two growing seasons with 49 wetlands sampled between June-October 2001 and an additional 30 wetlands sampled between June-October 2002. Wetlands were sampled in the panhandle (n = 13), north (n = 15), central (n = 25), and south (n = 26) wetland regions. Sample wetlands were situated in 3 *a priori* land use categories (reference n = 29, agricultural n = 24, and urban n = 26).

Samples were collected using a U.S. Standard 30 mesh D-frame net. One sweep covered 0.5 m<sup>2</sup> and was measured as 1 net width by 2 net lengths wide, which was repeated 3 times at each location to ensure adequate sampling coverage. Sweeps were always conducted over areas which had not recently been trampled by the field crew. Twenty sweeps were proportioned among major vegetation zones throughout each sample wetland. Sample wetlands generally had only one vegetation zone, although some wetlands did have two or three distinct vegetation zones. Vegetation zones were defined by clear changes in the dominant species. When herbaceous plants were included in the sweep area, the bottom of the net was swept from the substrate up the plant stalks. In areas with woody plant structure, the bottom of the net was swept from the substrate up the tree trunk and pieces of woody debris were brushed to remove attached macroinvertebrates. The contents from the sweeps were collected in a 3.8 L plastic jar and preserved with buffered formalin at a rate of 10% of the sample volume. Appendix A provides a more detailed description of field methods and preservation.

Macroinvertebrate identification was completed at the FDEP Central Laboratory, Tallahassee, Florida, following standard operating procedures (FDEP Standard Operating

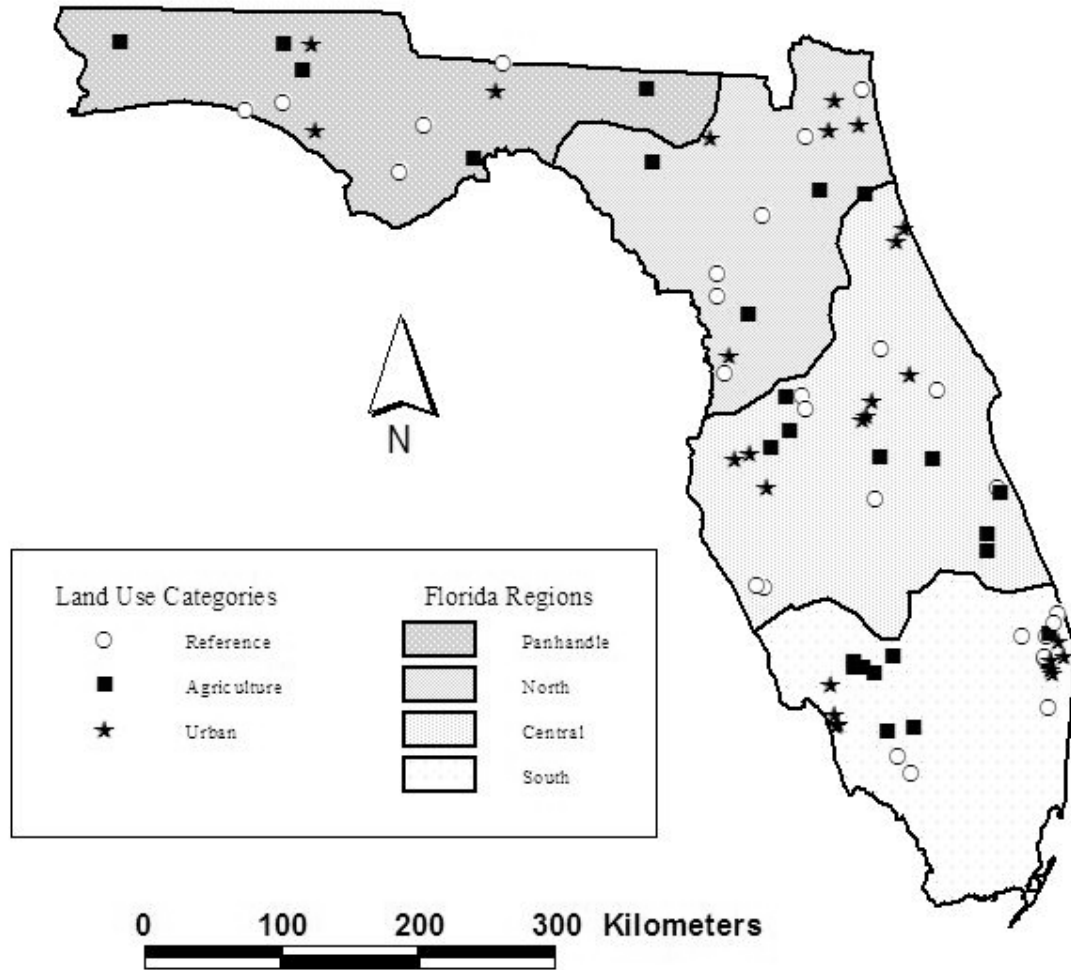


Figure 2-4. Macroinvertebrates were sampled at 79 isolated depressional forested wetlands. The state of Florida was separated into four wetland regions (Lane 2000). Sample wetlands were designated by surrounding land use: ○ reference, ■ agricultural, or ★ urban.

Procedure #IZ-06 <http://www.dep.state.fl.us/labs/sop>). Macroinvertebrate samples were sieved, washed, and placed on a pan with 24 individually numbered cells. Eight of the cells were randomly selected and combined in a second numbered tray. A single cell was randomly selected from the second tray for enumeration and identification of macroinvertebrates. When fewer than 100 individuals were encountered in the sample, a second cell was randomly selected from the second tray, and all of the individuals were enumerated and identified. Identification was to the lowest taxonomic level possible.

## Data Analysis

### *Water and Soil Parameters*

Water and soil parameters within three *a priori* land use categories were compared using Fisher's LSD pair wise comparison in Minitab (Version 13.1, Minitab Statistical Software). The non-parametric Mann-Whitney U-Test was used to discern differences among medians of low ( $LDI < 2.0$ ) and high ( $LDI \geq 2.0$ ) LDI groups for the water and soil parameters. An LDI break of 2.0 corresponds to a break in the LDI coefficients of undeveloped versus developed land uses (Table 2-3).

### *Summary Statistics*

Summary statistics for each assemblage included richness (R), evenness (E), and Shannon diversity (H). For the diatom and macrophyte assemblages summary statistics were calculated at the species level, and for the macroinvertebrate assemblage summary statistics were calculated at the genus level. Richness was defined as the total number of distinct taxa encountered within the sample wetland. Evenness has been described as the fraction of maximum possible diversity in a wetland and was calculated as the Shannon diversity index value divided by the natural log of taxa richness:

$$E = H / \log (R) \quad (2-3)$$

(McCune and Grace 2002). The Shannon diversity index has been described as measuring the "information content" of a sample unit where maximum diversity yields maximum uncertainty (McCune and Grace 2002). For Shannon diversity calculations (H), the sample unit was an individual isolated depressional forested wetland:

$$H = -\sum p_i * \log(p_i) \quad (2-4)$$

$$p_i = n_i / N \quad (2-5)$$

where  $n_i$  was the number of occurrences of taxon  $i$ , and  $N$  was the total number of occurrences of all taxa at a wetland. For the diatom and macroinvertebrate assemblages, the number of occurrence represented the enumeration of the laboratory identified sample and the total number of occurrences represented the sum of the number of occurrences of all taxa. For the macrophyte assemblage, the number of occurrences represented the number of quadrats a species occurred in, and the total number of occurrences represented the sum of the total number of quadrats of all of the species identified.

For the diatom and macroinvertebrate assemblage, Simpson's index (S) was also calculated, and the equation:

$$S = 1 - \sum (p_i * p_i) \quad (2-6)$$

where  $p_i$  was defined in Equation 2-5 as the number of occurrences of taxon  $i$  ( $n_i$ ) divided by the total number of occurrences of all taxa at a wetland ( $N$ ). For the macrophyte assemblage, first- and second-order jackknife estimators of species richness ( $Jack_1$  and  $Jack_2$ , respectively) and Whittaker's beta diversity ( $\beta W$ ) were calculated. First- and second-order jackknife estimators were calculated as estimates of true species richness (Colwell and Coddington 1994). Jackknife estimators are used to gauge the thoroughness

of sampling intensity and the inherent variability of the target system. Assuming that the sampling effort only measured a portion of the ecosystem, jackknife estimators of species richness provide estimates of actual species richness. The equation for first-order jackknife estimators of species richness is based on the number of species observed ( $S$ ), the number of species occurring in only one sample unit ( $r_1$ ) (where one sample unit represents one quadrat), and the number of sample units ( $n$ ) (quadrats):

$$\text{Jack}_1 = S + [(r_1 * (n-1)) / n] \quad (2-7)$$

The second-order jackknife estimator ( $\text{Jack}_2$ ) also incorporated the number of species occurring in exactly two sample units ( $r_2$ ), where a sample unit was defined as a quadrat:

$$\text{Jack}_2 = S + [(r_1 * (2n-3)) / n] - [(r_2 * (n-2) * 2) / (n * (n-1))] \quad (2-8)$$

These estimators of total species richness have shown useful in predicting actual species richness when only a small area of the total ecosystem has been sampled (McCune and Grace 2002).

Whittaker's beta diversity ( $\beta_w$ ) was computed as a calculation of overall beta diversity, or the compositional change represented in a sample. Whittaker's beta diversity was calculated as the number of species at a particular forested wetland ( $S_c$ ) divided by the average species richness per quadrat ( $S$ ) minus one:

$$\beta_w = [S_c / S] - 1 \quad (2-9)$$

The resulting value for Whittaker's beta diversity was described as the "number of distinct communities" (McCune and Grace 2002). When  $\beta_w$  equals zero, all of the sample units contain all of the species. Some multivariate methods strongly depend on beta diversity, and as a general rule beta diversity greater than five is considered high (McCune and Grace 2002).

Summary statistic means within *a priori* land use categories were compared with Fisher's Least Significant Difference (LSD) pair wise comparison test using Minitab (Version 13.1, Minitab Statistical Software). The strength of using Fisher's LSD is in the comparison of unequal group sizes (Ott and Longnecker 2001; Minitab 2000). Sample wetlands were divided into two groups based on Landscape Development Intensity (LDI) index values including low LDI ( $LDI < 2.0$ ) and high LDI ( $LDI \geq 2.0$ ) groups. Comparisons were made using the non-parametric Mann-Whitney U-Test in Minitab (Ott and Longnecker 2001).

Overall calculations of beta and gamma diversity were calculated for sample wetlands in the three *a priori* land use categories (reference, agricultural, urban). Gamma diversity was calculated as the overall number of taxa encountered at all sample wetlands per *a priori* land use category. A higher value of gamma diversity for an *a priori* land use category suggests a greater difference among the species composition of wetlands within that *a priori* land use category, assuming equal sample sizes among *a priori* land use categories. Beta diversity was calculated as *a priori* category gamma diversity divided by the average site taxa richness.

### ***Regional Compositional Analysis***

The Multi-Response Permutation Procedure (MRPP) was used to test the similarity of community composition for each assemblage among the four Florida

wetland regions (further application in Zimmerman et al. 1985; McCune et al. 2000; McCune and Grace 2002). MRPP is a nonparametric technique which tests for no difference between groups (the null hypothesis) and is available in PCORD (Version 4.1 from MJM Software, Gleneden Beach, Oregon). It is an appropriate procedure for ecological community data as it does not require distributional assumptions of normality and homogeneity of variances. The Sørensen distance measure was used to calculate the average weighted within-group distance.

MRPP provides a test statistic (T), p-value, and chance-corrected within-group agreement (A), which describes within-group similarity. When A equals one, all items are identical within groups, and when A equals zero, differences within-groups equal that expected by chance. Most values of A are less than 0.1 in community ecology (McCune and Grace 2002). MRPP was calculated across all groups (panhandle versus north versus central versus south) as well as for multiple pair wise comparisons (panhandle versus north, panhandle versus central, panhandle versus south, north versus central, north versus south, and central versus south) for the Florida wetland regions (Lane 2000).

### ***Community Composition***

Community composition of each assemblage was summarized in a non-metric multidimensional scaling (NMDS) ordination to relate changes in community composition with environmental gradients. NMDS is an ordination technique designed to compress multi-dimensional space, and is particularly agreeable with ecological data because it does not rely on linear relationships among variables. This has been described as a compensation for the “zero-truncation problem” through the use of ranked distances and the application of many distance measure (McCune and Grace 2002). The “zero-truncation problem” refers to the extraordinary number of zeros in community ecology data sets. Other ordination techniques (i.e. Principal Components Analysis, PCA) depend upon a value for each measured variable for each sample unit, interpreting shared zeros as a positive relationship. NMDS is useful for presence/absence or abundance data sets, where many species are not present, or receive a zero in the species by site matrix. By ranking the variables, NMDS caters to non-parametric, community ecology data sets.

NMDS explored the dissimilarities of the community composition of sample wetlands for each assemblage. The Sørensen (Bray-Curtis) distance measure was used for ordination. The dimensionality was chosen based on an initial six dimensional run in autopilot mode, which suggested an optimal three dimensional solution for the each community composition dataset. To find the optimal three dimensional solution, 50 runs with real data and 50 randomized runs were performed with the instability criterion set at 0.00001 and the maximum number of iterations to reach a stable solution set at 500. This procedure was repeated 20 times to insure stability and reproducibility in results. The final run was completed with the starting point set as the results from the best experimental with dimensional run, considered the run with the lowest stress and best overall fit.

Water and soil parameters, LDI, latitude, and longitude were correlated with the NMDS ordination axes with Pearson correlation coefficients. To improve normality and decrease skewness, 11 water and soil parameters were log (base 10) transformed, including water parameters (dissolved oxygen concentration, temperature, color,

turbidity, specific conductivity, ammonia-nitrogen concentration, nitrate/nitrite-nitrogen concentration, total Kjeldahl nitrogen (TKN) concentration, and total phosphorus (TP) concentration) and soil parameters (TKN and TP concentration). Water pH was not transformed as it is already on a log scale (pH is calculated as the log base 10 of the hydronium ion concentration). The remaining parameters were measured as percentages, and were transformed by taking the arcsine square root, including soil moisture and soil organic matter.

### **Metric Development**

In the context of this study, metrics were defined as biological attributes which have a consistent and predictable response to anthropogenic activities (Karr and Chu 1997). Metrics were summarized in five main categories:

1. Tolerance metrics (determined with indicator species or established index values)
2. Autecological (metrics that explore a previously described relationship between taxa and an environmental gradient)
3. Community structure (metrics that explore taxonomic structure)
4. Community balance (metrics with calculated values, such as evenness or dominance)
5. Functional group (metrics related to feeding behavior)

Appendix C provides tables of candidate metrics for each assemblage, including 59 candidate diatom metrics (Table C-1), 68 candidate macrophyte metrics (Table C-2), and over 100 candidate macroinvertebrate metrics (Table C-3). Candidate metrics were calculated at the statewide scale for both the diatom and macroinvertebrate assemblages, as sample sizes were limited for regional metric development, particularly in the north and panhandle wetland regions. Candidate macrophyte metrics were calculated at both the regional scale and statewide.

Metrics were calculated in multiple forms including richness (R) and proportion (P) metrics. The richness metric (R) simply represents a count of the taxa present within a metric category. The proportion metric (P) was calculated as the richness metric (R) divided by the total species richness (N) for each sample wetland:

$$P_i = R_i / N_i \quad (2-10)$$

Candidate metrics were accepted if they showed a constant and predictable change along the LDI (Brown and Vivas 2005) according to the strength and significance of the Spearman's correlation coefficient (Analyze-It software v. 1.67 for Microsoft Excel). The Spearman rank correlation tests for an association between two related variables, and is a non-parametric alternative to the Pearson correlation coefficient. Scatter plots were constructed for each candidate metric versus LDI to ensure correlations were visually distinguishable. Candidate metrics were subjected to a Mann-Whitney U-test to detect differences between low and high LDI groups.

### *Indicator Species Analysis*

For each assemblage, sample wetlands were categorized into two LDI groups and analyzed with Indicator Species Analysis (ISA) in PCORD. ISA evaluates the abundance and faithfulness of taxa in a defined group (McCune and Grace 2002). ISA can be used to detect and describe the value of taxa indicative of environmental conditions. It requires sites be assigned into groups and species data on the abundance or presence of taxa in each group. These groups are commonly defined by categorical environmental variables, levels of disturbance, experimental treatments, presence and absence of a target species, or habitat types (McCune and Grace 2002). Mathematical equations are available in Dufrêne and Legendre (1997) and McCune and Grace (2002). The calculated indicator species values are based on two standards, faithfulness and exclusion. Faithfulness is defined mathematically by a particular taxa always being present in a particular group. The perfect indicator taxon is exclusive to a group, meaning it would never occur in other groups. Indicator values range from 0 (no indication) to 100 (a perfect indication of a particular group).

ISA was conducted to determine sensitive and tolerant indicator taxa separately for each species assemblage. Statewide ISAs were run for the diatom and macroinvertebrate assemblages. ISA was conducted for each wetland region (panhandle, north, central, and south) as well as statewide for the macrophyte assemblage. Since correlations between LDI and each species assemblage were uncertain, iterative ISAs were calculated for each species assemblage. ISA depends on the predetermined categorization of sites, and sites were divided into groups based upon the calculated LDI scores. ISA was run for each LDI break from 1.0 to 7.0 at each 0.5 increment. For example, at an LDI break of 1.0, all sites with a calculated LDI of 1.0 were assigned to Group A, and all sites with a calculated LDI > 1.0 were assigned to Group B, and an ISA calculation completed. Then, for a separate iteration, sites were divided in Group A (LDI < 1.5) and Group B (LDI ≥ 1.5) and an ISA calculation was completed. Next, sites were assigned to Group A (LDI < 2.0) and Group B (LDI ≥ 2.0) and an ISA calculation was completed. This process of assigning sites into groups based on their calculated LDI scores was repeated at LDI breaks of 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, and 7.0.

The idea behind the iterative ISA calculations was to broaden the application of the LDI to detect changes in species composition among wetland sites for different species assemblages and for different anthropogenic influences which may be specific or more dramatic to one species assemblage. The final species lists for tolerant and sensitive indicator species were determined for the LDI break yielding the greatest number of indicator species and the strongest significance based on the Monte Carlo randomization technique. Calculated indicator values were tested for statistical significance using a Monte Carlo randomization technique with 1000 randomized runs. Indicator taxa categorized as tolerant taxa were associated with the higher LDI group (Group B); indicator taxa deemed sensitive taxa were associated with the lower LDI group (Group A).

In the macrophyte assemblage, the Spearman rank correlation was used to assess differences between statewide and regional indicator species lists for each of the four wetland regions. To test for equal application of the statewide indicator species list for each wetland region, the non-parametric Kruskal-Wallis test was run with Analyse-It



software (Ott and Longnecker 2001). Distributional differences were analyzed between *a priori* categories for both tolerant and sensitive indicator taxa among wetland regions.

### ***Diatom Metrics***

Diatom metrics (Appendix C Table C-1) were adopted from previous studies and could be categorized in three categories including tolerance, community composition, and autecological metrics (Bahls 1993; van Dam et al. 1994; McCormick and Cairns 1994; Stevenson 2001; Fore and Grafe 2002; Lane et al. 2002; USEPA 2002b; Lane 2003). Tolerance metrics were created with ISA. Community composition metrics included richness, evenness, and diversity calculations. Autecological metrics were based on available research that correlated individual diatoms with morphology, behavior, and the physical and chemical water environment. Diatom species were assigned ecological indicator values using a coded checklist of autecological relationships (Bahls 1993; van Dam et al. 1994). The ecological indicator values from van Dam et al. (1994) included categorizing diatoms according to water preference (moisture tolerance), nitrogen metabolism, pH, salinity, dissolved oxygen, saprobic condition, and trophic status. Pollution tolerance classification values were taken from Bahls' (1993) analysis of diatoms in Montana streams. Metrics based on morphology and motility were assigned from Stevenson (Lane 2003).

### ***Macrophyte Metrics***

Macrophyte metrics (Appendix C Table C-2) were adopted from previous studies and included tolerance, exotic species, Floristic Quality Assessment Index (FQAI), longevity and plant growth form (native perennial), and wetland status metrics (Adamus 1996; Kantrud and Newton 1996; Galatowitsch et al. 1999a; Gernes and Helgen 1999; Carlisle et al. 1999; Fennessy et al. 2001; Mack 2001; and Lane 2003). Tolerance metrics were calculated with ISA. Each species was categorized as native or exotic and annual or perennial (Godfrey and Wooten 1981; Tobe et al. 1998; Wunderlin 1998; USDA NRCS 2002; and Wunderlin and Hansen 2003). The exotic species metric was calculated as the proportion of species that were exotic to Florida divided by the number of species identified at each particular isolated depressional forested wetland. The timeline for determining the exotic status of a species was set near the beginning of European settlement in North America. The percent native perennial species metric was calculated as the number of native perennial species encountered divided by the wetland species richness. Wienhold and Van der Valk (1989), Ehrenfeld and Schneider (1991), and Lane (2003) determined that disturbance often favors annual species over perennial species and promotes the invasion of nonnative perennials in wetlands. Galatowitsch et al. (2000) found that while native perennial cover was reduced in wetlands impacted by cultivation, the occurrence of introduced perennials rather than annuals increased in stormwater impacted wetlands.

The wetland status metric was calculated as the percent of plants classified as obligate or facultative wetland indicator species divided by the number of species at each wetland. Wetland indicator status classifications was from FDEP's vegetative index as presented in section 62-340.450, F.A.C., which can be found in Tobe et al. (1998), USDA

NRCS (2002), and Wunderlin and Hansen (2003). There are five potential species classifications, including obligate, facultative wetland, facultative, upland, and invisible. In some cases, when the Florida wetland indicator status was not available, the National Wetlands Inventory wetland indicator status for the United States was used.

### *Macroinvertebrate Metrics*

Candidate metrics for the macroinvertebrate assemblage (Appendix C Table C-3) were adopted from previous studies and could be classified in four categories, including tolerance, community structure, community balance, and functional group metrics (Lenat 1993; Lenat and Barbour 1994; Kerans and Karr 1994; Wallace et al. 1996; Barbour et al. 1996b; Gerristen and White 1997; Danielson 1998a; Leslie et al. 1999; Galatowitsch et al. 1999a; Smogor and Angermeier 2001; Helgen 2001; Cummins and Merritt 2001; USEPA 2002c; Lane et al. 2002; Lane 2003; Griffith et al. 2003; and Butcher et al. 2003). In addition to ISA, other tolerance candidate metrics were calculated. Many of the established tolerance metrics were created for flowing waters, which complicated the application of established tolerance values (ex. the Florida Index from Beck 1954 and Barbour et al. 1996b; and the Hilsenhoff Biotic Index from Hilsenhoff 1987), since the wetlands sampled in this study were isolated from flowing surface waters (except in extreme high water events when some surface flow may be detectable).

Community structure metrics included richness measures (Danielson 1998a), for example the number of distinct species or specified taxonomic units such as the number of families, genera, or species in a sample. Examples of taxa richness metrics include total taxa richness, Ephemeroptera richness, number of Coleoptera species, or number of Insecta species.

The use of community balance metrics included some measure of abundance or relative abundance in an attempt to measure the evenness of the macroinvertebrate community (Lenat and Barbour 1994). Examples of community balance metrics include the Shannon diversity index or the percent contribution of the most abundant taxon (Lenat and Barbour 1994).

Macroinvertebrate taxa were grouped based on their functional relationships that overlap taxonomic categorization, including functional feeding groups, habitat groups, and voltinism groups (life-cycle patterns). Cummins and Merritt (2001) suggest using ratios of numerical abundance or, more favorably, biomass of the various functional groups as indicators of ecosystem attributes, essentially considering the functional groups as surrogates of ecosystem condition. Functional feeding group metrics were based on the morphological structures and behaviors responsible for food acquisition by particular taxa at a site (Resh and Jackson 1993; Danielson 1998a). For example, herbivores consume algae and plant material, while predators consume animals, omnivores eat both plant and animal materials, and detritivores consume decomposed particulate material (Helgen 2001).

## Florida Wetland Condition Index

Candidate metrics were selected for inclusion in the Florida Wetland Condition Index (FWCI) if they satisfactorily met three criteria:

- Metrics were correlated with the LDI according to the strength and significance of the Spearman's correlation coefficient
- Metrics displayed visually distinguishable correlations with LDI in scatter plots
- Metrics showed significant differences between low ( $LDI < 2.0$ ) and high ( $LDI \geq 2.0$ ) LDI groups tested with the Mann-Whitney U-test.

An FWCI was constructed for each assemblage, including the diatom FWCI, the macrophyte FWCI, and the macroinvertebrate FWCI. Each FWCI was composed of individual metrics specific to the assemblage, which were scaled and added together. Metric scoring was based on an approach modified from the SCI, a Florida based biological index of the macroinvertebrate assemblage used to discern stream condition (Fore 2004). Metrics with a skewed distribution were log transformed to improve the distribution. The 5<sup>th</sup> to 95<sup>th</sup> percentile values of each metric were normalized from 0 to 10, with 10 always representing the best biological wetland condition. The selected metrics, FWCI, and LDI were correlated with water and soil parameters using Spearman's correlation coefficient.

In order to determine whether the FWCI provided comparable scores for wetlands with similar community composition within each assemblage, an agglomerative cluster analysis in PCORD was used to determine wetland clusters (McCune and Grace 2002). The dissimilarity matrix was constructed using the Sørensen distance measure and the flexible beta linkage method ( $\beta = -0.25$ ), which is a flexible clustering setting designed to reduce chaining in the dendrogram. The resulting dendrogram was pruned to maintain the smallest number of significantly different clusters based on Fisher's LSD pair wise comparison ( $p < 0.05$ ). To compare differences in wetland condition between the three species assemblages, metrics selected for inclusion in the three FWCI among different species assemblages were compared using the Pearson correlation coefficient (Analyse-it Software).

## CHAPTER 3 RESULTS

### Quantifying Anthropogenic Influence

Three independent quantitative measures of anthropogenic activity were calculated for the 118 isolated depressional forested wetlands including the Landscape Development Intensity (LDI) index, the Wetland Rapid Assessment Procedure (WRAP), and the Minnesota disturbance index (DI). Individual scores for each wetland are presented in Appendix D. Potential LDI scores ranged from a minimum of 1.0 to a maximum of 10.0 representing a highly impacted wetland. Actual LDI scores ranged from 1.0 at 26 wetlands to 7.2 at two wetlands including SU8 and CU6, two urban wetlands surrounded by transportation and commercial land uses. The mean LDI score for *a priori* reference wetlands was 1.1 ( $\sigma = 0.2$ ). *A priori* agricultural and urban wetlands had similar mean LDI scores at 4.5 ( $\sigma = 1.4$ ) and 4.8 ( $\sigma = 1.5$ ), respectively.

Scores for the WRAP potentially range from 0.0 to 3.0, with the lowest score of 0.0 representing a severely impacted wetland. Actual WRAP scores ranged from 3.0 at seven *a priori* reference wetlands to 0.5 at two urban wetlands including SU8 and PU10. The mean WRAP score for *a priori* reference wetlands was 2.8 ( $\sigma = 0.2$ ), with *a priori* agricultural and urban wetlands having similar mean scores of 1.6 ( $\sigma = 0.5$ ) and 1.4 ( $\sigma = 0.4$ ), respectively.

The third gradient of anthropogenic influence, the Minnesota DI ranges from zero to 17 with unimpacted wetlands receiving a score of zero. Actual Minnesota DI scores ranged from zero at 15 *a priori* reference wetlands to 17 at three *a priori* agricultural wetlands. The mean Minnesota DI score for *a priori* reference wetlands was 1.6 ( $\sigma = 2.1$ ). *A priori* agricultural and urban wetlands had higher mean Minnesota DI scores at 11.3 ( $\sigma = 5.4$ ) and 12.2. ( $\sigma = 3.6$ ), respectively.

Comparisons among the three measures of anthropogenic influence showed strong and significant correlations (Pearsons correlation coefficient  $|r| \geq 0.84$ ,  $p < 0.01$ ) (Table 3-1). WRAP and the Minnesota DI had the greatest correlation coefficient ( $r = 0.89$ ). The comparisons between LDI and WRAP and LDI and the Minnesota DI also had large correlation coefficients (0.87 and 0.84, respectively). The LDI index was selected for further analysis and metric selection for the development of the Florida Wetland Condition Index (FWCI) because it is an objective and repeatable measure of anthropogenic activity and it is not based on the subjective scoring of an assessor.

### Water and Soil Parameters

Physical and chemical properties of 75 water samples and 118 soil samples were analyzed for isolated depressional forested wetlands. Table 3-2 shows mean values for water and soil parameters for the three *a priori* land use categories (reference, agricultural, and urban). Means with similar letters were not significantly different (Fisher's LSD pair wise comparison,  $\alpha = 0.05$ ), including water temperature, water

Table 3-1. Comparison among three measures of anthropogenic influence, including the Landscape Development Intensity (LDI) index, the Wetland Rapid Assessment Procedure (WRAP), and the Minnesota disturbance index (DI).

	LDI	WRAP
WRAP	0.87	
DI	0.84	0.89

Values are Pearson correlation coefficients.  
All correlations were significant ( $p < 0.01$ ).

nitrate/nitrite-nitrogen, and soil TKN, which were not significantly different among *a priori* land use categories.

Of the 14 water and soil parameters measured, eight water and three soil parameters showed significant difference ( $p < 0.05$ ) among reference, agricultural and/or urban wetlands, categorized by the three *a priori* land use categories. Reference wetlands had significantly different dissolved oxygen ( $x = 2.9$  mg O<sub>2</sub>/L), turbidity ( $x = 3.8$  NTU), water pH ( $x = 5.2$ ), and water column TP ( $x = 0.08$  mg P/L), than agricultural and urban wetlands. Water ammonia-nitrogen (mg N/L), water TKN (mg N/L), soil moisture, and soil TP (mg P/g soil) were significantly different between reference and agricultural wetlands. Specific conductivity was significantly different between reference ( $x = 81$  umhos/cm) and urban wetlands ( $x = 231$  umhos/cm). The water color of urban wetlands ( $x = 198$  PCU) was significantly different from reference ( $x = 285$  PCU) and agricultural ( $x = 346$  PCU) wetlands. Soil organic matter was significantly different between agricultural ( $x = 30\%$ ) and urban wetlands ( $x = 41\%$ ).

Measures of four water and two soil parameters were significantly different ( $p < 0.05$ ) between sample wetlands in low (LDI  $< 2.0$ ) and high (LDI  $\geq 2.0$ ) LDI groups (Table 3-3). Dissolved oxygen levels were higher in less developed wetlands ( $x = 2.9$  mg O<sub>2</sub>/L) as compared to wetlands surrounded by higher development intensity ( $x = 1.8$  mg O<sub>2</sub>/L), and wetlands surrounded by lower development intensity had lower water column pH ( $x = 5.2$ ) than wetlands in higher development intensity landscapes ( $x = 6.3$ ). Low LDI wetlands had significantly less TP in both the water column ( $x = 0.08$  mg P/L) and soil ( $x = 0.39$  mg P/L), than the high LDI wetlands. The physical measure of turbidity showed that wetlands surrounded by low development intensity had lower turbidity ( $x = 3.8$  NTU) than those surrounded by higher development intensity ( $x = 13.2$  NTU). Additionally, low LDI wetlands had higher soil moisture ( $x = 59$ ) than high LDI wetlands ( $x = 50$ ).

Table 3-2. Water and soil parameters among three *a priori* land use categories, including reference, agricultural, and urban wetlands.

	Reference				Agricultural				Urban			
	n	x	$\sigma$	95% CI	n	x	$\sigma$	95% CI	n	x	$\sigma$	95% CI
<b>Water Parameters</b>												
Dissolved oxygen (mg/L)	29	2.9	1.7	(2.3 to 3.6) <sup>a</sup>	18	1.6	0.9	(1.2 to 2.1) <sup>b</sup>	24	1.9	1.1	(1.4 to 2.4) <sup>b</sup>
Temperature (°C)	29	26.2	2.8	(25.1 to 27.3) <sup>a</sup>	18	25.2	1.9	(24.2 to 26.2) <sup>a</sup>	24	24.9	2.4	(23.9 to 25.9) <sup>a</sup>
Color (PCU)	30	285	178	(218 to 352) <sup>a</sup>	19	346	204	(248 to 445) <sup>a</sup>	26	198	129	(146 to 251) <sup>b</sup>
Turbidity (NTU)	30	3.8	4.2	(2.3 to 5.4) <sup>a</sup>	19	17.7	40.7	(0.0 to 37.3) <sup>b</sup>	26	9.5	11.9	(4.7 to 14.3) <sup>b</sup>
pH	30	5.2	1.2	(4.8 to 5.6) <sup>a</sup>	19	6.2	0.8	(5.8 to 6.6) <sup>b</sup>	26	6.4	1.0	(5.9 to 6.8) <sup>b</sup>
Specific conductivity (umhos/cm)	10	81	48	(46 to 115) <sup>a</sup>	10	136	134	(39 to 232) <sup>ab</sup>	13	231	175	(126 to 337) <sup>b</sup>
Ammonia-nitrogen (mg N/L)	30	0.15	0.33	(0.03 to 0.28) <sup>a</sup>	19	0.33	0.57	(0.06 to 0.61) <sup>b</sup>	26	0.19	0.27	(0.08 to 0.30) <sup>ab</sup>
Nitrate/nitrite-nitrogen (mg N/L)	26	0.09	0.37	(0.00 to 0.24) <sup>a</sup>	15	0.01	0.01	(0.00 to 0.01) <sup>a</sup>	21	0.02	0.03	(0.00 to 0.03) <sup>a</sup>
TKN nitrogen (mg N/L)	30	1.93	1.24	(1.47 to 2.39) <sup>a</sup>	19	3.17	2.20	(2.11 to 4.23) <sup>b</sup>	26	1.84	1.06	(1.41 to 2.27) <sup>ab</sup>
Total phosphorus (mg P/L)	30	0.08	0.11	(0.04 to 0.12) <sup>a</sup>	19	0.81	1.38	(0.15 to 1.47) <sup>b</sup>	26	0.24	0.26	(0.13 to 0.34) <sup>b</sup>
<b>Soil Parameters</b>												
Moisture (%)	38	61	20	(54 to 67) <sup>a</sup>	39	46	17	(40 to 51) <sup>b</sup>	41	55	20	(48 to 61) <sup>ab</sup>
Organic matter (%)	38	40	25	(32 to 48) <sup>ab</sup>	39	30	17	(24 to 35) <sup>a</sup>	41	41	28	(32-50) <sup>b</sup>
TKN nitrogen (mg N/g soil)	38	6.76	3.68	(5.55 to 7.97) <sup>a</sup>	39	5.53	3.30	(4.46 to 6.59) <sup>a</sup>	41	6.71	4.75	(5.21 to 8.20) <sup>a</sup>
Total phosphorus (mg P/g soil)	38	0.38	0.28	(0.29 to 0.47) <sup>a</sup>	39	0.91	1.27	(0.50 to 1.32) <sup>b</sup>	41	0.53	0.31	(0.43 to 0.63) <sup>ab</sup>

\*Minimum Detection Limits: Ammonia-nitrogen 0.010 mg N/L; Nitrate/nitrite-nitrogen 0.004 mg N/L; TKN nitrogen 0.060 mg N/L; Total phosphorus 0.015 mg P/L

x = mean;  $\sigma$  = standard deviation; 95% CI = 95% confidence interval

Categories with dissimilar letters were significantly different (Fisher's LSD pair wise comparison,  $\alpha=0.05$ ).

Table 3-3. Water and soil parameters among LDI groups. The Low LDI Group includes wetlands with an LDI < 2.0, and the High LDI Group includes wetlands with an LDI ≥ 2.0.

	Low LDI Group				High LDI Group				W <sup>^</sup>	p <sup>`</sup>
	n	x	σ	95% CI	n	x	σ	95% CI		
Water parameters										
Dissolved oxygen (mg/L)	30	2.9	1.7	(2.2 to 3.5)	41	1.8	1.0	(1.5 to 2.1)	367	<0.01*
Temperature (°C)	30	26.1	2.8	(25.1 to 27.2)	41	25.0	2.2	(24.4 to 25.7)	453	0.06
Color (PCU)	31	292	179	(226 to 358)	44	255	177	(202 to 309)	568	0.21
Turbidity (NTU)	31	3.8	4.1	(2.3 to 5.3)	44	13.2	28.1	(4.6 to 21.7)	907	0.02*
pH	31	5.2	1.1	(5.0 to 5.6)	44	6.3	0.9	(6.0 to 6.6)	1063	<0.01*
Specific conductivity (umhos/cm)	10	81	48	(46 to 115)	23	190	163	(119 to 260)	165	0.05
Ammonia-nitrogen (mg N/L)*	31	0.15	0.33	(0.03 to 0.27)	44	0.25	0.43	(0.12 to 0.38)	853	0.07
Nitrate/nitrite-nitrogen (mg N/L)*	26	0.09	0.37	(0.00 to 0.24)	36	0.01	0.03	(0.01 to 0.02)	450	0.77
TKN nitrogen (mg N/L)*	31	1.99	1.27	(1.53 to 2.46)	44	2.37	1.75	(1.84 to 2.90)	705	0.81
Total phosphorus (mg P/L)*	31	0.08	0.11	(0.04 to 0.12)	44	0.48	0.96	(0.19 to 0.77)	1057	<0.01*
Soil parameters										
Moisture (%)	45	59	19	(54 to 65)	73	50	20	(46 to 55)	1163	<0.01*
Organic matter (%)	45	40	24	(33 to 48)	73	35	24	(29 to 41)	1357	0.11
TKN nitrogen (mg N/g soil)	45	6.88	3.66	(5.78 to 7.98)	73	6.00	4.15	(5.03 to 6.97)	1366	0.13
Total phosphorus (mg P/g soil)	45	0.39	0.27	(0.31 to 0.48)	73	0.74	0.97	(0.51 to 0.96)	2140	<0.01*

\*Minimum Detection Limits: Ammonia-nitrogen 0.010 mg N/L; Nitrate/nitrite-nitrogen 0.004 mg N/L; TKN nitrogen 0.060 mg N/L; Total phosphorus 0.015 mg P/L

<sup>^</sup>W = Mann-Whitney U-test statistic.

<sup>`</sup>p = significance value.

## Diatom Assemblage

Statewide 50 wetlands were sampled with diatoms identified at the species level represented 98% of the sample. Five diatom species were identified at 50% or more of the sample wetlands ( $n \geq 25$ ) including *Pinnularia subcapitata* (at 66% of the wetlands), *Eunotia bilunaris* (60%), *Nitzschia palea debilis* (60%), *Eunotia incisa* (54%), and *Gomphonema gracile* (50%). The three diatoms identified most often included *Eunotia naegelii*, *Eunotia incisa*, and *Nitzschia palea debilis*. Of the diatoms encountered, 94 taxa (44%) occurred at a minimum of 5% of the sample wetlands ( $n \geq 3$ ); forty-one percent of the taxa identified (87 taxa) were encountered in only one wetland.

In the panhandle ecoregion, 10 wetlands were sampled with four reference, four agricultural, and two urban wetlands hosting 73 diatom taxa. In the north ecoregion 10 wetlands were sampled (four reference, two agricultural, and four urban) with 94 taxa encountered. The central ecoregion included 13 sample wetlands (five reference, four agricultural, and four urban) with 112 taxa sampled. The south ecoregion included 17 sample wetlands (six reference, five agricultural, and six urban) with 147 taxa identified.

### *Summary Statistics*

Richness (R), evenness (E), Shannon diversity (H), and Simpson's index (S) were calculated for each sample wetland (Appendix E). Table 3-4 summarizes the richness, evenness, and diversity calculations of the diatom assemblage by *a priori* land use category. Species richness ranged from nine taxa at CR6 (embedded within upland pine forest) and CU1 (bordered on three sides by an arboretum and on the remaining side by a paved road) to 39 taxa at CU3 (surrounded by commercial and residential land uses). Species evenness ranged from 0.57 at NU5 (surrounded by residential and transportation), to 0.89 at CA6 (surrounded by citrus groves). Shannon diversity ranged from 1.41 at NU5 to 2.95 at SR5 (surrounded by marsh and flooded flatwoods). Simpson's index was highest at SR5 and SU2 (adjacent to a recently built public school and transportation) at 0.93, and lowest at NU5 at 0.58. Richness, evenness, Shannon diversity, and Simpson's index were not significantly different among the three *a priori* land use categories (Table 3-4) or between LDI groups (Table 3-5). Beta and gamma diversity were similar among *a priori* land use categories. Beta and gamma diversity were higher for the high LDI group (beta diversity of 8.2 and gamma diversity of 167) and lower for the low LDI group (beta diversity of 7.5 and gamma diversity of 145).

### *Compositional Analysis*

MRPP was calculated across all groups (panhandle versus north versus central versus south) as well as for multiple pair wise comparisons (panhandle versus north, panhandle versus central, panhandle versus south, north versus central, north versus south, and central versus south) in order to test the similarity of diatom taxa composition among the wetland regions. Table 3-6 shows the results for the MRPP tests, including the test statistic (T), the chance-corrected within-group agreement (A, a measure of within group similarity), and the significance value (p). The global comparison among all wetlands and the three *a priori* categories showed that diatom community composition



Table 3-4. Diatom richness, evenness, and diversity among *a priori* land use categories.

	Reference	Agricultural	Urban
Species richness (R)	19 (8) <sup>a</sup>	19 (6) <sup>a</sup>	22 (8) <sup>a</sup>
Species evenness (E)	0.74 (0.09) <sup>a</sup>	0.75 (0.08) <sup>a</sup>	0.73 (0.10) <sup>a</sup>
Shannon diversity (H)	2.13 (0.49) <sup>a</sup>	2.18 (0.34) <sup>a</sup>	2.21 (0.49) <sup>a</sup>
Simpson's index (S)	0.80 (0.09) <sup>a</sup>	0.82 (0.07) <sup>a</sup>	0.81 (0.10) <sup>a</sup>
Beta diversity	6.9	6.1	5.8
Gamma diversity	132	117	126

Values represent the mean  $\pm$  standard deviation.

Categories with dissimilar letters are significantly different (Fisher's LSD pair wise comparison,  $\alpha = 0.05$ ).

at the species level was significantly different ( $\alpha = 0.05$ ). Within the pair wise comparisons, only the panhandle versus south and north versus south comparisons had significantly different diatom community composition for all land use types. Reference wetlands in the south wetland region had a significantly different diatom community composition compared to wetlands in both the panhandle and north ecoregions. Similarly, diatom community composition among pair wise comparisons of agricultural wetlands was significantly different for the panhandle versus south ecoregions. Only the north and south ecoregions had significantly different diatom community composition among urban wetlands.

### ***Community Composition***

Figure 3-1 shows a two dimensional bi-plot of the NMS axes used to explore diatom community composition with overlays of significant (transformed) environmental variables, including LDI and six water parameters: DO (log), water turbidity (log), pH, specific conductivity (log), TKN (log), and TP (log). Table 3-7 provides the Pearson r-

Table 3-5. Mean diatom summary statistics between LDI groups.

	Low LDI	High LDI	W <sup>^</sup>	p <sup>`</sup>
Species richness (R)	19 (8)	20 (7)	484	0.61
Species evenness (E)	0.73 (0.09)	0.74 9(0.09)	497	0.80
Shannon diversity (H)	2.13 (0.47)	2.20 (0.43)	486	0.64
Simpson's index (S)	0.80 (0.09)	0.82 (0.08)	480	0.55
Beta diversity	7.5	8.2		
Gamma diversity	145	167		

Values represent the mean (standard deviation).

<sup>^</sup>W = Mann-Whitney U-test statistic.

<sup>`</sup>p = significance value.

Table 3-6. Similarity of diatom community composition using MRPP.

	Sites (n)	T <sup>^</sup>	A <sup>`</sup>	p <sup>#</sup>
<b>All wetlands</b>				
All regions (P vs N vs C vs S)	50	-2.2	0.01	0.03*
Panhandle vs north	20	0.5	-0.19	0.67*
Panhandle vs central	23	0.9	-0.03	0.81*
Panhandle vs south	27	-2.5	0.06	0.02*
North vs central	23	-0.7	0.02	0.20*
North vs south	27	-3.9	0.09	0.00*
Central vs south	30	-1.7	0.04	0.06*
<b>Reference wetlands</b>				
All regions (P vs N vs C vs S)	18	-1.3	0.09	0.11*
Panhandle vs north	8	-0.7	0.07	0.21*
Panhandle vs central	8	0.8	-0.07	0.76*
Panhandle vs south	10	-2.1	0.16	0.03*
North vs central	8	0.9	-0.08	0.82*
North vs south	10	-2.4	0.17	0.02*
Central vs south	10	0.3	-0.02	0.58*
<b>Agricultural wetlands</b>				
All regions (P vs N vs C vs S)	16	0.1	-0.01	0.52*
Panhandle vs north	6	0.6	-0.06	0.70*
Panhandle vs central	9	0.1	-0.01	0.51*
Panhandle vs south	9	-2.4	0.16	0.02*
North vs central	7	1.5	-0.19	0.93*
North vs south	7	0.2	-0.02	0.52*
Central vs south	10	-0.3	0.02	0.38*
<b>Urban wetlands</b>				
All regions (P vs N vs C vs S)	16	-0.7	0.05	0.23*
Panhandle vs north	6	-0.9	0.15	0.18*
Panhandle vs central	6	0.9	-0.07	0.83*
Panhandle vs south	8	-0.3	0.02	0.37*
North vs central	8	-0.1	0.01	0.37*
North vs south	10	-1.9	0.11	0.05*
Central vs south	10	1.1	-0.05	0.86*

\*A high |T| value and significant p-value (p<0.05) suggests a difference in species composition

<sup>^</sup>T = the MRPP test statistic

<sup>`</sup>A = the chance corrected within-group agreement

<sup>#</sup>p = the significance value

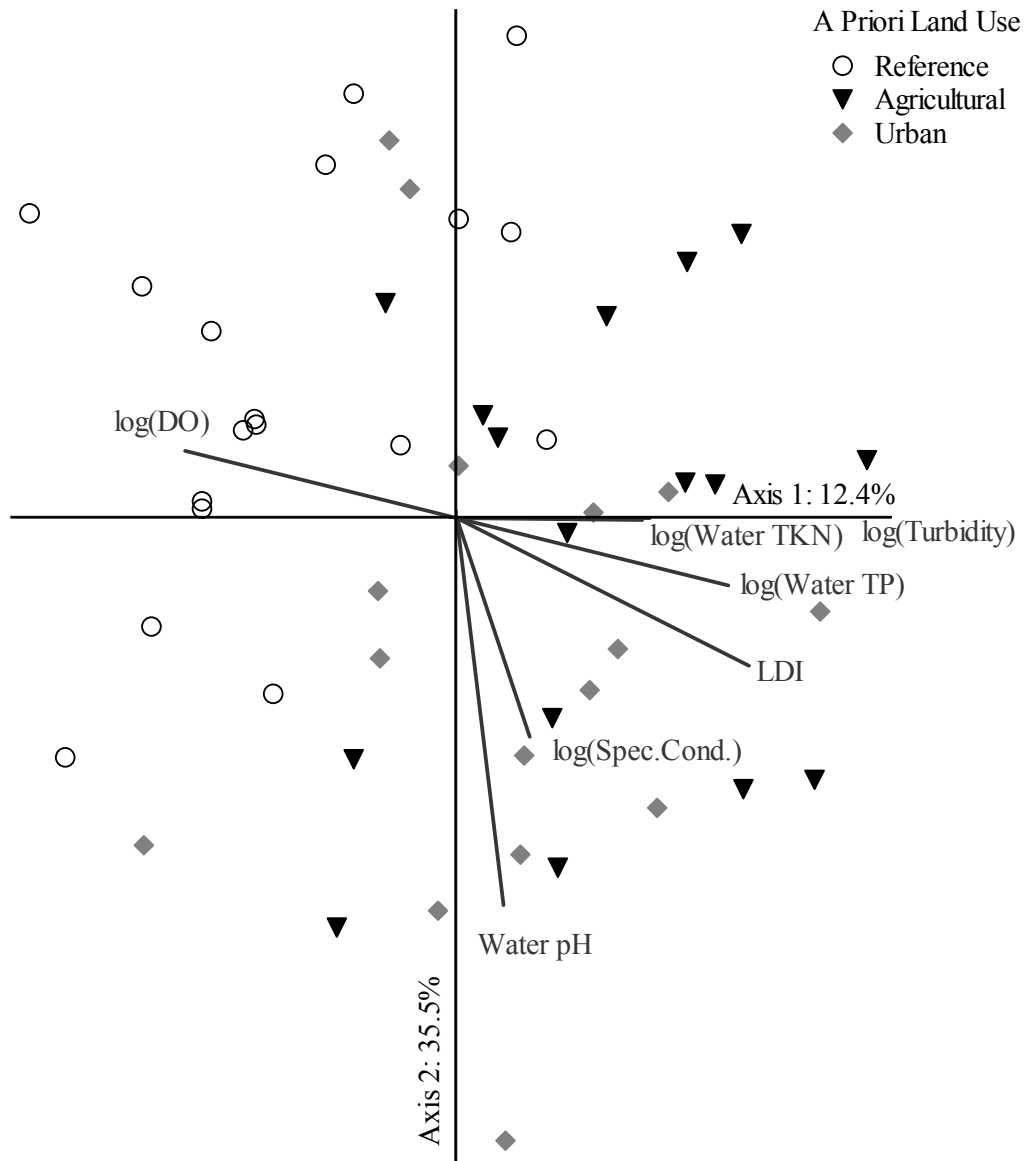


Figure 3-1. NMS ordination bi-plot of 50 wetlands in diatom species space with an overlay of environmental parameters. LDI, dissolved oxygen-DO (log), turbidity (log), water column pH, specific conductivity-Spec.Cond. (log), water TKN (log), and water TP (log), shown as radiating vectors, were significantly correlated with the NMS axes based on diatom community composition. The length of the vector represents the strength of the correlation, and the angle represents the direction of maximum change. Axis 1 explained 12.4% variance, axis 2 explained 35.5% variance, and axis 3 (not shown) represented an additional 26.7% variance.

Table 3-7. Pearson r-squared correlation coefficients between environmental parameters and NMS ordination axes based on diatom community composition.

	Axis 1	Axis 2	Axis 3
Incremental $r^2$	12.4%	35.5%	26.7%
Cumulative $r^2$	12.4%	47.9%	74.6%
Latitude	0.06	0.12	0.16
Longitude	0.03	0.05	0.04
LDI	0.35	0.18	0.05
DO (log)	0.33	0.08	0.09
Temperature (log)	0.03	0.08	0.07
Color (log)	0.15	0.09	0.17
Turbidity (log)	0.22	0.01	0.00
pH	0.06	0.47	0.45
Spec.Cond. (log)	0.09	0.27	0.06
Ammonia-N (log)	0.13	0.00	0.03
Nitrate/nitrite-N (log)	0.01	0.01	0.05
TKN (log)	0.23	0.00	0.01
Water TP (log)	0.33	0.08	0.00
%Soil moisture (arcsine sqroot)	0.11	0.00	0.08
Soil TP (log)	0.07	0.05	0.02

squared correlation coefficients between the environmental variables and the NMS ordination axes. A three dimensional solution was constructed with an overall stress of 16.3 and a final stability of 0.00001, which is borderline high but an acceptable stress limit for a useful ordination with community data (Kruskal 1964; Clarke 1993; McCune and Grace 2002). Axis 1 explained 12.4% of the variance and was correlated with LDI, DO (log), water TKN (log), and water TP (log). Axis 2 explained 35.5% variance and was correlated with pH and specific conductivity (log). Axis 3 explained an additional 26.7% variance and was correlated with water column pH.

### ***Metric Selection***

The pool of potential candidate metrics was streamlined to reduce the redundancy of selected metrics. Metrics selected for inclusion represented two of the metric categories, including tolerance metrics and autecological metrics. In general, at least one metric was selected from each of the autecological categories according to which metric was most highly correlated with LDI. If two metrics were calculated from the same set of taxa representing redundant metrics only one metric was included (i.e. metrics based on the same autecological category were not included, for example if Dissolved Oxygen Class 1 were included, no other metric based on Dissolved Oxygen Class would be included). Seven metrics which were significant according to the Spearman's correlation coefficient ( $|r| \geq 0.46$ ,  $p < 0.01$ ) (Table 3-8), showed visually distinguishable trends with

Table 3-8. Spearman's correlation coefficients for seven diatom metrics with LDI.

Diatom Metrics	Spearman's r
Tolerant Indicator Species	0.65
Sensitive Indicator Species	-0.60
Pollution Class 1	0.52
Nitrogen Class 3	0.48
Saprobity Class 4	0.48
pH Class 3	0.48
Dissolved Oxygen Class 1	-0.46

All correlations were significant ( $p < 0.01$ )

LDI, and were significant for the Mann-Whitney U-test between LDI groups ( $p < 0.10$ ) (Table 3-9) were selected for inclusion in the diatom FWCI. Metrics selected for the FWCI were calculated as the percent of species richness using presence/absence data.

Tolerance metrics included Tolerant Indicator Species and Sensitive Indicator Species. The five autecological metrics included Pollution Class 1 (very tolerant to pollution), Nitrogen Uptake Metabolism Class 3 (need periodically elevated concentrations of organically bound nitrogen), Saprobity Class 4 (inhabit aquatic environments with an oxygen saturation between 10-25% and a biological oxygen demand of approximately 13-22 mg O<sub>2</sub>/L), pH Class 3 (circumneutral, mainly occurring at pH values around 7), and Dissolved Oxygen Class 1 (requiring continuously high dissolved oxygen concentrations near 100%). Pollution Class was established by Bahls (1993), and Nitrogen Uptake Metabolism, Saprobity, pH, and Dissolved Oxygen Classes were defined by van Dam et al. (1994). Tolerant Indicator Species, Pollution Class 1, Nitrogen Uptake Metabolism Class 3, Saprobity Class 4, and pH Class 3 increased with increasing LDI. Sensitive Indicator Species and Dissolved Oxygen Class 1 decreased with increasing LDI. Table 3-9 shows that all of the selected metrics significantly differentiated between the high (LDI  $\geq 2.0$ ) and low (LDI  $< 2.0$ ) LDI groups ( $p < 0.01$ ).

Table 3-9. Comparisons among diatom metrics and the diatom FWCI for LDI groups.

Metric	Low LDI	High LDI	W <sup>^</sup>	p <sup>`</sup>
Tolerant Indicator Species	1.4 (2.5)	35.5 (15.8)	309.0	<0.001
Sensitive Indicator Species	36.6 (25.0)	18.0 (18.5)	719.0	<0.001
Pollution Class 1	3.7 (6.0)	7.6 (10.1)	337.0	<0.001
Nitrogen Class 3	7.7 (15.5)	20.3 (21.5)	361.0	0.003
Saprobity Class 4	2.7 (4.5)	25.4 (25.6)	373.5	0.006
pH Class 3	18.3 (22.2)	14.1 (16.6)	344.0	0.001
Dissolved Oxygen Class 1	69.3 (24.3)	39.9 (23.8)	658.0	0.004
Diatom FWCI	55.3 (11.1)	45.4 (27.1)	718.0	<0.001

Values represent the mean (standard deviation).

W<sup>^</sup> = the Mann-Whitney U-Test statistic

p<sup>`</sup> = the significance value.

### Tolerance metrics

The lists of Tolerant and Sensitive Indicator Species were determined using Indicator Species Analysis (ISA) using species-level abundance data for the diatom assemblage. Tolerant diatom indicator species were established at an LDI break of 4.75, and included 17 species representing nine genera. Table 3-10 lists the diatom Tolerant Indicator Species. The three tolerant indicator species with the highest indicator values were all in the genera *Navicula*, including *N. minima*, *N. confervacea*, and *N. mutica*. Figure 3-2 shows the percent Tolerant Indicator Species increased with increasing LDI. The two wetlands with the highest percent Tolerant Indicator Species in the high LDI group included SU1 (a wetland surrounded by residential land use and a golf course, which was previously embedded in pasture) and CU5 (surrounded by transportation and stormwater retention basins). Four agricultural and urban wetlands and 10 reference wetlands had zero percent tolerant diatom indicator species.

Diatom Sensitive Indicator Species were selected at an LDI break of 1.25, correlating to a break in the natural and developed land uses. Table 3-11 lists the 18 Sensitive Indicator Species. The five Sensitive Indicator Species with the highest indicator values included *Eunotia naegelii*, *E. rhomboidea*, *Frustulia rhomboides*, *Anomoeoneis brachysira*, and *Desmogonium rabenhorstianum*. Figure 3-3 shows that the percent Sensitive Indicator Species decreased with increasing development intensity in the surrounding landscape. Six agricultural and 2 urban wetlands hosted no sensitive indicator species.

Table 3-10. Diatom Tolerant Indicator Species.

Tolerant Indicator Species	Indicator Value
<i>Cyclotella pseudostelliger</i>	20.0
<i>Diploneis elliptica</i>	19.7
<i>Navicula confervacea</i>	45.3
<i>Navicula minima</i>	48.0
<i>Navicula mutica</i>	40.5
<i>Navicula recens</i>	12.9
<i>Navicula subminuscula</i>	12.5
<i>Neidium alpinum</i>	20.0
<i>Nitzschia subacicularis</i>	18.1
<i>Pinnularia braunii</i>	22.6
<i>Pinnularia divergentissima</i>	13.3
<i>Stauroneis kriegeri</i>	13.3

All reported indicator values were significant ( $p < 0.10$ ).

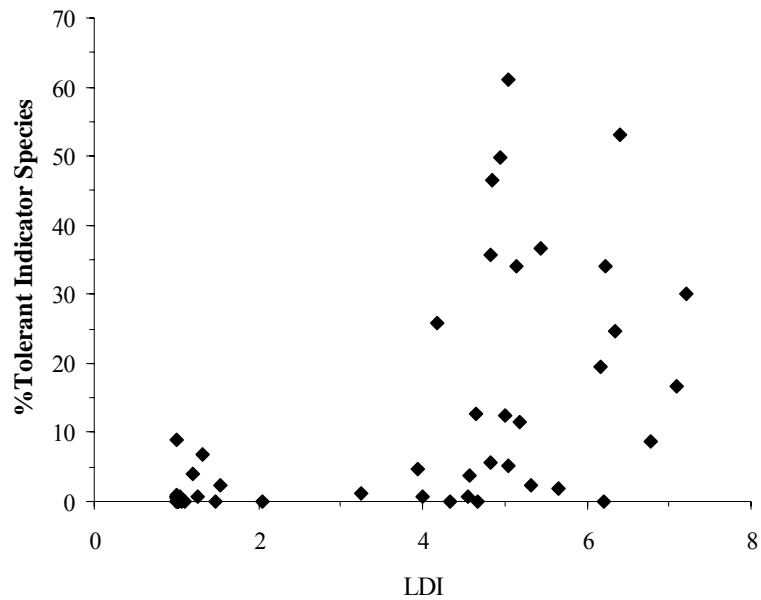


Figure 3-2. Percent diatom Tolerant Indicator Species increased with increasing development intensity (LDI).

Table 3-11. Diatom Sensitive Indicator Species.

Indicator Species	Indicator Value
<i>Anomoeoneis brachysira</i>	39.2
<i>Cymbella microcephala</i>	12.5
<i>Desmogonium rabenhorstianum</i>	33.5
<i>Encyonema silesiacum</i>	24.4
<i>Eunotia flexuosa</i>	12.5
<i>Eunotia glacialis</i>	17.0
<i>Eunotia intermedia</i>	28.1
<i>Eunotia naegelii</i>	59.2
<i>Eunotia pectinalis undulate</i>	26.9
<i>Eunotia rhomboidea</i>	45.9
<i>Frustulia rhomboids</i>	41.6
<i>Frustulia rhomboides crassinervia</i>	18.7
<i>Navicula capitatoradiata</i>	11.5
<i>Navicula subtilissima</i>	12.5
<i>Nitzschia nana</i>	17.0
<i>Nitzschia paleacea</i>	18.7
<i>Pinnularia streptoraphe</i>	18.7
<i>Rhopalodia gibba</i>	18.7

All reported indicator values were significant ( $p < 0.10$ ).

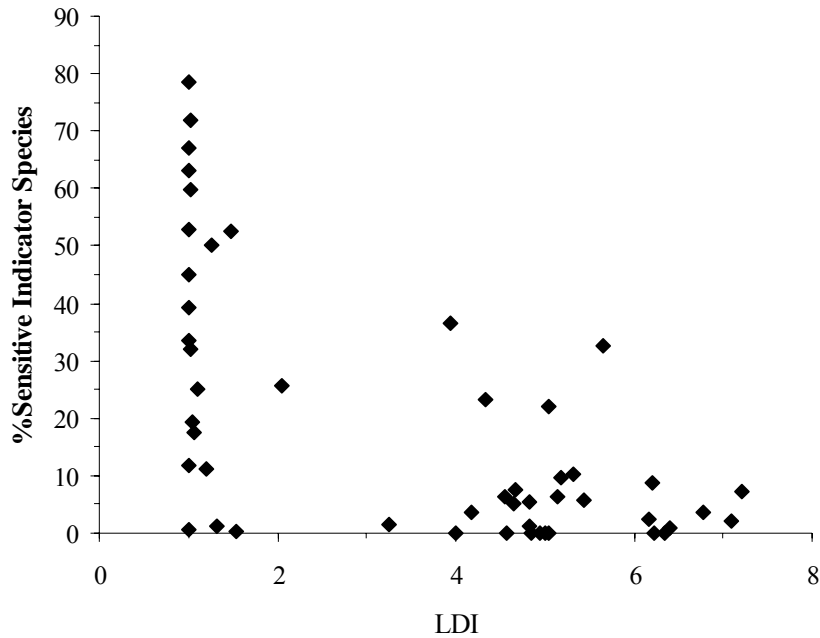


Figure 3-3. Percent diatom sensitive indicator species decreased with increasing development intensity (LDI).

#### *Autecological metrics*

Between 56-69% of diatoms identified received scores based on established autecological relationships (Bahls 1993; van Dam et al. 1994). Five metrics based on scoring diatoms from a coded checklist describing their autecology were incorporated in the diatom FWCI, including the proportion of diatoms in Pollution Class 1, Nitrogen Uptake Metabolism Class 3, Saprobity Class 4, pH Class 3, and Dissolved Oxygen Requirement Class 1. Figure 3-4 shows that the proportion of diatoms in Pollution Class 1 increased with increasing development intensity in the surrounding landscape. Diatoms in Pollution Class 1 were very tolerant to pollution, as compared to Pollution Class 2 (moderately tolerant) or Pollution Class 3 (sensitive to pollution) (Bahls 1993). In general a background level of approximately 10% of the diatoms belonging to Pollution Class 1 distinguishes low ( $LDI < 2.0$ ) and high ( $LDI \geq 2.0$ ) LDI wetlands. The wetland with the greatest percent of diatoms in Pollution Class 1 was CA3 (LDI 4.9, pollution class 1 = 88%), a wetland that received waters dispersed from a pullet farm operation.

Figure 3-5 shows that the proportion of diatoms in Nitrogen Uptake Metabolism Class 3 increased with increasing development intensity in the surrounding landscape. Membership in Nitrogen Uptake Metabolism Class 3 was defined by facultative nitrogen-heterotrophic taxa, those needing periodically elevated concentrations of organically bound nitrogen (van Dam et al. 1994). Four wetlands in the low LDI ( $LDI < 2.0$ ) group that had higher than anticipated percent diatoms in Nitrogen Uptake Metabolism Class 3 included SR4, SR5, SR6, and SU3. The three southern reference wetlands (SR4, SR5, and SR6) were located within state or federal lands protected as part of the Florida Everglades. The urban outlier, SU3 was surrounded by nearly 100 m of marsh that has received nutrient enriched waters since the mid 1970s.



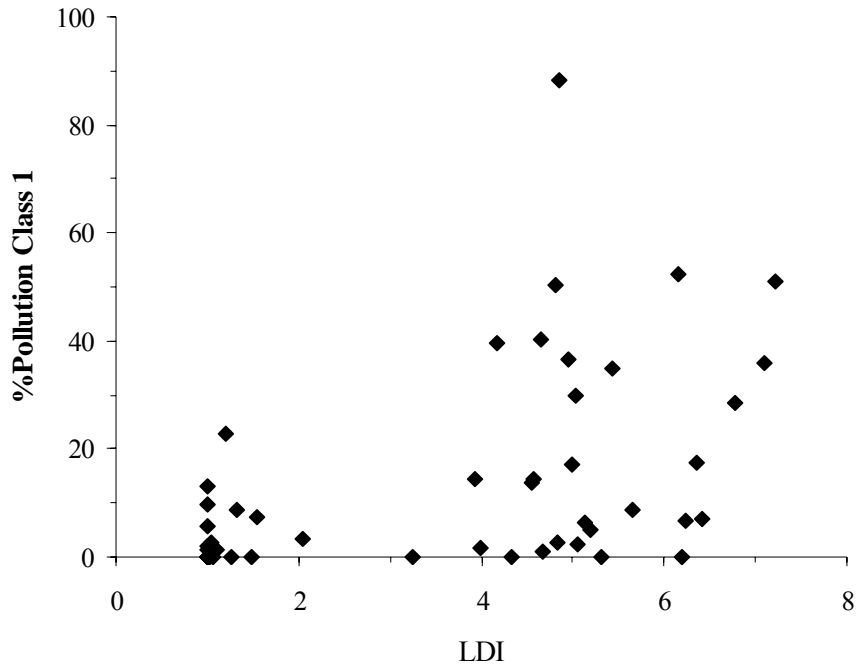


Figure 3-4. Pollution Tolerance Class 1 diatoms increased with increasing development intensity (LDI).

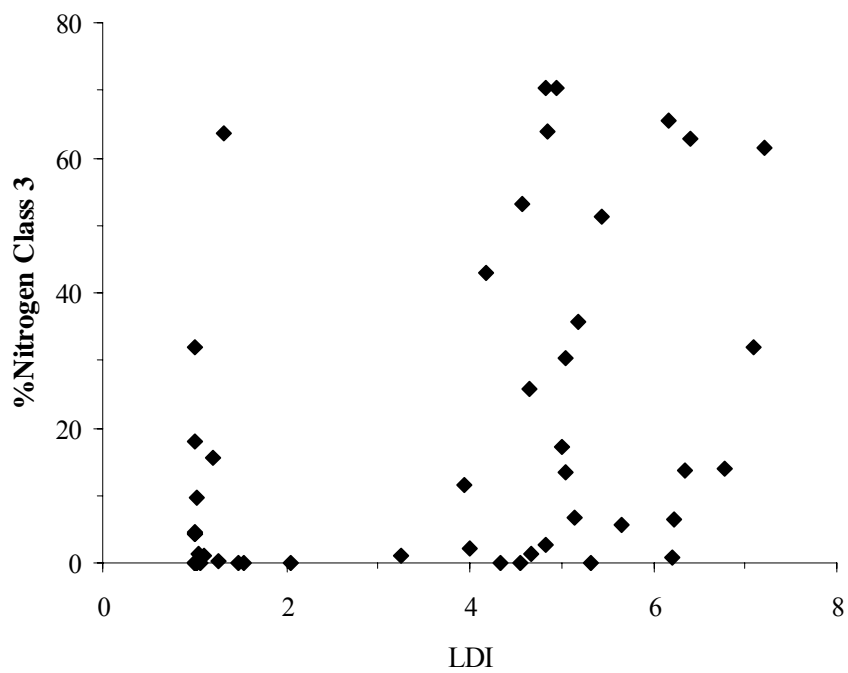


Figure 3-5. Nitrogen Uptake Metabolism Class 3 diatoms increased with increasing development intensity (LDI).

The percent of diatoms in Saprobity Class 4 increased with increasing landscape development intensity (Figure 3-6). Diatoms characterized as belonging to Saprobity Class 4 included meso- to poly-saprobous species (that inhabit aquatic environments with an oxygen saturation between 10-25% and a biological oxygen demand (BOD520) of 13-22 mg/L) (van Dam et al. 1994). Eighty-five percent of the wetlands in the low LDI group had less than 5% of diatoms in Saprobity Class 4. CA3 (a wetland receiving waters dispersed from a pullet farm operation) hosted the greatest percent of diatoms in Saprobity Class 4 (saprobity class 4 = 52%).

Figure 3-7 shows that the percent of diatoms in pH Class 3 increased with increasing LDI. Diatoms categorized in pH Class 3 were described as circumneutral (mainly occurring at pH values of approximately 7) (van Dam et al. 1994). In the low LDI group, the wetland with the highest percent diatoms in pH Class 3 was SU4, a wetland surrounded by low intensity residential, previously in pasture (pH Class 3 = 89%).

Diatoms requiring continuously high dissolved oxygen concentrations of approximately 100% saturation (Dissolved Oxygen Class 1) decreased with increasing development intensity in the surrounding landscape (Figure 3-8). Six wetlands with low LDI scores (LDI < 2.0) hosted a smaller percent of diatoms requiring high dissolved oxygen than anticipated. These were the same six wetlands that hosted a higher percent pH Class 3 diatoms (Figure 3-7).

### ***Diatom Wetland Condition Index***

The seven metrics described above were scored and added together to create the diatom FWCI. Appendix F provides detailed instructions on metric scoring. Figure 3-9 shows the relationship between the diatom FWCI and LDI. Potential scores for the diatom FWCI ranged from 0-70, with higher values representing wetlands surrounded by undeveloped landscapes. Actual scores ranged from 8 at CA3 (an agricultural wetland receiving inputs from a spray field associated with pullet farm wastes), to 69 at SR2 (a wetland surrounded by flooded flatwoods and marsh). The next highest scoring wetlands received diatom FWCI scores 2 points lower than SR2, with a score of 67 at both NR3 and SR1.

Diatom FWCI scores varied regionally, with the highest scores in each wetland region including PR4 (65), NR3 (67), CR6 (65), and SR2 (69), in the panhandle, north, central, and south wetland regions, respectively. The lowest scores in the panhandle and north wetland regions were for urban wetlands embedded in residential land use, including PU4 (11) and NU2 (24). Wetlands surrounded by agricultural land uses received the lowest scores in the central and south wetland regions, including CA3 (8) and SA4 (16). The diatom FWCI was robustly correlated with the LDI index (Spearman correlation  $|r| = 0.64$ ,  $p < 0.001$ ). A Kruskal-Wallis test between median diatom FWCI score values suggested a significant difference ( $H = 20.7$ ,  $p < 0.001$ ) among wetlands in the three *a priori* land use categories.

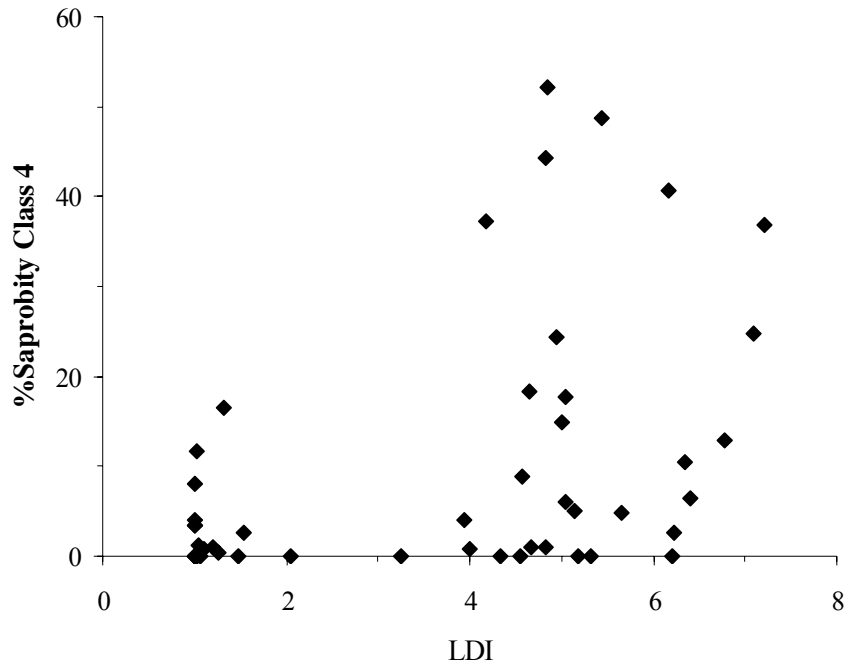


Figure 3-6. Saprobity Class 4 diatoms increased with increasing development intensity (LDI).

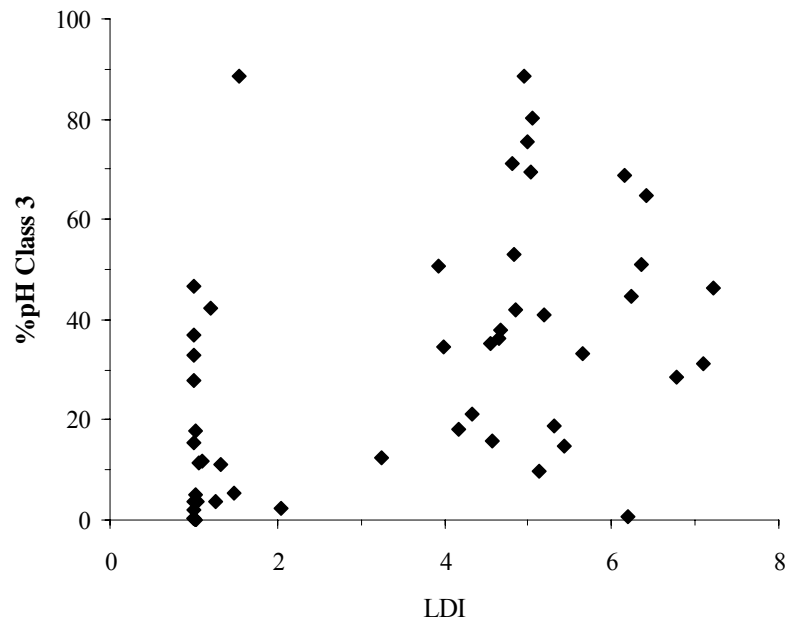


Figure 3-7. The pH Class 3 diatoms increased with increasing development intensity (LDI).

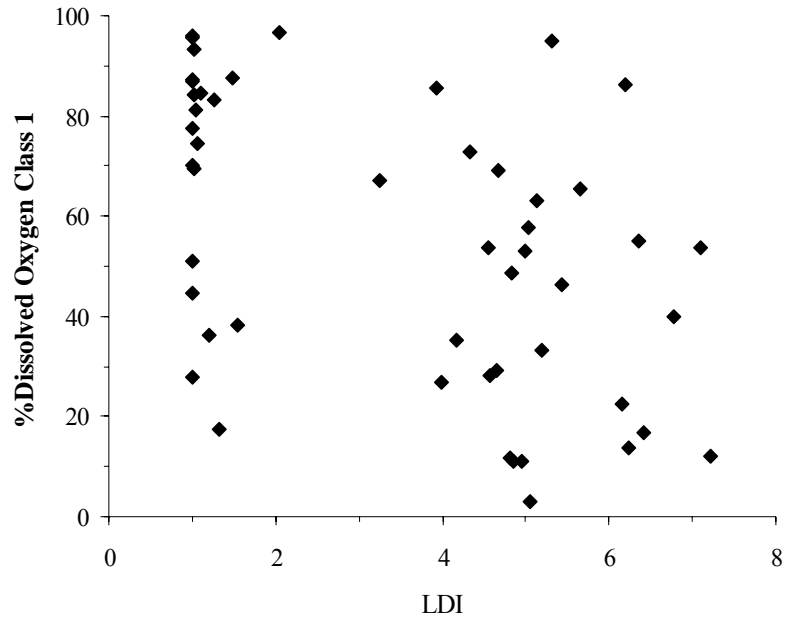


Figure 3-8. Dissolved Oxygen Class 1 diatoms decreased with increasing development intensity (LDI).

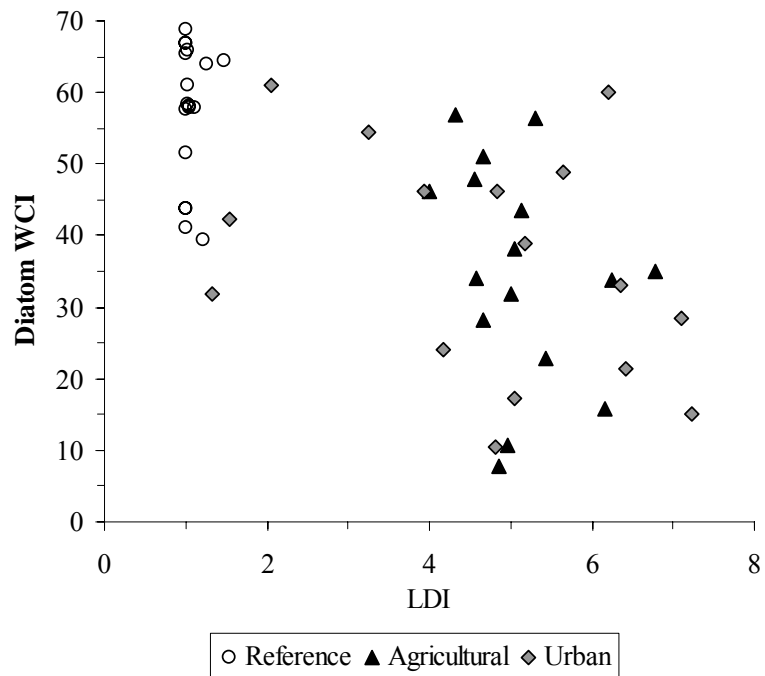


Figure 3-9. Diatom FWCI scores decreased with increasing development intensity (LDI). Sample wetlands are designated by *a priori* land use category: reference, agricultural, or urban.

### Cluster Analysis

Cluster analysis determined four categories based on diatom community composition. Using site descriptions, clusters were explained by wetland regions and *a priori* land use categories including: 1: wetlands in the panhandle to central wetland regions surrounded by low development land use activities; 2: wetlands occurring in mixed wetland regions surrounded by low development land use activities; 3: wetlands within the southern Everglades; and 4: wetlands within mixed wetland regions surrounded by high development land use activities. Figure 3-10 shows that based on the diatom FWCI scores, clusters 1 and 2 were not significantly different from one another, but were significantly different from both cluster 3 and cluster 4 ( $p < 0.05$ ). Clusters 3 and 4 were significantly different from each other. Table 3-12 provides means and standard deviations for diatom FWCI scores and LDI of the four diatom based clusters. Cluster 4 had significantly different diatom FWCI and LDI scores than all other clusters.

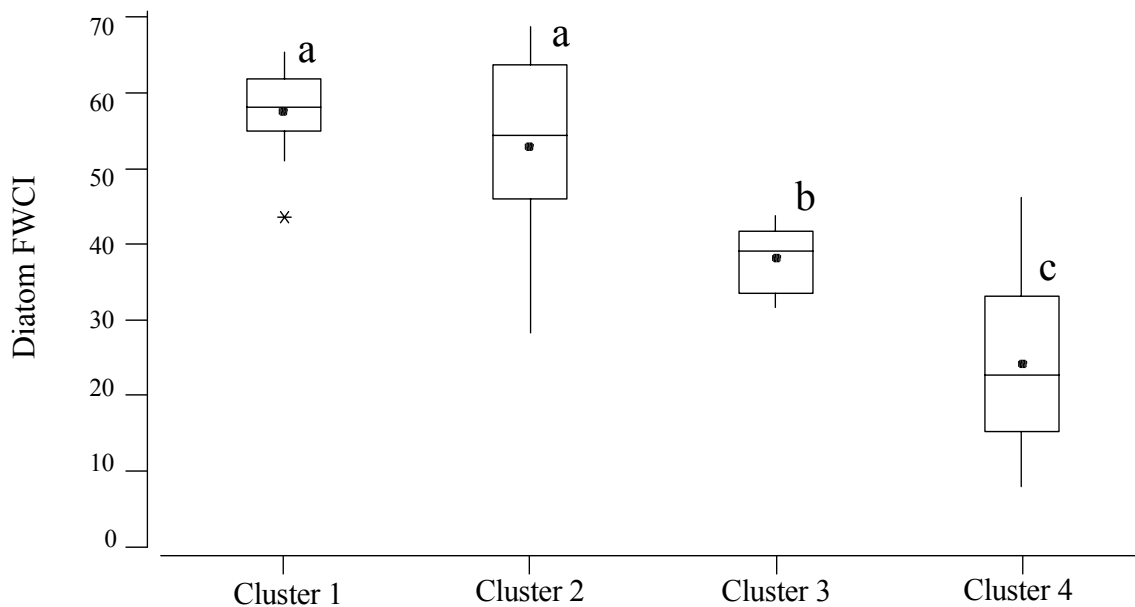


Figure 3-10. Diatom FWCI scores for wetland clusters based on diatom community composition. Boxes represent the interquartile range; solid circles represent the mean; middle lines represent the median; whiskers represent the range; asterisks represent outliers ( $> \pm 2$  standard deviations). Clusters with similar letters were not significantly different (Fisher's LSD,  $p < 0.05$ ).

Table 3-12. Mean diatom FWCI and LDI values for wetland clusters based on diatom community composition.

	Cluster*			
	1	2	3	4
Diatom FWCI	57.6 (6.4) <sup>a</sup>	52.9 (11.6) <sup>a</sup>	38.1 (4.4) <sup>b</sup>	24.1 (11.7) <sup>c</sup>
LDI	2.9 (2.1) <sup>a</sup>	2.8 (2.0) <sup>a</sup>	2.4 (1.9) <sup>a</sup>	5.3 (1.4) <sup>b</sup>

\* Clusters with similar letters were not significantly different (Fisher's LSD, p<0.05).  
Values represent mean (standard deviation).

## Macrophyte Assemblage

Statewide, 118 wetlands were sampled with 605 species, representing 323 genera and 126 families identified. The most abundant species was *Taxodium ascendens* found rooted within the vegetation quadrats at 93% of the study wetlands. The second most abundant species was *Myrica cerifera* found in 64% of the study wetlands. The most common fern was *Woodwardia virginica* found at 53% of the wetlands; the most common vine was *Toxicodendron radicans* also found at 53% of the wetlands; and the most common graminoid was *Panicum hemitomon* found at 50% of the wetlands. Of the species encountered, 130 species (22%) occurred at a minimum of 5% of the sample wetlands ( $n \geq 6$ ). Approximately one-third of the species identified (202 species or 33.5%) were rooted in the vegetation quadrats at only one wetland. In the panhandle wetland region, 28 wetlands were sampled hosting 328 species, representing 191 genera and 90 families. In the north wetland region 31 wetlands were sampled with 306 species (180 genera and 89 families) encountered. The central wetland region included 31 wetlands with 329 species (202 genera and 94 families) sampled. The south wetland region had 28 sample wetlands with 266 species (in 180 genera and 89 families) identified.

### Summary Statistics

Species richness (R), first and second order jackknife estimators (Jack1 and Jack2, respectively), species evenness (E), Shannon diversity (H), and Whittaker's beta diversity ( $\beta W$ ) were calculated based on the macrophyte assemblage for each sample wetland (Appendix E). Species richness ranged from 13 species at NA3 (embedded in silvicultural land use), to 77 species at NA12 (surrounded with pasture and row crops). The greatest estimates of species richness were 99 species and 114 species at NA12, for first and second order jackknife estimators, respectively. Sampled species richness at NA12 was 77 species. The lowest estimates of actual species richness were for NR1 (surrounded by pine flatwoods), with 11 and 15 species estimated with first and second order jackknife estimators, respectively. Sampled species richness for NR1 was 14 species. Species evenness ranged from 0.71 at PR7 (a large, deep wetland on a private conservation tract), to 0.93 at PU4 (surrounded by residential land use including housing and a community park). Shannon diversity ranged from 1.8 to 3.9 at two agricultural wetlands, NA3 (surrounded by silviculture) and NA12 (surrounded by pasture and row crops), respectively, similar to species richness. Whittaker's beta diversity ranged from a low of 0.2 at PU9 (surrounded by residential land use), to a high of 9.4 at CR5 (located in a fragmented state park within highly developed Seminole County).

Table 3-13 summarizes comparisons of mean richness and diversity calculations by *a priori* land use category. Agricultural wetlands had the greatest species richness followed closely by urban wetlands. This same trend was evident for species evenness, with agricultural wetlands having greater species evenness. Diversity indices yielded similar results, with reference wetlands having lower Shannon diversity and Whittaker's beta diversity than both agricultural and urban wetlands. Beta and gamma diversity were calculated for *a priori* land use categories, with urban wetlands having the highest beta diversity and agricultural wetlands the highest gamma diversity. Only Whittaker's beta

Table 3-13. Mean macrophyte richness, evenness, and diversity among *a priori* land use categories.

	Reference	Agricultural	Urban
Species richness (R)	32 (11) <sup>a</sup>	37 (14) <sup>a</sup>	36 (10) <sup>a</sup>
Species evenness (E)	0.85 (0.04) <sup>a</sup>	0.87 (0.04) <sup>a</sup>	0.86 (0.03) <sup>a</sup>
Shannon diversity (H)	2.9 (0.3) <sup>a</sup>	3.1 (0.4) <sup>a</sup>	3.1 (0.3) <sup>a</sup>
Whittaker's Beta diversity ( $\beta$ W)	4.0 (2.9) <sup>a</sup>	4.8 (1.7) <sup>b</sup>	4.3 (1.7) <sup>ab</sup>
Beta diversity	9.5	10.4	10.5
Gamma diversity	304	383	378

Categories with similar letters were not significantly different (Fisher's LSD,  $\alpha=0.05$ ). Values represent mean (standard deviation).

diversity was significantly different among *a priori* land use categories (Fisher's LSD pair wise comparison,  $\alpha = 0.05$ ). Species richness and Whittaker's beta diversity were not significantly different between low (LDI < 2.0) and high (LDI  $\geq$  2.0) LDI groups (Table 3-14); whereas, species evenness and Shannon diversity were significantly different ( $p < 0.05$ ) between LDI groups (Mann-Whitney U-Test).

### ***Compositional Analysis***

MRPP was calculated across all wetland regions (panhandle versus north versus central versus south) as well as for multiple pair wise comparisons (panhandle versus north, panhandle versus central, panhandle versus south, north versus central, north versus south, and central versus south). Table 3-15 shows the results for the MRPP tests, including the test statistic (T), chance-corrected within-group agreement (A), and significance value (p). Only two of the MRPP comparisons (agricultural wetlands in the panhandle and north, and central and south wetland regions) were not significant at the

Table 3-14. Mean macrophyte richness, evenness, and diversity between LDI groups.

	Low LDI	High LDI	W <sup>^</sup>	p <sup>`</sup>
Species richness (R.)	33 (10)	37 (12)	2120	0.07
Species evenness (E)	0.85 (0.04)	0.87 (0.04)	2028	0.02*
Shannon diversity (H)	2.9 (0.3)	3.1 (0.4)	2062	0.03*
Whittaker's Beta diversity ( $\beta$ W)	4.1 (1.9)	4.5 (1.7)	2131	0.08
Beta diversity	10.2	13.8		
Gamma diversity	338	510		

\* Indicates significance at  $\alpha < 0.05$

W<sup>^</sup> = Mann-Whitney U-test statistic

p<sup>`</sup> = significance value

Values represent mean (standard deviation).



Table 3-15. Macrophyte community composition similarity among wetland regions with MRPP.

	Sites (n)	T <sup>^</sup>	A <sup>`</sup>	p <sup>#</sup>
<b>All wetlands</b>				
All regions (P vs N vs C vs S)	118	-26.8	0.06	0.00*
Panhandle vs north	59	-7.1	0.03	0.00*
Panhandle vs central	59	-13.1	0.04	0.00*
Panhandle vs south	56	-23.6	0.09	0.00*
North vs central	62	-7.9	0.03	0.00*
North vs south	59	-24.2	0.09	0.00*
Central vs south	59	-11.8	0.04	0.00*
<b>Reference wetlands</b>				
All regions (P vs N vs C vs S)	37	-12.5	0.12	0.00*
Panhandle vs north	17	-5.2	0.08	0.00*
Panhandle vs central	19	-6.8	0.09	0.00*
Panhandle vs south	17	-8.1	0.14	0.00*
North vs central	20	-4.7	0.06	0.00*
North vs south	18	-8.5	0.14	0.00*
Central vs south	20	-7.1	0.07	0.00*
<b>Agricultural wetlands</b>				
All regions (P vs N vs C vs S)	40	-6.6	0.05	0.00*
Panhandle vs north	22	-0.7	0.01	0.21
Panhandle vs central	19	-2.8	0.03	0.01*
Panhandle vs south	19	-8.1	0.10	0.00*
North vs central	21	-2.0	0.02	0.04*
North vs south	21	-7.7	0.09	0.00*
Central vs south	18	-1.4	0.02	0.09
<b>Urban wetlands</b>				
All regions (P vs N vs C vs S)	41	-15.3	0.14	0.00*
Panhandle vs north	20	-6.0	0.07	0.00*
Panhandle vs central	21	-9.0	0.10	0.00*
Panhandle vs south	20	-11.1	0.17	0.00*
North vs central	21	-3.3	0.03	0.00*
North vs south	20	-10.5	0.15	0.00*
Central vs south	21	-8.6	0.09	0.00*

\*A high |T| value and significant p-value (p<0.05) suggests a difference in species composition.

T<sup>^</sup> = the MRPP test statistic

A<sup>`</sup> = the chance corrected within-group agreement

p<sup>#</sup> = the significance value.

$\alpha = 0.05$  level, suggesting that there were regionally significant differences among species composition across wetland regions and within *a priori* land use categories.

### ***Community Composition***

Macrophyte community composition was summarized in two separate NMDS ordinations to relate changes in macrophyte community composition with environmental variables. Figure 3-11 shows a two dimensional bi-plot of the NMDS axes used to explore the dissimilarities of macrophyte community composition with overlays of significant environmental variables, including soil TP (log), LDI, latitude, and longitude. Table 3-16 provides the Pearson correlation coefficients between environmental variables and NMDS ordination axes. A three dimensional solution was constructed with an overall stress of 20.8 and a final stability of 0.06, which is a fairly high stress value but considered useful for ordinations with community data sets (Kruskal 1964; Clarke 1993; McCune and Grace 2002). Axis 1 explained 29.1% variance and was correlated with latitude and longitude; axis 2 explained 34.2% additional variance and was correlated with LDI and soil TP (log). Axis 3 explained an additional 12.1% of the variance and was not significantly correlated with any of the measured soil parameters, LDI, latitude, or longitude.

A second NMS ordination was completed using the macrophyte species composition at the 75 sample wetlands with measured water chemical and physical parameters. Figure 3-12 shows the bi-plot from the NMS ordination. The final stress was 16.6 with a final instability of 0.004. The ordination explained a cumulative 77.4% of the variance in wetland macrophyte community composition. Table 3-17 shows the Pearson r-squared correlation coefficient values for the environmental parameters and the three ordination axes. Axis 1 explained 31.3% of the variance and was correlated with latitude and longitude. Axis 2 was correlated with LDI, water pH, water TP (log), soil TP (log), and DO (log), and explained 25.7% additional variance. Axis 3 explained an additional 20.4% of the variance and was correlated with water pH and soil moisture (arcsine square root). Water ammonia-nitrogen concentration (log), water nitrate/nitrite-nitrogen concentration (log), water TKN-nitrogen concentration (log), water temperature (log), water color (log), and water turbidity (log) were not strongly correlated with the NMDS ordination axes.

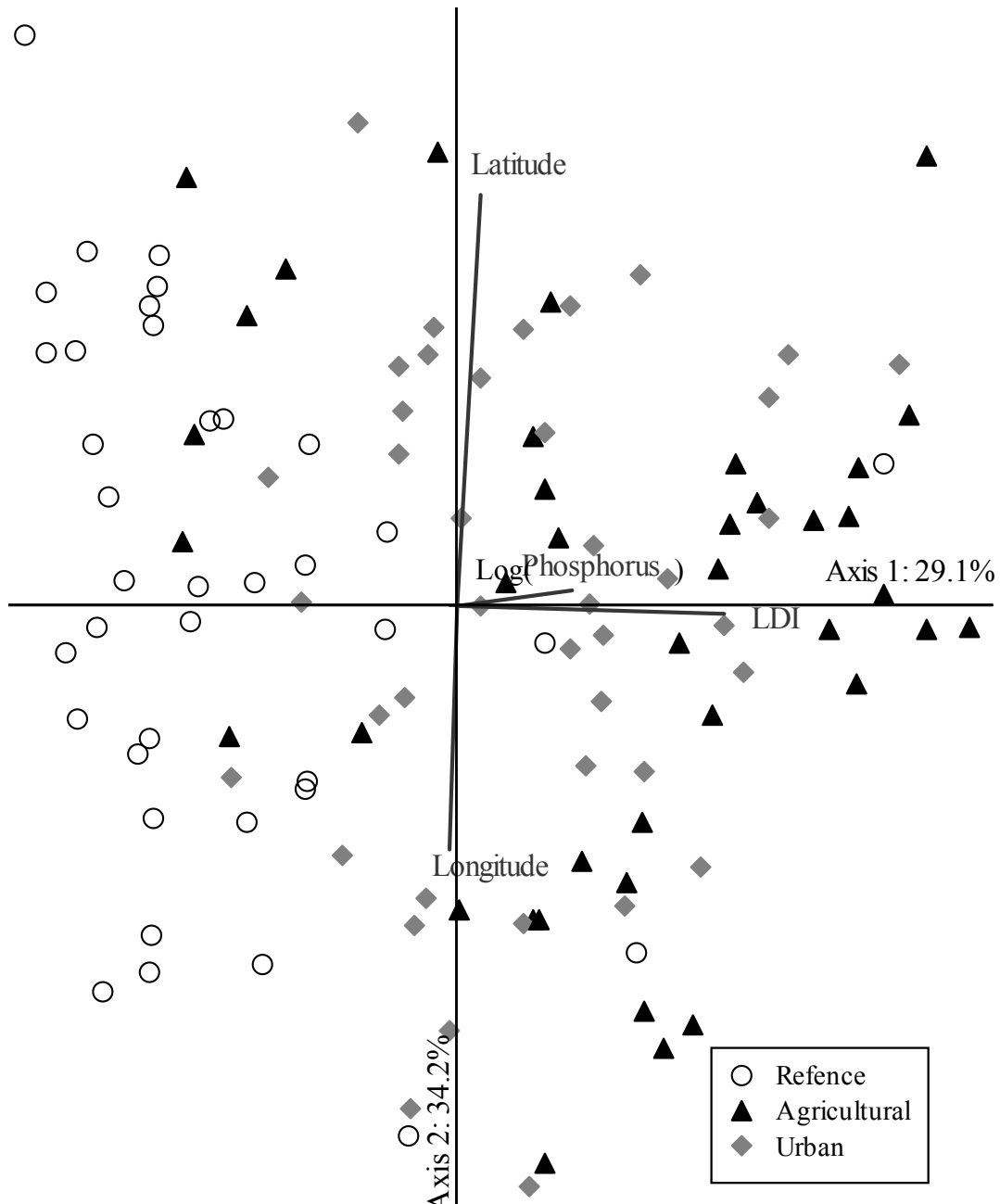


Figure 3-11. NMDS ordination bi-plot of 118 sample wetlands in macrophyte species space with an overlay of environmental parameters. Latitude, longitude, LDI, and log(soil TP), shown as radiating vectors, were significantly correlated with the NMS axes. Vector length represents the strength of the correlation, and the angle represents the direction of maximum change. Axis 1 explained 29.1% variance, axis 2 explained 34.2% additional variance, and axis 3 (not shown) represented an additional 12.1% variance.

Table 3-16. Pearson correlation coefficients between environmental variables and NMDS axes based on macrophyte community composition at 118 wetlands.

	Axis 1	Axis 2	Axis 3
Incremental $r^2$	29.1%	34.2%	12.1%
Cumulative $r^2$	29.1%	63.3%	75.4%
Latitude	0.71	0.04	0.00
Longitude	0.42	0.02	0.00
LDI	0.01	0.46	0.01
Arcsine Sqrt (Soil moisture)	0.00	0.11	0.04
Log (Soil TKN)	0.03	0.06	0.00
Log(Soil TP)	0.03	0.20	0.00

### *Metric Selection*

The pool of potential candidate metrics was streamlined to reduce the redundancy of selected metrics. If two metrics were calculated from the same set of taxa representing redundant metrics only one metric was included (i.e. metrics based on the same category were not included, for example if percent Obligate Species was included, no other metric based on wetland indicator status). Six metrics that were significant for the Spearman's correlation coefficient ( $|r| > 0.50$ ,  $p < 0.001$ ) (Table 3-18), showed visually distinguishable trends with LDI, and were significant for the Mann-Whitney U-test between LDI groups ( $p < 0.001$ ) (Table 3-19) were selected for inclusion in the macrophyte FWCI. Metrics selected for the FWCI were calculated as the percent of species richness using presence/absence data.

Metrics selected for inclusion were percent Tolerant Indicator Species, percent Sensitive Indicator Species; Floristic Quality Assessment Index (FQAI); percent Exotic Species; percent Native Perennial Species; and percent Wetland Status Species (including both obligate and facultative wetland species). The percent of Tolerant Indicator Species and percent Exotic Species increased with increasing development intensity. Whereas, percent Sensitive Indicator Species, FQAI, percent Native Perennial Species, and percent Wetland Status Species values decreased with increased landscape development. Table 3-19 shows that all six metrics significantly differentiated between low ( $LDI < 2.0$ ) and high ( $LDI \geq 2.0$ ) LDI groups.

### *Tolerance metrics*

Macrophyte Tolerant and Sensitive Indicator Species lists were determined using Indicator Species Analysis (ISA) for each of the four wetland regions and statewide using species-level abundance data. Macrophyte Tolerant Indicator Species were established at an LDI break of 4.0. Table 3-20 provides a list of Tolerant Indicator Species comparing regional and statewide analyses. The same random seed number was used for each ISA for statewide and regional analyses.

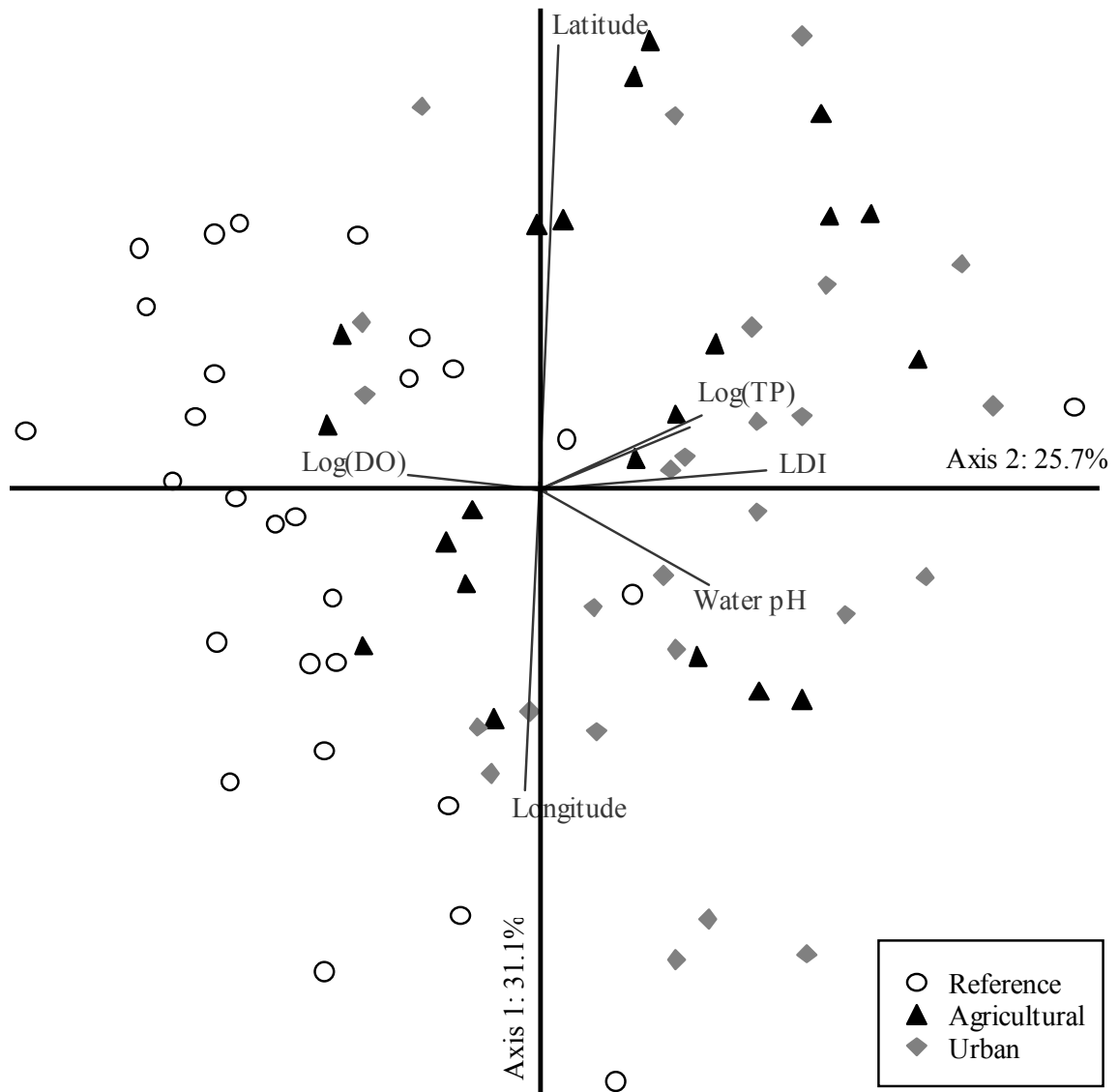


Figure 3-12. NMDS ordination bi-plot of 75 sample wetlands in macrophyte species space with an overlay of environmental parameters. Latitude, longitude, LDI, TP (log), water pH, and DO (log), shown as radiating vectors, were significantly correlated with the NMDS axes. Vector length represents the strength of the correlation, and the angle represents the direction of maximum change. Axis 1 explained 31.3% variance, axis 2 explained 25.7% additional variance, and axis 3 (not shown) represented an additional 20.4% variance.

Table 3-17. Pearson correlations between environmental variables and NMDS axes based on macrophyte community composition at 75 wetlands.

	Axis 1	Axis 2	Axis 3
Incremental $r^2$	31.3%	25.7%	20.4%
Cumulative $r^2$	31.3%	57.0%	77.4%
Latitude	0.68	0.03	0.17
Longitude	0.46	0.03	0.06
LDI	0.03	0.35	0.11
Water parameters			
DO (log)	0.02	0.20	0.05
Temperature (log)	0.02	0.05	0.00
Color (log)	0.14	0.00	0.05
Turbidity (log)	0.03	0.06	0.03
pH	0.15	0.26	0.35
Ammonia-N (log)	0.01	0.04	0.00
Nitrate/nitrite-N (log)	0.06	0.00	0.06
TKN (log)	0.02	0.01	0.01
TP (log)	0.11	0.25	0.06
Soil parameters			
Moisture (arcsine sqrt)	0.00	0.00	0.24
TKN (log)	0.00	0.00	0.16
TP (log)	0.09	0.23	0.01

Table 3-18. Spearman correlations between six statewide macrophyte metrics with LDI.

Metric	Spearman's r
Percent Tolerant Indicator Species	0.75
Percent Sensitive Indicator Species	-0.66
FQAI	-0.71
Percent Exotic Species	0.65
Percent Native Perennial Species	-0.63
Percent Wetland Status Species	-0.55

All correlations were significant ( $p < 0.01$ ).

Table 3-19. Comparisons among six statewide macrophyte metrics between LDI groups.

Metric	Low LDI	High LDI	W <sup>^</sup>	p <sup>`</sup>
Percent Tolerant Indicator Species	7.8 (7.8)	31.2 (14.7)	1116.5	<0.001
Percent Sensitive Indicator Species	39.5 (16.7)	9.4 (10.1)	3665.0	<0.001
FQAI	4.81 (0.62)	3.62 (0.80)	3771.0	<0.001
Percent Exotic Species	3.0 (3.6)	14.3 (10.6)	1379.0	<0.001
Percent Native Perennial Species	92.7 (4.5)	79.7 (12.0)	3453.0	<0.001
Percent Wetland Status Species	72.0 (9.8)	54.1 (12.5)	3612.0	<0.001

Values represent the mean (standard deviation)

W<sup>^</sup> = the Mann-Whitney U-Test statistic

p<sup>`</sup> = the significance value

In total the ISA reported 69 statewide Tolerant Indicator Species, and less for each wetland region with 7, 28, 7, and 12 for the panhandle, north, central, and south wetland regions, respectively. The statewide ISA produced 69 Tolerant Indicator Species with an additional seven species included on regional lists, but not the statewide list. No species occurred on the Tolerant Indicator Species lists statewide and in all four wetland regions. Three species occurred on the statewide Tolerant Indicator Species list and on three of the wetland region lists, including *Commelina diffusa* (north, central, and south), *Cynodon dactylon* (panhandle, north, central), and *Diodia virginiana* (panhandle, north, south). Seven species occurred on the Tolerant Indicator Species list statewide and in two wetland regions, and 24 were listed both statewide and in one wetland region. Thirty-five of the 69 statewide Tolerant Indicator Species (51%) were not listed in any wetland region. In total, the wetland regions shared more than two-thirds of their listed species with the statewide list: panhandle (100%), north (93%), central (86%), and south (67%). Two species were unique to the north, one to the central, and four to the south wetland region Tolerant Indicator Species lists.

Figure 3-13 shows the scatterplots of the percent Tolerant Indicator Species versus LDI. The percent Tolerant Indicator Species increased with increasing development intensity. For the statewide Tolerant Indicator Species, CA2 (surrounded by pasture; tolerant = 72%) had the highest percent statewide Tolerant Indicator Species. Three wetlands in the low LDI group with high percents Tolerant Indicator Species included SA8 (a suburban park receiving run-off from a pasture; 32%), CA8 (in a preserve but receiving run-off from dairy activities; 26%), and PR7 (located on a private conservation tract; 25%). In the regional ISA calculations, a wetland in the north wetland region, NA1 (surrounded by pasture; north tolerant = 47%) had the largest percent Tolerant Indicator Species.

Macrophyte Sensitive Indicator Species were established at an LDI break of 2.0. Table 3-21 provides a list of statewide and regional Sensitive Indicator Species. The statewide Sensitive Indicator Species list included 61 species of which 16 were not listed in any of the wetland regions. Two species occurred on all statewide and wetland region lists, including *Eriocaulon decangulare* and *Panicum erectifolium*. Similarly, six species occurred on the statewide list and three wetland region lists including *Andropogon*

Table 3-20. Statewide and regional macrophyte Tolerant Indicator Species.

	Statewide	Panhandle	North	Central	South
No. of Tolerant Species	69	7	28	7	12
<i>Acer rubrum</i>	28.5		53.7		
<i>Alternanthera philoxeroides</i>	11.3				
<i>Amaranthus spinosus</i>	10.9		21.4		
<i>Ampelopsis arborea</i>	18.6				
<i>Aster carolinianus</i>	9.5				
<i>Axonopus fissifolius</i>	9.1				
<i>Blechnum serrulatum</i>				42.4	
<i>Boehmeria cylindrica</i>	37.9	57.9	43.7		
<i>Carex longii</i>	30.6		54.3		
<i>Centella asiatica</i>					52.0
<i>Colocasia esculenta</i>	5.5				
<i>Commelina diffusa</i>	44.8		50.0	73.3	44.1
<i>Cuphea carthagenensis</i>	28.0		28.6		50.0
<i>Cynodon dactylon</i>	29.4	35.7	35.7	28.1	
<i>Cyperus croceus</i>	9.1				
<i>Cyperus lanceolatus</i>	7.3				
<i>Cyperus polystachyos</i>	13.1				
<i>Cyperus retrorsus</i>	18.2		35.7	20.0	
<i>Cyperus virens</i>	11.3		42.9		
<i>Digitaria ciliaris</i>	9.1				
<i>Diodia virginiana</i>	37.2	50.0	40.5		50.0
<i>Dioscorea bulbifera</i>	7.3				
<i>Echinochloa colona</i>	5.5				
<i>Eclipta prostrata</i>	17.9				
<i>Eupatorium capillifolium</i>	37.9		44.1		
<i>Galium hispidulum</i>	5.5				
<i>Galium tinctorium</i>	22.6		51.8	26.7	
<i>Hymenachne amplexicaulis</i>	9.1				25.0
<i>Hypericum mutilum</i>			28.6		
<i>Juncus effusus</i>	22.1		42.9		
<i>Kyllinga brevifolia</i>	7.3				
<i>Leersia hexandra</i>	7.3			20.0	
<i>Lepidium virginicum</i>	5.5				
<i>Ligustrum sinense</i>	10.2				
<i>Lonicera japonica</i>	12.7				
<i>Ludwigia peruviana</i>	17.7				
<i>Ludwigia repens</i>	14.3		21.4		
<i>Luziola fluitans</i>	5.5				
<i>Lygodium japonicum</i>	11.9				



Table 3-20. Continued.

	Statewide	Panhandle	North	Central	South
<i>Melaleuca quinquenervia</i>					36.2
<i>Melothria pendula</i>	20.8		28.6		
<i>Micranthemum umbrosum</i>	5.5				
<i>Momordica charantia</i>	10.2				
<i>Oxalis corniculata</i>	18.5		28.6		
<i>Parthenocissus quinquefolia</i>	34.6		40.5		52.0
<i>Paspalum notatum</i>	24.4		30.7		
<i>Paspalum urvillei</i>	18.5	35.7	28.6		
<i>Phyla nodiflora</i>	15.2				32.1
<i>Phyllanthus urinaria</i>	16.7		21.4		
<i>Phytolacca americana</i>	26.1	36.7			
<i>Polygonum hydropiperoides</i>	21.1	57.1			
<i>Polygonum punctatum</i>	28.0	42.9	44.7		
<i>Polypremum procumbens</i>	9.5				
<i>Proserpinaca palustris</i>	5.5				
<i>Richardia brasiliensis</i>	5.5				
<i>Rubus argutus</i>	25.1		47.1		
<i>Rubus trivialis</i>	17.7				
<i>Sabal palmetto</i>					66.7
<i>Sacciolepis indica</i>	7.3				
<i>Sambucus canadensis</i>	24.6		30.7		
<i>Sapium sebiferum</i>	19.4				
<i>Saururus cernuus</i>			40.5		
<i>Senna obtusifolia</i>	9.1				
<i>Sesbania vesicaria</i>	5.5				
<i>Setaria parviflora</i>	7.3				
<i>Sida rhombifolia</i>	20.8		35.7		
<i>Smilax pumila</i>	7.3		21.4		
<i>Solanum carolinense</i>	9.1				
<i>Solidago stricta</i>	5.5				
<i>Sporobolus indicus</i>	7.3				
<i>Stenotaphrum secundatum</i>	9.5			26.7	
<i>Toxicodendron radicans</i>	38.0				
<i>Trifolium repens</i>	7.3		21.4		
<i>Urena lobata</i>					52.0
<i>Vitis rotundifolia</i>	36.7				48.5
<i>Wedelia trilobata</i>	5.5				25.0

Values represent Indicator Values.

All reported Indicator Values were significant ( $p < 0.10$ ).

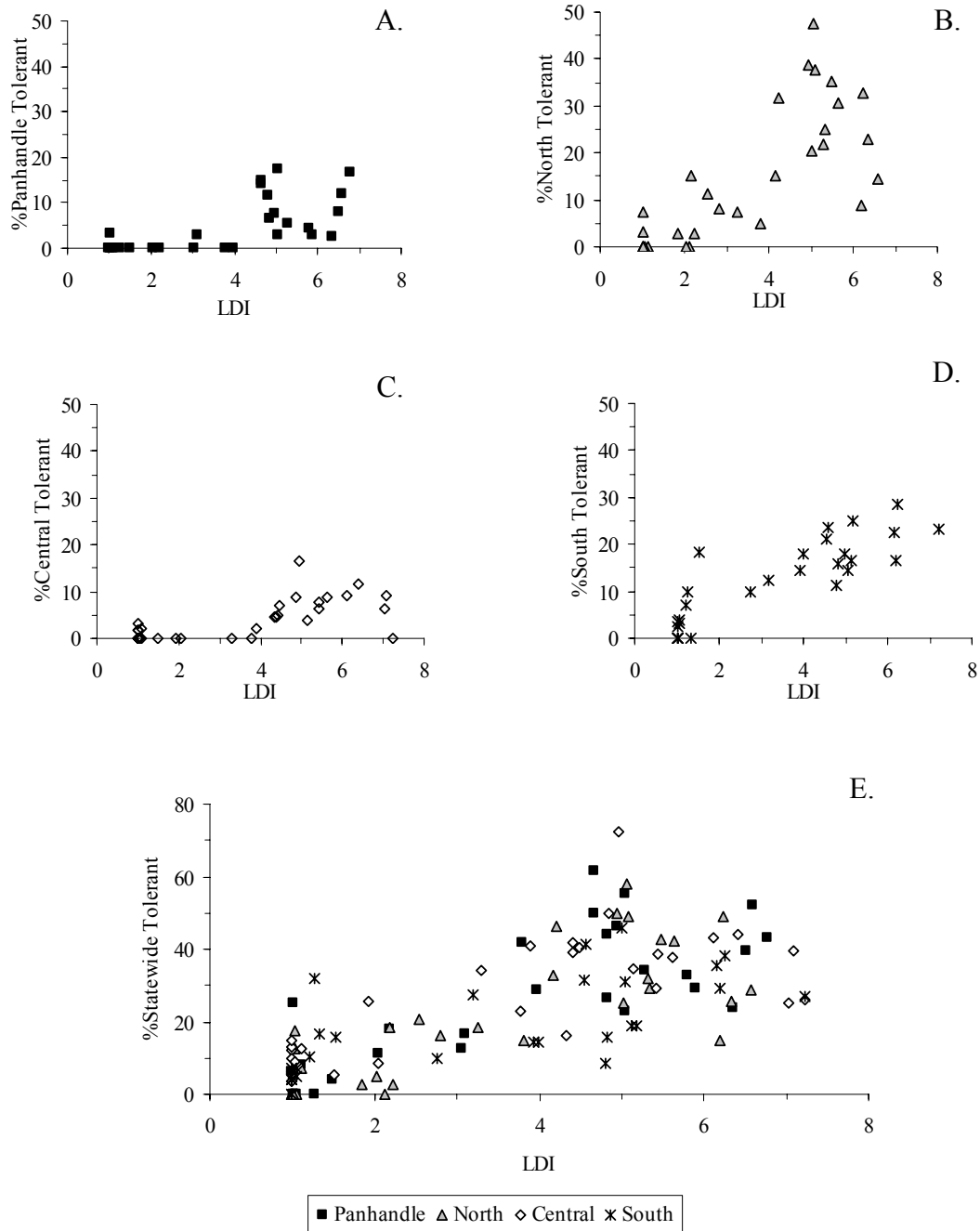


Figure 3-13. Macrophyte Tolerant Indicator Species increased with increasing development intensity (LDI). A) panhandle Tolerant Indicator Species at panhandle study wetlands. B) north Tolerant Indicator Species at north study wetlands. C) central Tolerant Indicator Species at central study wetlands. D) south Tolerant Indicator Species at south study wetlands. E) Statewide Tolerant Indicator Species at all 118 study wetlands.

Table 3-21. Statewide and regional macrophyte Sensitive Indicator Species.

	Statewide	Panhandle	North	Central	South
No. of Sensitive Species	61	28	19	24	13
<i>Amphicarpum muhlenbergianum</i>	19.3		52.4		
<i>Andropogon virginicus</i>	46.9	62.5	66.7	66.5	
<i>Aristida beyrichiana</i>	8.6	45.5			
<i>Aristida patula</i>			22.2		
<i>Aristida purpurascens</i>	32.9	50.0	22.2	53.5	
<i>Carex verrucosa</i>				25.0	
<i>Cladium jamaicense</i>	22.3				60.6
<i>Coelorachis rugosa</i>	7.3				
<i>Cyperus haspan</i>	11.0			33.3	
<i>Drosera brevifolia</i>	7.3				
<i>Erianthus giganteus</i>	17.8				33.3
<i>Eriocaulon compressum</i>	17.1	25.0	33.3		
<i>Eriocaulon decangulare</i>	37.8	37.5	22.2	53.5	33.3
<i>Eupatorium leptophyllum</i>	14.6			25.0	
<i>Eupatorium mohrii</i>	8.6				
<i>Fuirena scirpoidea</i>	26.9	25.0		25.0	36.2
<i>Gaylussacia frondosa</i>		25.0			
<i>Gratiola ramosa</i>	18.3			41.7	
<i>Hypericum chapmanii</i>	8.6	45.5			
<i>Hypericum fasciculatum</i>	38.2			53.5	44.4
<i>Hypericum myrtifolium</i>	17.8		47.7		
<i>Hyptis alata</i>	10.8				
<i>Ilex glabra</i>	46.2	53.9	78.6	45.9	
<i>Ilex myrtifolia</i>	17.1	71.2			
<i>Ipomoea sagittata</i>	11.0				
<i>Lachnanthes caroliniana</i>	39.6	71.2		40.2	
<i>Lachnocaulon anceps</i>		25.0			
<i>Lobelia floridana</i>		25.0			
<i>Lophiola aurea</i>	12.2	62.5			
<i>Ludwigia linifolia</i>	7.3				
<i>Lycopodiella alopecuroides</i>	9.8	25.0	22.2		
<i>Lyonia lucida</i>	24.4				
<i>Nymphaea odorata</i>	6.2		22.2		
<i>Nymphoides aquatica</i>	12.2				
<i>Panicum ensifolium</i>	13.1				
<i>Panicum erectifolium</i>	41.5	50.0	33.3	45.2	36.2
<i>Panicum hemitomon</i>	40.4			79.2	
<i>Panicum rigidulum</i>	17.1	25.0			
<i>Panicum tenerum</i>	14.6				33.3

Table 3-21. Continued.

	Statewide	Panhandle	North	Central	South
<i>Pinus elliotii</i>	33.9	56.2		37.0	25.0
<i>Pinus palustris</i>	8.6				
<i>Pluchea foetida</i>	10.1			25.0	
<i>Pluchea rosea</i>	15.5			33.3	
<i>Polygala cymosa</i>	28.0	37.5	29.3	33.3	
<i>Polygala lutea</i>	7.3				
<i>Proserpinaca pectinata</i>	12.4				
<i>Rhexia alifanus</i>	13.4	70.3			
<i>Rhexia lutea</i>	13.4	45.5	22.2		
<i>Rhexia mariana</i>	23.1		47.7	45.2	
<i>Rhexia petiolata</i>		25.0			
<i>Rhus copallinum</i>		33.1			
<i>Rhynchospora corniculata</i>					25.0
<i>Rhynchospora filifolia</i>		37.5			
<i>Rhynchospora inundata</i>	17.2		22.2		
<i>Rhynchospora microcarpa</i>				28.8	
<i>Rhynchospora wrightiana</i>			22.2		
<i>Sabatia bartramii</i>	7.3				
<i>Sagittaria graminea</i>	13.9			41.7	
<i>Sagittaria lancifolia</i>	17.2				36.2
<i>Salix caroliniana</i>					44.4
<i>Sarracenia minor</i>			22.2		
<i>Scleria baldwinii</i>	7.3				
<i>Scleria Georgiana</i>	7.3				
<i>Scleria triglomerata</i>	7.3	25.0			
<i>Serenoa repens</i>	22.3	37.5	57.6		
<i>Spartina bakeri</i>	7.3			25.0	
<i>Stillingia aquatica</i>	13.4				36.2
<i>Syngonanthus flavidulus</i>	12.2		22.2		
<i>Utricularia purpurea</i>	8.6				25.0
<i>Vaccinium corymbosum</i>	20.9	33.1		33.3	
<i>Xyris ambigua</i>	11.0				
<i>Xyris caroliniana</i>	7.3	25.0			
<i>Xyris elliotii</i>	18.3			50.0	
<i>Xyris jupicai</i>	8.6			25.0	

Values represent Indicator Values.

All reported Indicator Values were significant ( $p < 0.10$ ).

*virginicus*, *Aristida purpurascens*, *Ilex glabra*, and *Polygala cymosa* (statewide, panhandle, north, and central); and *Fuirena scirpoidea* and *Pinus elliottii* (statewide, panhandle, central, and south). All four wetland regions shared over three-quarters of their species with the statewide list (panhandle = 79%, north = 84%, central = 92%, and south = 85%). Six species were unique to the panhandle Sensitive Indicator Species analysis, three to the north, two to the central, and two to the south wetland region. Figure 3-14 shows that the percent Sensitive Indicator Species, statewide and regionally, decreased with increasing development intensity.

All of the indicator species metrics were significantly correlated with the LDI index. Table 3-22 shows Spearman correlations calculated with both statewide and regional Indicator Species lists for each region. Regional Indicator Species metrics had a stronger correlation value, though all metrics were significantly correlated with LDI ( $p < 0.01$ ). Two exceptions included the panhandle Tolerant Indicator Species correlations (statewide Tolerant Indicator Species,  $r = 0.73$ , panhandle Tolerant Indicator Species,  $r = 0.72$ ) and central (statewide Tolerant Indicator Species,  $r = 0.74$ , central Tolerant Indicator Species,  $r = 0.68$ ).

Shrub and tree species were included in the ISA for both Tolerant and Sensitive Indicator Species metrics. Metrics developed based on the macrophyte community composition included woody species rooted with the sampling quadrats, as structure was thought to play an important role in the biological condition of isolated forested wetlands. Excluding the tree and shrub layers would underscore their importance in providing structure for the isolated depressional forested wetlands. However, trees comprised only a small percentage of the Tolerant and Sensitive Indicator Species lists (Tables 3-20 and 3-21). Three percent of the statewide Tolerant Indicator Species were trees, 9% were shrubs, 14% vines, and 74% herbaceous (including herbs, sedges, grasses, etc.). The two statewide tree Tolerant Indicator Species included the hardwood *Acer rubrum* and exotic *Sapium sebiferum* (Table 3-20). The six statewide shrub Tolerant Indicator Species were of the genera *Aster* (a climbing species), an exotic *Ligustrum*, an exotic *Ludwigia*, *Rubus*, and *Sambucus*. Of the statewide Sensitive Indicator Species, 3% were trees, 15% shrubs, 2% vines, and 80% herbaceous.

#### *Floristic Quality Assessment Index metric*

Figure 3-15 shows wetland FQAI scores decreased with increasing landscape development intensity. Wetlands in the panhandle (maximum modified FQI = 6.25) and north (maximum FQAI = 5.95) wetland regions had higher FQAI scores versus wetlands in the central (maximum FQAI = 4.93) and south (maximum FQAI = 5.24) wetland regions. Statewide the FQAI was significantly correlated with LDI ( $|r| = 0.71$ ,  $p < 0.001$ ; Table 3-18); and there was a significant difference between the mean FQAI scores between low ( $LDI < 2.0$ ) and high ( $LDI \geq 2.0$ ) LDI groups ( $W = 3771.0$ ,  $p = < 0.001$ ; Table 3-19). CU6, an urban wetland in the high LDI group in the central wetland region surrounded by recently developed and under construction sections of residential land use (FQAI = 4.43; LDI = 7.2), had a higher FQAI score than anticipated. The wetland with the lowest FQAI score was NA1, an agricultural wetland surrounded by pasture (FQAI = 2.06).

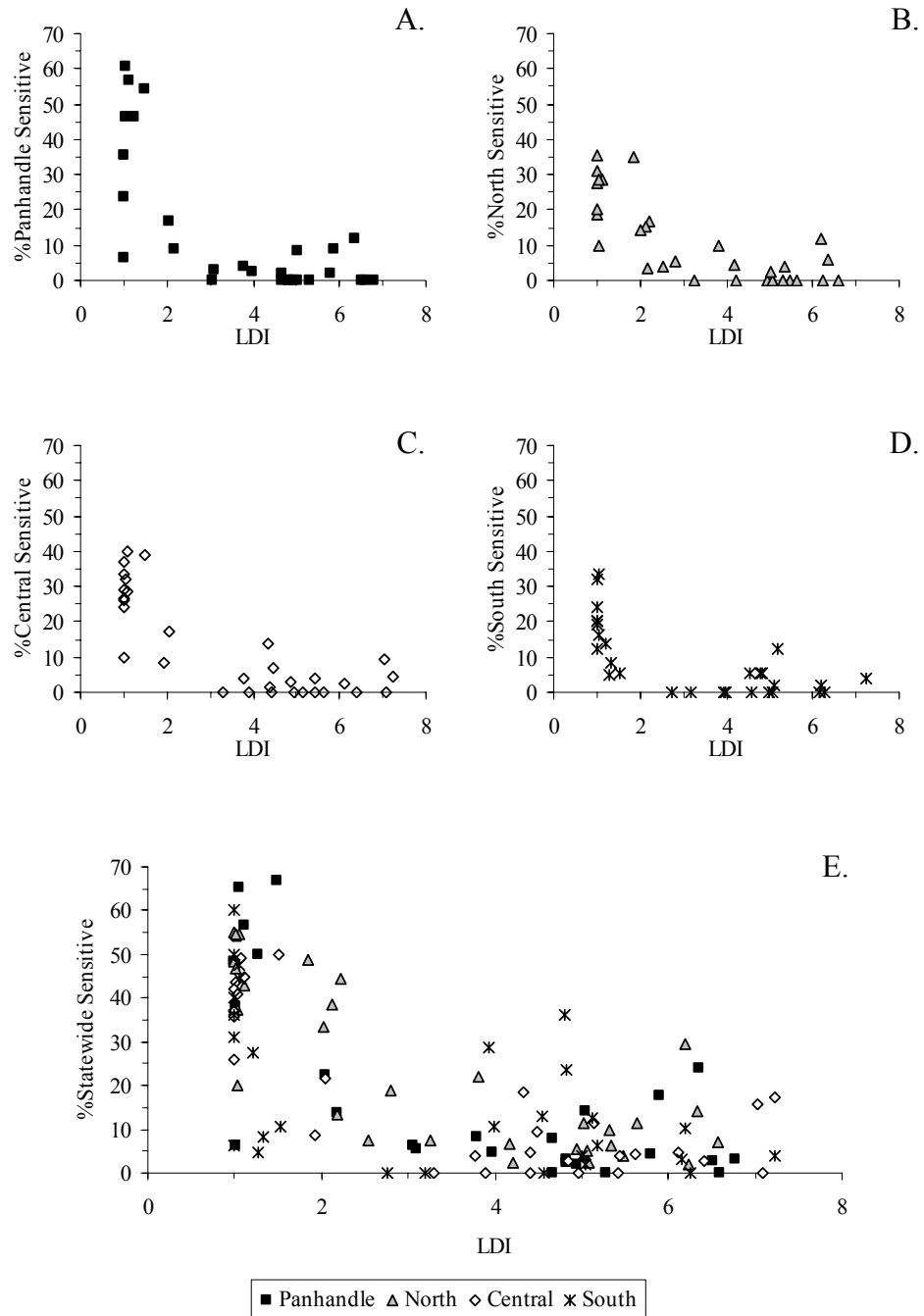


Figure 3-14. Macrophyte Sensitive Indicator Species decreased with increasing development intensity (LDI). A) panhandle Sensitive Indicator Species at panhandle study wetlands. B) north Sensitive Indicator Species at north study wetlands. C) central Sensitive Indicator Species at central study wetlands. D) south Sensitive Indicator Species at south study wetlands. E) Statewide Sensitive Indicator Species at all 118 study wetlands.

Table 3-22. The percent of statewide and regional macrophyte Indicator Species metrics were significantly correlated with LDI ( $p < 0.01$ ).

Wetland Region	n=	%Statewide ISA Spearman's r	%Regional ISA Spearman's r
Statewide			
Tolerant Indicator Species	118	0.75	
Sensitive Indicator Species	118	-0.66	
Panhandle			
Tolerant Indicator Species	28	0.73	0.72
Sensitive Indicator Species	28	-0.66	-0.68
North			
Tolerant Indicator Species	31	0.79	0.80
Sensitive Indicator Species	31	-0.76	-0.78
Central			
Tolerant Indicator Species	31	0.74	0.68
Sensitive Indicator Species	31	-0.67	-0.72
South			
Tolerant Indicator Species	28	0.78	0.86
Sensitive Indicator Species	28	-0.60	-0.71

Values represent Spearman's correlation coefficients, all reported correlations significant ( $P < 0.01$ ).

#### *Exotic Species metric*

Statewide, the percent Exotic Species was significantly correlated with development intensity in the surrounding landscape ( $r = 0.65$ ,  $p < 0.001$ ; Table 3-18). Figure 3-16 shows that the percent of exotic species increased with increasing LDI in each wetland region. The north wetland region hosted the wetland with the greatest percent Exotic Species, NA1 with 52.6% Exotic Species. NA1 was surrounded by a research facility growing experimental pasture grass species, biasing the high percent Exotic Species present at this study wetland. The wetland with the second highest percent Exotic Species was SU8 (38.5%), a wetland embedded in urban land use (residential and commercial). One apparent irregularity in south wetland region low LDI group was SU4 a wetland surrounded by low density residential land use, previously embedded in pasture with 18.4% Exotic Species. All remaining wetlands in the low LDI group ( $n = 40$ ) had less than 10% Exotic Species. Statewide, the percent Exotic Species was significantly different between low and high LDI groups ( $W = 1379.0$ ,  $p < 0.001$ ; Table 3-19). Table 3-23 lists the 113 Exotic Species encountered throughout Florida and identifies the wetland region(s) in which each species was found. Only six Exotic Species were found in all four wetland regions including *Commelina diffusa*, *Cuphea carthagenensis*, *Cynodon dactylon*, *Kyllinga brevifolia*, *Ludwigia peruviana*, and *Paspalum notatum*. Fourteen Exotic Species occurred in three of the four wetland regions.

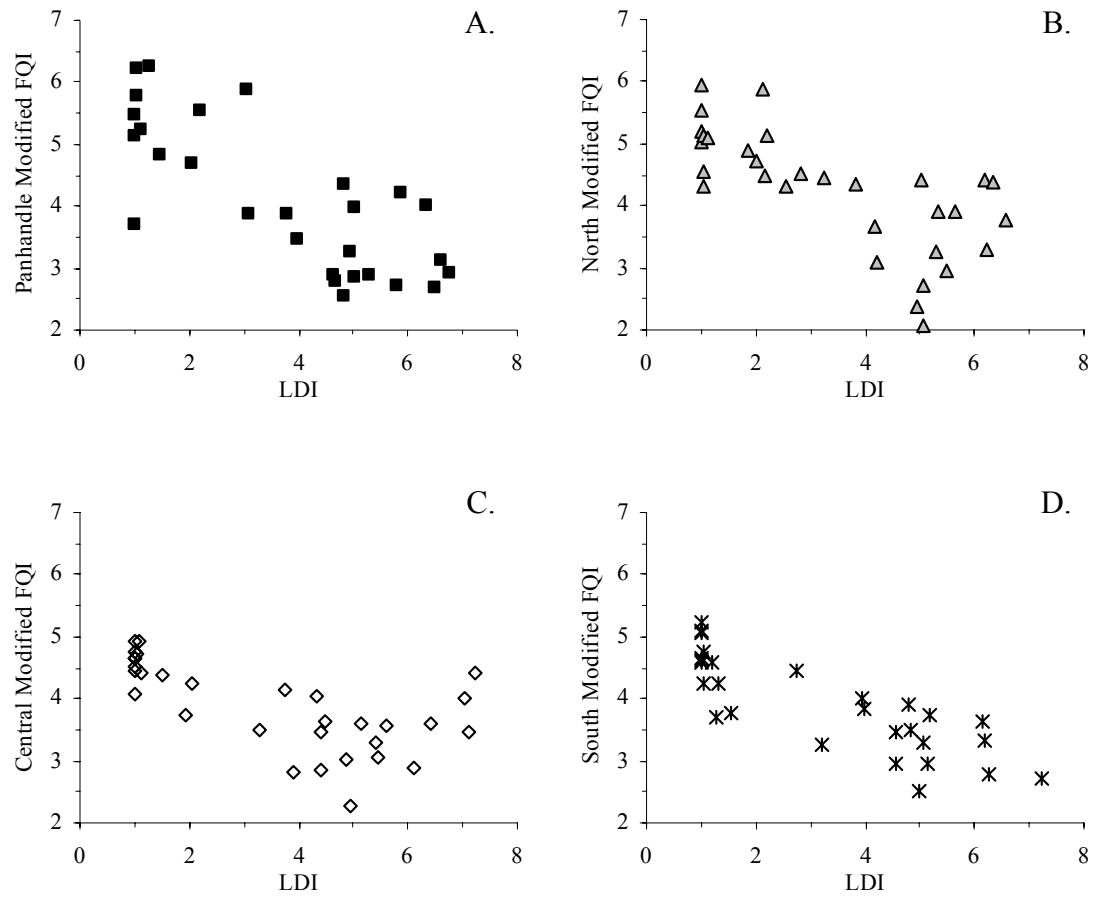


Figure 3-15. FQAI scores decreased with increasing development intensity (LDI). A) FQAI scores at panhandle study wetlands. B) FQAI scores at north study wetlands. C) FQAI scores at central study wetlands. D) FQAI scores at south study wetlands.



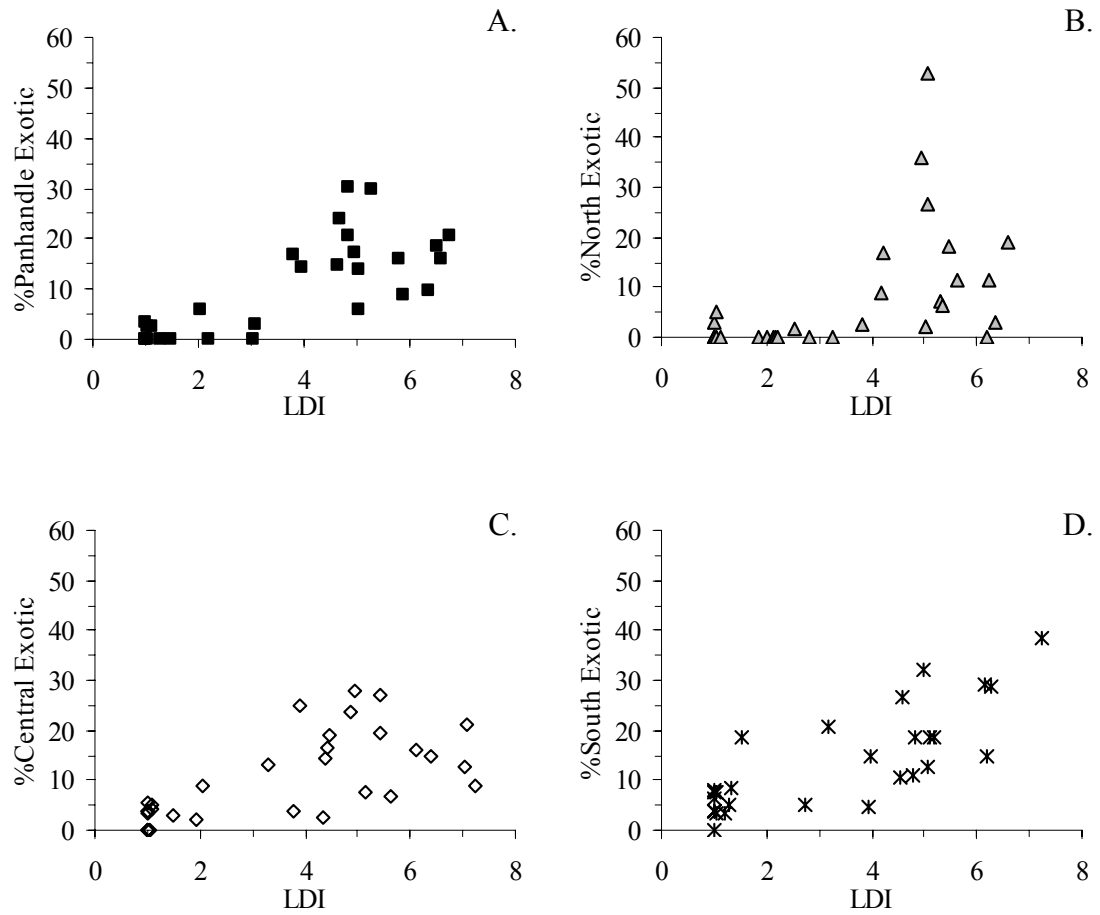


Figure 3-16. Exotic Species increased with increasing development intensity (LDI). A) Exotic Species at panhandle study wetlands. B) Exotic Species at north study wetlands. C) Exotic Species at central study wetlands. D) Exotic Species at south study wetlands.

Table 3-23. Macrophyte Exotic Species identified at 118 study wetlands throughout the four Florida wetland regions.

Exotic Species	P	N	C	S	Exotic Species	P	N	C	S
<i>Albizia julibrissin</i>	★		★		<i>Kyllinga brevifolia</i>	★	★	★	★
<i>Aloe vera</i>		★			<i>Lantana camara</i>				★
<i>Alternanthera philoxeroides</i>	★	★	★		<i>Ligustrum japonicum</i>	★			
<i>Alternanthera sessilis</i>			★		<i>Ligustrum lucidum</i>		★		
<i>Amaranthus blitum</i>		★	★		<i>Ligustrum sinense</i>	★	★		
<i>Amaranthus spinosus</i>	★	★			<i>Lindernia crustacea</i>				★
<i>Ardisia crenata</i>	★				<i>Lolium perenne</i>		★		
<i>Begonia cucullata</i>		★	★		<i>Lonicera japonica</i>	★	★	★	
<i>Bischofia javanica</i>				★	<i>Ludwigia peruviana</i>	★	★	★	★
<i>Blechum pyramidatum</i>				★	<i>Lygodium japonicum</i>	★	★		★
<i>Bromus catharticus</i>		★			<i>Lygodium microphyllum</i>		★		★
<i>Callisia repens</i>				★	<i>Macroptilium lathyroides</i>			★	
<i>Chenopodium album</i>			★		<i>Melaleuca quinquenervia</i>				★
<i>Chenopodium ambrosioides</i>		★			<i>Melia azedarach</i>			★	
<i>Cinnamomum camphora</i>	★	★	★		<i>Melochia corchorifolia</i>	★		★	★
<i>Citrus Xaurantium</i>				★	<i>Momordica charantia</i>			★	★
<i>Colocasia esculenta</i>	★	★			<i>Morrenia odorata</i>			★	
<i>Commelina diffusa</i>	★	★	★	★	<i>Morus alba</i>		★		
<i>Conyza bonariensis</i>	★				<i>Murdannia nudiflora</i>				★
<i>Cuphea carthagenensis</i>	★	★	★	★	<i>Nandina domestica</i>	★			
<i>Cyclosporum leptophyllum</i>				★	<i>Nephrolepis cordifolia</i>			★	★
<i>Cynodon dactylon</i>	★	★	★	★	<i>Oeceoclades maculata</i>				★
<i>Cyperus iria</i>	★				<i>Oxalis debilis</i>		★		
<i>Cyperus lanceolatus</i>	★	★	★		<i>Paederia foetida</i>		★	★	
<i>Desmodium incanum</i>			★		<i>Panicum maximum</i>			★	
<i>Digitaria bicornis</i>	★				<i>Panicum repens</i>	★	★		★
<i>Dioscorea bulbifera</i>		★	★		<i>Paspalidium geminatum</i>				★
<i>Duchesnea indica</i>	★				<i>Paspalum acuminatum</i>			★	
<i>Echinochloa colona</i>	★	★		★	<i>Paspalum notatum</i>	★	★	★	★
<i>Echinochloa crusgalli</i>		★			<i>Paspalum urvillei</i>	★	★	★	
<i>Eichhornia crassipes</i>			★		<i>Phalaris angusta</i>		★		
<i>Eleusine indica</i>	★	★			<i>Phyllanthus tenellus</i>				★
<i>Eragrostis atrovirens</i>				★	<i>Phyllanthus urinaria</i>	★	★	★	
<i>Eugenia uniflora</i>				★	<i>Plantago lanceolata</i>	★			
<i>Hedychium coronarium</i>			★		<i>Pouzolzia zeylanica</i>			★	
<i>Hedyotis corymbosa</i>			★	★	<i>Pueraria montana</i>	★			
<i>Hemarthria altissima</i>				★	<i>Rhodomyrtus tomentosa</i>				★
<i>Hymenachne amplexicaulis</i>			★	★	<i>Rhoeo discolor</i>				★
<i>Imperata cylindrica</i>			★		<i>Richardia brasiliensis</i>		★	★	
<i>Ipomoea indica</i>	★				<i>Richardia scabra</i>	★			
<i>Ipomoea quamoclit</i>	★				<i>Rumex crispus</i>	★	★		
<i>Kummerowia striata</i>	★				<i>Rumex obtusifolius</i>		★	★	

Table 3-23. Continued.

Exotic Species	P	N	C	S	Exotic Species	P	N	C	S
<i>Rumex pulcher</i>		★			<i>Tradescantia zebrina</i>				★
<i>Sacciolepis indica</i>			★	★	<i>Trifolium repens</i>	★	★		
<i>Salvinia minima</i>		★	★		<i>Urena lobata</i>			★	★
<i>Sapium sebiferum</i>	★	★	★		<i>Urtica dioica</i>	★			
<i>Schinus terebinthifolius</i>			★	★	<i>Verbena bonariensis</i>	★			
<i>Senna obtusifolia</i>	★		★	★	<i>Verbena brasiliensis</i>	★		★	
<i>Senna pendula</i>				★	<i>Viburnum odoratissimum</i>		★		
<i>Solanum tampicense</i>				★	<i>Vicia sativa</i>	★			
<i>Solanum viarum</i>			★	★	<i>Wedelia trilobata</i>				★
<i>Sonchus asper</i>			★		<i>Xanthosoma sagittifolium</i>	★			
<i>Sorghum bicolor</i>		★			<i>Xyris jupicai</i>	★		★	★
<i>Spermocoe verticillata</i>				★	<i>Youngia japonica</i>		★		
<i>Sporobolus indicus</i>	★	★		★	<i>Yucca aloifolia</i>			★	
<i>Thelypteris dentata</i>		★			<i>Zea mays</i>	★			
<i>Tradescantia fluminensis</i>	★								

Ecoregions determined by Lane (2000): P = panhandle wetland region; N = north wetland region; C = central wetland region; S = south wetland region.

#### *Native Perennial Species metric*

Of the 605 macrophyte species identified, 427 (71%) were classified as native perennials. Figure 3-17 shows that the percent Native Perennial Species decreased with increasing development intensity. The Native Perennial Species metric was significantly correlated with LDI (Spearman  $|r| = 0.63$ ,  $p < 0.001$ ; Table 3-18). Statewide there was a significant difference between the percent Native Perennial Species between low and high LDI groups ( $W = 3453.0$ ,  $p < 0.001$ ; Table 3-19).

#### *Wetland Status Species metric*

Fifty-six percent of the macrophyte species identified were included in the Wetland Status Species metric, including 160 species designated as obligate and 180 species designated as facultative wetland species. Figure 3-18 shows that the percent Wetland Status Species decreased with increasing development intensity in each wetland region. The percent Wetland Status Species was significantly different between LDI groups ( $W = 3612.0$ ,  $p < 0.001$ ; Table 3-19); and significantly correlated statewide with the LDI index (Spearman  $|r| = 0.55$ ,  $p < 0.001$ ; Table 3-18).

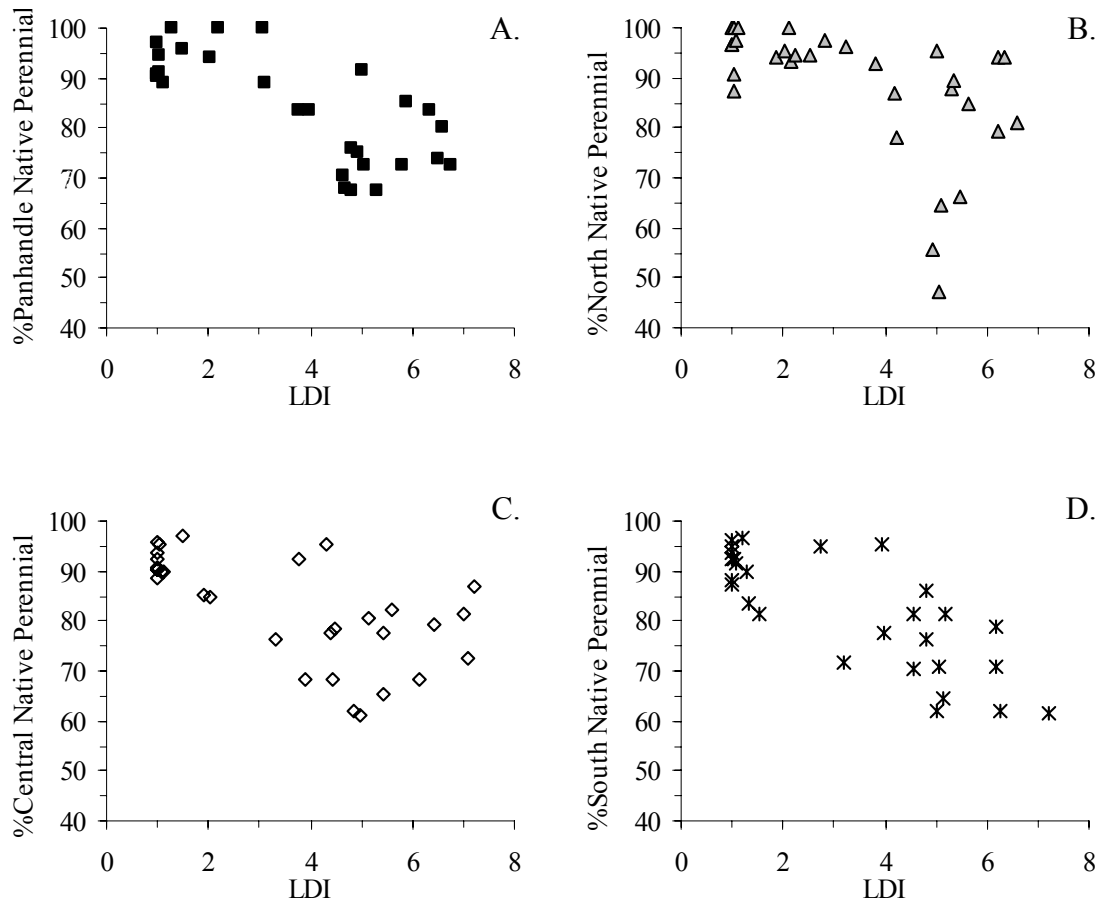


Figure 3-17. The percent Native Perennial Species decreased with increasing development intensity (LDI). A) panhandle study wetlands. B) north study wetlands. C) central study wetlands. D) south study wetlands.

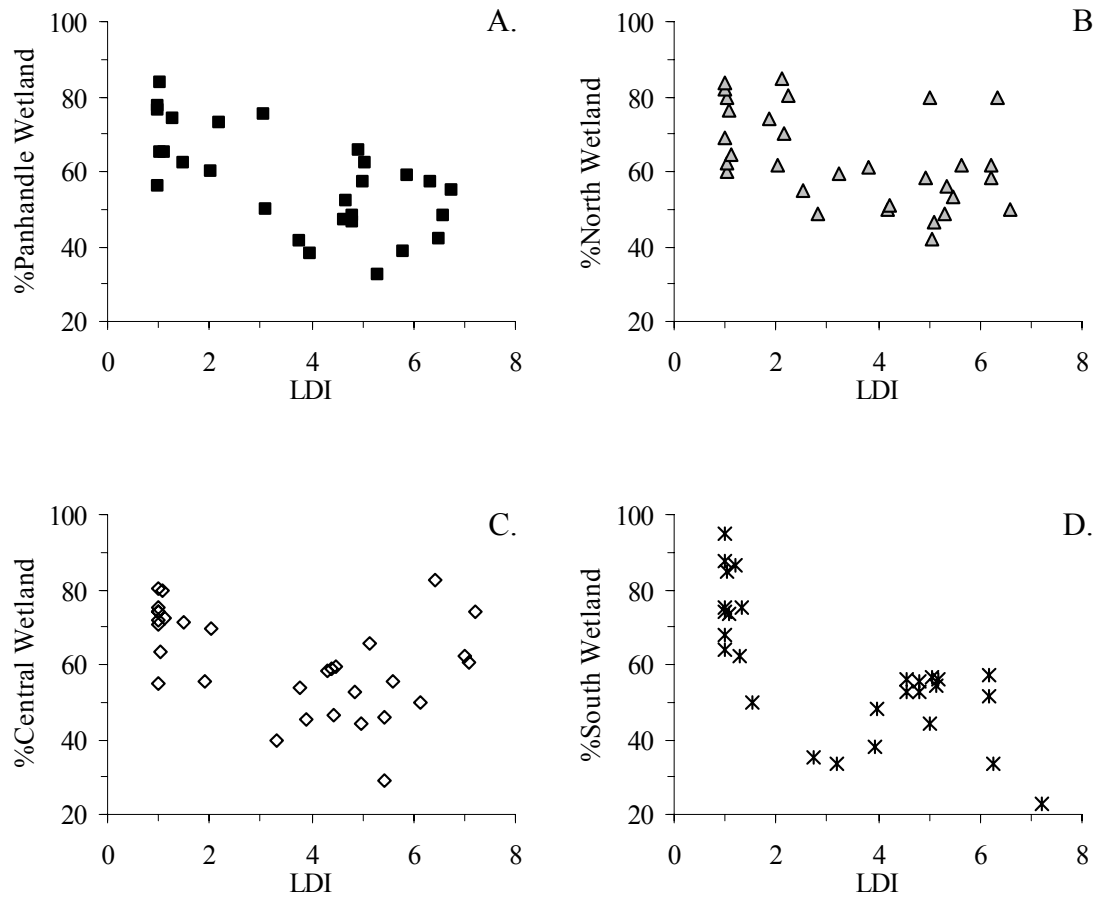


Figure 3-18. The percent Wetland Status Species decreased with increasing development intensity (LDI). This trend was consistent for the (A) panhandle, (B) north, (C) central, and (D) south wetland regions.

### ***Macrophyte Florida Wetland Condition Index***

The six metrics described above were included in the macrophyte FWCI. Appendix F provides detailed instructions on metric scoring. Figure 3-19 shows that both statewide and regional macrophyte FWCI scores decrease with increasing development intensity. Table 3-24 compares the overall macrophyte FWCI calculated statewide and regionally for the low LDI group ( $LDI < 2.0$ ). A comparable statewide macrophyte FWCI should equally score reference wetlands in each wetland region. However, the south wetland region had significantly different overall macrophyte FWCI scores for the low LDI group compared to the panhandle, north, and central wetland regions. When calculated statewide, five of the six metrics had one or more wetland region with significantly different metric scores. The north and central wetland regions had significantly different scores for the statewide Tolerant Indicator Species metric, whereas the north and south wetland regions had significantly different scores for the statewide Sensitive Indicator Species metric. The panhandle and north wetland regions were not significantly different from each other, but were significantly different from the central and south wetland regions for FQAI scores; suggesting that the panhandle and north wetland regions hosted more species with a narrower set of ecological conditions found in reference wetlands. The south wetland region had significantly different statewide Exotic Species metric scores than the other three wetland regions. The scores for the statewide Native Perennial Species metric were significantly different for the north wetland region. When the macrophyte FWCI was scored regionally, there was not a significant difference between mean scores for the low LDI group (Table 3-24). The only regionally scored metric with significantly different mean scores for the low LDI group was the regional Tolerant Indicator Species metric for the south wetland region which was only significantly different from the panhandle wetland region.

Table 3-25 shows similar results for the high LDI group ( $LDI \geq 2.0$ ). For the statewide macrophyte FWCI wetlands in the north wetland region had significantly different macrophyte FWCI scores, suggesting the north wetland region high LDI wetlands had higher ecological integrity than wetlands of other wetland regions. Five of the six metrics calculated statewide had at least one wetland region with significantly different mean scores in the high LDI group. Only the Wetland Status Species metric did not have significantly different scores for both statewide and regional calculations among wetland regions. For the regional macrophyte FWCI calculations, the north and south wetland regions had significantly different mean macrophyte FWCI scores. Only the regional Tolerant Indicator Species metric for the panhandle and south wetland regions had significantly different mean scores for all of the regionally calculated metrics.

Correlations between macrophyte FWCI and the six component metrics with LDI were strong ( $|r| > 0.50$ ,  $p < 0.01$ ) for all of the metrics statewide and regionally (Table 3-26), except for the central wetland region Wetland Status Species metric ( $|r| = 0.39$ ,  $p = 0.03$ ). This metric was still significantly correlated at a more flexible significance level ( $p < 0.05$ ). Three of the four wetland regions, including the panhandle, north, and south wetland regions had stronger FWCI correlations with LDI than both the statewide and central wetland region FWCI. The regional macrophyte FWCI was significantly correlated with the LDI index (Spearman's  $|r| = 0.73$ ,  $p < 0.001$ ). A Kruskal-Wallis test

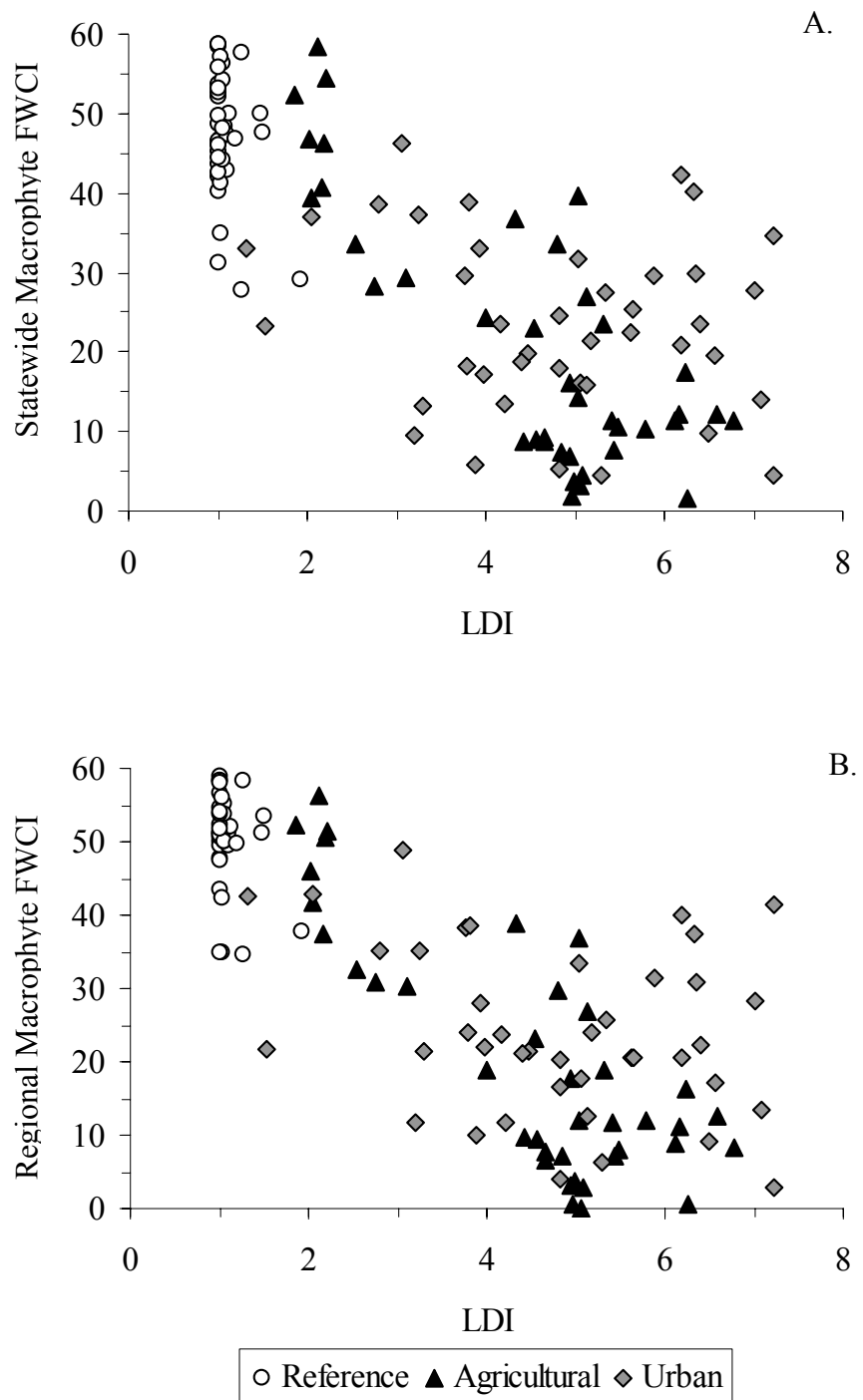


Figure 3-19. Macrophyte FWCI scores decreased with increasing development intensity (LDI). A) Statewide FWCI. B) Regional FWCI.

Table 3-24. Macrophyte FWCI and component metrics scored statewide and regionally for study wetlands in the low LDI group (LDI &lt; 2.0).

	Panhandle	North	Central	South
Statewide				
Macrophyte FWCI	50.3 (8.6) <sup>a</sup>	51.6 (8.4) <sup>a</sup>	45.9 (3.4) <sup>ab</sup>	42.2 (9.4) <sup>b</sup>
State Tolerant	8.1 (2.5) <sup>ab</sup>	8.4 (2.2) <sup>a</sup>	6.6 (1.2) <sup>b</sup>	6.8 (2.3) <sup>ab</sup>
State Sensitive	8.8 (2.6) <sup>ab</sup>	9.0 (1.3) <sup>a</sup>	8.6 (0.7) <sup>ab</sup>	6.7 (2.9) <sup>b</sup>
FQAI Score	8.5 (2.2) <sup>a</sup>	8.1 (1.4) <sup>a</sup>	6.6 (0.9) <sup>b</sup>	6.4 (1.7) <sup>b</sup>
Exotic Species	9.3 (1.0) <sup>a</sup>	9.4 (1.1) <sup>a</sup>	8.4 (1.3) <sup>a</sup>	6.6 (1.9) <sup>b</sup>
Native Perennial Species	8.6 (0.9) <sup>ab</sup>	9.1 (1.0) <sup>a</sup>	8.3 (0.6) <sup>b</sup>	7.9 (1.1) <sup>b</sup>
Wetland Status Species	7.0 (2.0) <sup>a</sup>	7.5 (1.9) <sup>a</sup>	7.4 (1.6) <sup>a</sup>	7.7 (2.3) <sup>a</sup>
Regional				
Macrophyte FWCI	50.9 (7.2) <sup>a</sup>	51.3 (7.9) <sup>a</sup>	51.4 (4.0) <sup>a</sup>	46.8 (9.8) <sup>a</sup>
Regional Tolerant	9.6 (1.0) <sup>a</sup>	9.2 (1.3) <sup>ab</sup>	9.0 (1.5) <sup>ab</sup>	7.7 (2.7) <sup>b</sup>
Regional Sensitive	8.2 (2.6) <sup>a</sup>	8.5 (1.7) <sup>a</sup>	8.6 (1.6) <sup>a</sup>	6.9 (2.4) <sup>a</sup>
FQAI Score	7.6 (2.3) <sup>a</sup>	7.9 (1.4) <sup>a</sup>	8.7 (1.1) <sup>a</sup>	7.7 (2.0) <sup>a</sup>
Exotic Species	9.3 (1.0) <sup>a</sup>	9.5 (1.1) <sup>a</sup>	8.3 (1.4) <sup>a</sup>	8.3 (1.9) <sup>a</sup>
Native Perennial Species	8.3 (1.0) <sup>a</sup>	9.2 (0.9) <sup>a</sup>	9.1 (0.7) <sup>a</sup>	8.7 (1.2) <sup>a</sup>
Wetland Status Species	8.0 (2.1) <sup>a</sup>	7.0 (2.5) <sup>a</sup>	7.8 (1.9) <sup>a</sup>	7.6 (2.1) <sup>a</sup>

Values represent the mean (standard deviation).

Wetland regions with similar letters were not significantly different (p<0.05)

Table 3-25. Macrophyte FWCI and component metrics scored statewide and regionally for study wetlands in the high LDI group (LDI ≥ 2.0).

	Panhandle	North	Central	South
Statewide				
Macrophyte FWCI	20.4 ± 13.1 <sup>a</sup>	29.4 ± 15.9 <sup>b</sup>	18.9 ± 11.0 <sup>a</sup>	17.6 ± 10.1 <sup>a</sup>
State Tolerant	2.1 ± 1.9 <sup>ab</sup>	3.3 ± 2.8 <sup>a</sup>	2.0 ± 1.6 <sup>b</sup>	3.3 ± 1.9 <sup>ab</sup>
State Sensitive	2.5 ± 2.0 <sup>ab</sup>	4.1 ± 2.4 <sup>a</sup>	2.2 ± 2.1 <sup>ab</sup>	2.9 ± 2.7 <sup>b</sup>
FQAI Score	3.2 ± 3.2 <sup>a</sup>	4.3 ± 2.8 <sup>a</sup>	2.8 ± 1.8 <sup>b</sup>	2.5 ± 1.8 <sup>b</sup>
Exotic Species	4.0 ± 3.0 <sup>a</sup>	6.3 ± 3.7 <sup>a</sup>	3.6 ± 2.4 <sup>a</sup>	2.7 ± 2.4 <sup>b</sup>
Native Perennial Species	5.2 ± 2.7 <sup>ab</sup>	6.6 ± 3.2 <sup>a</sup>	4.3 ± 2.6 <sup>b</sup>	3.9 ± 2.9 <sup>b</sup>
Wetland Status Species	3.3 ± 2.4 <sup>a</sup>	4.8 ± 2.6 <sup>a</sup>	4.0 ± 2.5 <sup>a</sup>	2.3 ± 1.8 <sup>a</sup>
Regional				
Macrophyte FWCI	21.3 ± 14.5 <sup>ab</sup>	27.1 ± 16.1 <sup>a</sup>	20.6 ± 12.8 <sup>ab</sup>	16.6 ± 9.6 <sup>b</sup>
Regional Tolerant	5.5 ± 3.7 <sup>a</sup>	3.9 ± 3.2 <sup>ab</sup>	4.2 ± 3.6 <sup>ab</sup>	1.8 ± 1.4 <sup>b</sup>
Regional Sensitive	1.3 ± 1.7 <sup>a</sup>	2.1 ± 2.4 <sup>a</sup>	1.6 ± 2.0 <sup>a</sup>	1.3 ± 1.8 <sup>a</sup>
FQAI	2.8 ± 2.8 <sup>a</sup>	4.4 ± 2.7 <sup>a</sup>	3.3 ± 2.5 <sup>a</sup>	2.9 ± 2.1 <sup>a</sup>
Exotic Species	3.9 ± 3.0 <sup>a</sup>	6.4 ± 3.6 <sup>a</sup>	3.2 ± 2.5 <sup>a</sup>	3.7 ± 2.9 <sup>a</sup>
Native Perennial Species	4.2 ± 3.2 <sup>a</sup>	6.8 ± 3.2 <sup>a</sup>	4.5 ± 2.9 <sup>a</sup>	4.3 ± 3.2 <sup>a</sup>
Wetland Status Species	3.7 ± 2.8 <sup>a</sup>	3.5 ± 3.3 <sup>a</sup>	3.7 ± 2.9 <sup>a</sup>	2.6 ± 1.8 <sup>a</sup>

Values represent the mean (standard deviation).

Wetland regions with similar letters were not significantly different (p<0.05).



Table 3-26. Spearman correlations between the macrophyte FWCI and component metrics with LDI.

	Spearman's r	p-value
Statewide		
Macrophyte FWCI	-0.73	<0.0001
Tolerant Indicator Species	0.75	<0.0001
Sensitive Indicator Species	-0.66	<0.0001
FQAI Score	-0.71	<0.0001
Exotic Species	0.65	<0.0001
Native Perennial Species	-0.63	<0.0001
Wetland Status Species	-0.55	<0.0001
Panhandle		
Macrophyte FWCI	-0.74	<0.0001
Tolerant Indicator Species	0.72	<0.0001
Sensitive Indicator Species	-0.68	<0.0001
FQAI Score	-0.68	<0.0001
Exotic Species	0.72	<0.0001
Native Perennial Species	-0.67	0.0001
Wetland Status Species	-0.60	0.0007
North		
Macrophyte FWCI	-0.74	<0.0001
Tolerant Indicator Species	0.80	<0.0001
Sensitive Indicator Species	-0.78	<0.0001
FQAI Score	-0.75	<0.0001
Exotic Species	0.65	<0.0001
Native Perennial Species	-0.65	<0.0001
Wetland Status Species	-0.55	0.0015
Central		
Macrophyte FWCI	-0.73	<0.0001
Tolerant Indicator Species	0.68	<0.0001
Sensitive Indicator Species	-0.72	<0.0001
FQAI Score	-0.68	<0.0001
Exotic Species	0.70	<0.0001
Native Perennial Species	-0.66	<0.0001
Wetland Status Species	-0.39	0.0309
South		
Macrophyte FWCI	-0.88	<0.0001
Tolerant Indicator Species	0.86	<0.0001
Sensitive Indicator Species	-0.71	<0.0001
FQAI Score	-0.86	<0.0001
Exotic Species	0.80	<0.0001
Native Perennial Species	-0.80	<0.0001
Wetland Status Species	-0.69	<0.0001

suggested a significant difference ( $H = 66.3$ ,  $p < 0.001$ ) among median regional macrophyte FWCI scores for study wetlands among the three *a priori* land use categories (reference, agricultural, urban).

### Cluster Analysis

Cluster analysis determined five categories of wetlands based on macrophyte community composition. Clusters were roughly defined by wetland regions and *a priori* land use categories, including: 1: northern reference; 2: southern reference; 3: northern developed land use; 4: southern developed land use; and 5: statewide pasture land use. Figure 3-20 shows that based on regional macrophyte FWCI scores, clusters 1 and 2 were not significantly different from one another, but were significantly different from clusters 3, 4, and 5 ( $p < 0.05$ ). Clusters 3 and 4 were not significantly different from each other. Cluster 5 was significantly different from all other clusters. Identical results were obtained using statewide macrophyte FWCI scores. Table 3-27 provides means and standard deviations for cluster statewide macrophyte FWCI scores, regional macrophyte FWCI scores, and LDI.

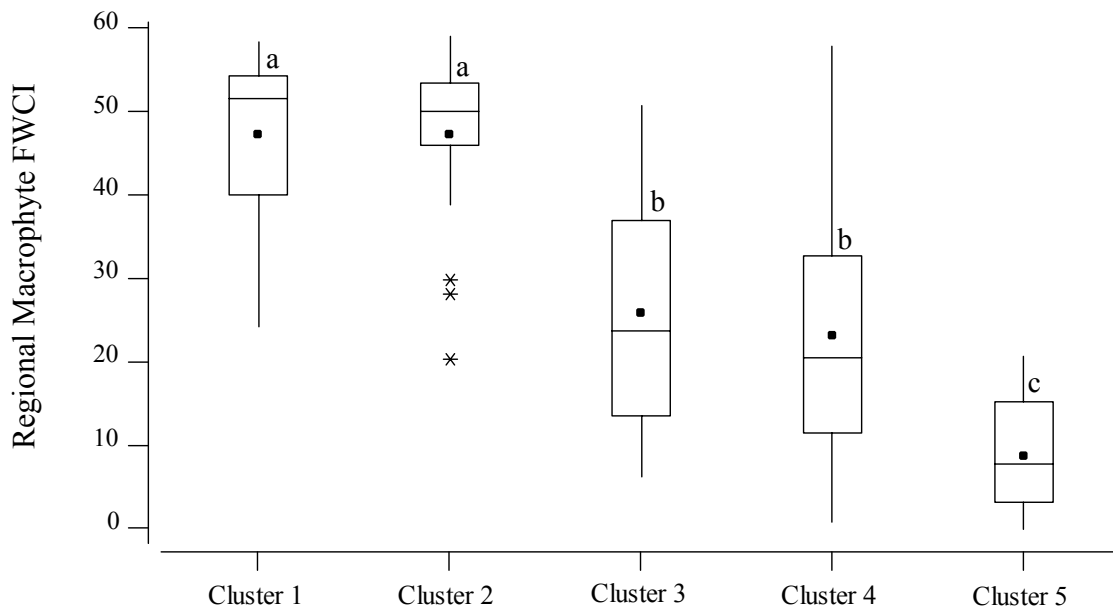


Figure 3-20. Regional macrophyte FWCI scores for five wetland clusters based on macrophyte community composition.

Table 3-27. Macrophyte FWCI scores and LDI values for wetland clusters based on macrophyte community composition.

Cluster	Statewide Macrophyte FWCI	Regional Macrophyte FWCI	LDI
1	46.8 (11.1) <sup>a</sup>	47.4 (9.9) <sup>a</sup>	2.3 (1.9) <sup>a</sup>
2	44.5 (7.7) <sup>a</sup>	47.4 (9.7) <sup>a</sup>	1.7 (1.3) <sup>a</sup>
3	24.9 (12.2) <sup>b</sup>	25.9 (12.6) <sup>b</sup>	4.4 (1.9) <sup>bc</sup>
4	21.9 (13.8) <sup>b</sup>	23.2 (16.2) <sup>b</sup>	4.0 (2.0) <sup>b</sup>
5	10.6 (7.0) <sup>c</sup>	8.8 (6.6) <sup>c</sup>	5.2 (0.5) <sup>c</sup>

Values represent mean (standard deviation).

Clusters with similar letters within columns were not significantly different ( $p < 0.05$ ).

## Macroinvertebrate Assemblage

Statewide 79 wetlands were sampled for the macroinvertebrate assemblage, with 118 species, 169 representing genera, 85 families, 24 orders, nine classes, and five phyla. The most common macroinvertebrate genera identified were *Polypedilum*, *Dero*, and *Goeldichironomous*, comprising 19%, 18%, and 8% of all the individual macroinvertebrates identified to the genus or lower taxonomic level, respectively. Four genera, *Polypedilum*, *Dero*, *Goeldichironomous*, and *Kiefferulus*, were found at over 50% of the study wetlands. Of the genera encountered, 81 genera (48%) occurred at a minimum of 5% of the sample wetlands ( $n \geq 4$ ). Approximately one-third of the genera identified (53 genera or 31%) were encountered at only one wetland.

The most common families identified included Chironomidae, Naididae, Enchytraeidae, and Culicidae, representing 39, 19, 4, and 4% of the individuals identified, and occurring at 99, 81, 52, and 56% of the study wetlands, respectively. Macroinvertebrates in the family Chironomidae were further divided into the subfamilies Chironominae (89% of Chironomidae), Tanypodinae (10% of Chironomidae), and Orthocladiinae (1% of Chironomidae). Six orders were found at over 50% of the wetlands sampled, including Diptera (47% of individuals identified to the order taxonomic level or lower), Tubificida (24%), Coleoptera (6%), Basommatophora (5%), Odonata (4%), and Hemiptera (3%). The most common classes of macroinvertebrates identified included Insecta (63%), Oligochaeta (24%), Gastropoda (6%), and Crustacea (5%), all occurring at over 50% of the study wetlands. Five phyla were identified, including Arthropoda, Annelida, Mollusca, Platyhelminthes, and Nemertea, with Arthropoda, Annelida, Mollusca found at 100, 92, and 65% of the wetlands sampled, respectively.

In the panhandle wetland region, 13 wetlands were sampled hosting 84 genera representing 48 families and 17 orders. In the north wetland region 15 wetlands were sampled with 87 genera (58 families and 20 orders) encountered. The central wetland region included 25 wetlands with 109 genera (60 families and 23 orders) identified. The south wetland region had 26 sample wetlands with 105 genera (in 60 families and 21 orders) identified.

### *Summary Statistics*

Richness (R), evenness (E), Shannon diversity (H), and Simpson's index (S) were calculated for each sample wetland (Appendix E). Richness ranged from one genus (*Pristina*) at PR4, a wetland embedded in a low-intensity silvicultural land use, to 26 genera at PA3, a wetland surrounded with row crops. Species evenness ranged from 0.00 at PR4 to 0.97 at PR5, a wetland surrounded by upland forest. Shannon diversity ranged from 0 at PR4 to 0.92 at CA7, a wetland surrounded by silvicultural operations and pasture with cattle. Simpson's index was highest at CR10 at 2.79. Table 3-28 summarizes the richness, evenness, and diversity calculations by *a priori* land use category (reference, agricultural, or urban). No significant differences were found in richness, evenness, Shannon diversity, or Simpson's index among the three *a priori* land use categories. Beta and gamma diversity were also similar among *a priori* land use categories. Table 3-29 shows that the no significant differences were found for richness,

Table 3-28. Macroinvertebrate richness, evenness, and diversity among *a priori* land use categories.

	Reference	Agricultural	Urban
Richness (R)	14 (6) <sup>a</sup>	14 (6) <sup>a</sup>	13 (5) <sup>a</sup>
Evenness (E)	0.69 (0.24) <sup>a</sup>	0.69 (0.12) <sup>a</sup>	0.68 (0.15) <sup>a</sup>
Shannon Diversity (H)	0.70 (0.26) <sup>a</sup>	0.72 (0.15) <sup>a</sup>	0.70 (0.17) <sup>a</sup>
Simpson's Index (S)	1.83 (0.75) <sup>a</sup>	1.81 (0.54) <sup>a</sup>	1.73 (0.55) <sup>a</sup>
Beta Diversity	8.0	7.8	8.5
Gamma Diversity	114	110	111

Categories with similar letters were not significantly different (Fisher's LSD,  $\alpha=0.05$ )

Values represent mean (standard deviation).

evenness, Shannon diversity, or Simpson's index between wetlands in low ( $LDI < 2.0$ ) and high ( $LDI \geq 2.0$ ) LDI groups. Beta and gamma diversity were higher for the high LDI groups with beta diversity at 10.9 and gamma diversity at 146 for the high LDI group and beta diversity at 8.7 and gamma diversity at 124 for the low LDI group wetlands.

### ***Compositional Analysis***

MRPP was used to test the similarity of macroinvertebrate genera composition across all wetland regions (panhandle versus north versus central versus south) as well as for multiple pair wise region comparisons (panhandle versus north, panhandle versus central, panhandle versus south, north versus central, north versus south, and central versus south). Among all wetlands, the comparison across all groups and the multiple pair wise comparisons suggested macroinvertebrate community composition at the genera level was significantly different (at the  $\alpha = 0.05$  level), with the exception of the pair wise comparison between the panhandle and north wetland regions (Table 3-30). The macroinvertebrate community composition of the panhandle and north wetland regions was not significantly different for all tests, including among all wetlands and for reference, agricultural, and urban wetlands independently.

Table 3-29. Macroinvertebrate richness, evenness, and diversity for LDI groups.

	Low LDI	High LDI	$W^{\wedge}$	$p^{\cdot}$
Richness (R)	14 (6)	13 (5)	661	0.33
Evenness (E)	0.68 (0.22)	0.69 (0.14)	687	0.47
Shannon Diversity (H)	0.70 (0.24)	0.71 (0.17)	695	0.52
Simpson's Index (S)	1.82 (0.71)	1.77 (0.56)	678	0.42
Beta Diversity	8.7	10.9		
Gamma Diversity	124	146		

$W^{\wedge}$  = Mann-Whitney U-Test statistic

$p^{\cdot}$  = significance value

Values represent mean (standard deviation).

Table 3-30. Macroinvertebrate community composition similarity among *a priori* land use categories and wetland regions.

	Sites (n)	T <sup>^</sup>	A <sup>`</sup>	p <sup>#</sup>
All Wetlands				
All regions (P vs N vs C vs S)	79	-7.1	0.10	0.00*
Panhandle vs north	28	0.5	-0.01	0.67
Panhandle vs central	38	-3.6	0.05	0.00*
Panhandle vs south	39	-8.5	0.13	0.00*
North vs central	40	-2.4	0.04	0.02*
North vs south	41	-6.6	0.10	0.00*
Central vs south	51	-3.2	0.04	0.00*
Reference wetlands				
All regions (P vs N vs C vs S)	29	-3.2	0.13	0.00*
Panhandle vs north	12	-0.2	0.01	0.35
Panhandle vs central	14	-0.7	0.03	0.21
Panhandle vs south	15	-3.8	0.17	0.00*
North vs central	14	-0.5	0.03	0.27
North vs south	15	-3.3	0.15	0.00*
Central vs south	17	-2.1	0.08	0.03*
Agricultural wetlands				
All regions (P vs N vs C vs S)	24	-2.7	0.12	0.01*
Panhandle vs north	8	-0.5	0.04	0.30
Panhandle vs central	12	-2.1	0.10	0.03*
Panhandle vs south	12	-3.3	0.18	0.00*
North vs central	12	-1.0	0.04	0.17
North vs south	12	-2.3	0.12	0.02*
Central vs south	16	-0.5	0.02	0.30
Urban wetlands				
All regions (P vs N vs C vs S)	26	-1.2	0.05	0.11
Panhandle vs north	8	-1.0	0.09	0.16
Panhandle vs central	12	-0.6	0.03	0.27
Panhandle vs south	12	-2.1	0.09	0.03*
North vs central	14	0.7	-0.03	0.75
North vs south	14	-1.6	0.07	0.07
Central vs south	18	-1.1	0.03	0.14

\*A high |T| value and significant p-value (p<0.05) suggests a difference in species composition

T<sup>^</sup> = the MRPP test statistic

A<sup>`</sup> = the chance corrected within-group agreement

p<sup>#</sup> = the significance value.

In reference wetlands, the south wetland region had a significantly different macroinvertebrate community composition as compared to all other wetland regions (panhandle versus south  $T = -3.2$ ,  $p = 0.00$ ; north versus south  $T = -3.3$ ,  $p = 0.00$ ; central versus south  $T = -2.1$ ,  $p = 0.03$ ). There were not significant differences in macroinvertebrate community composition between agricultural wetlands in any neighboring wetland regions. However, macroinvertebrate community composition in the south wetland region was significantly different from both the panhandle and north regions; as were the central and panhandle regions. The only wetland regions with significantly different macroinvertebrate community composition among urban wetlands were the panhandle and south regions.

### ***Community Composition***

Macroinvertebrate community composition was summarized in an NMDS ordination to relate changes in macroinvertebrate community composition with environmental variables. Figure 3-21 shows a two dimensional bi-plot of the NMDS axes. Overlays of significant environmental variables include water column pH, LDI, and latitude. Table 3-31 provides the Pearson's r-squared correlation coefficients between environmental variables and NMDS ordination axes. A three dimensional solution was constructed with an overall stress of 19.8 with a final stability of 0.04. Axis 1 explained 18.9% of the variance and was not correlated with any measured environmental parameter. Axis 2 explained 14.2% variance and was correlated with LDI; axis 3 explained 35.9% variance and was correlated with latitude and water column pH.

### ***Metric Selection***

The pool of potential candidate metrics was streamlined to reduce the redundancy of selected metrics. If two metrics were calculated from the same set of taxa representing redundant metrics only one metric was included. Six metrics that were significant for the Spearman's correlation coefficient with LDI ( $|r| > 0.30$ ,  $p < 0.01$ ) (Table 3-32), showed visually distinguishable trends with LDI, and were significant for the Mann-Whitney U-test between LDI groups ( $p < 0.001$ ) (Table 3-33) were selected for inclusion in the macrophyte FWCI. Table 3-32 provides the statewide Spearman correlation values between the six macroinvertebrate metrics and LDI, water column pH, dissolved oxygen, and TP. Correlations with additional measured environmental variables were used to corroborate meaningful ecological trends in metrics despite the Spearman's correlation coefficient ( $|r| > 0.30$ ) for metrics and LDI. Metrics selected for the macroinvertebrate FWCI were calculated based on the presence/absence of individual macroinvertebrates.

Macroinvertebrate metrics selected for inclusion in the FWCI represented tolerance, community balance, and functional group metrics. Tolerance metrics included the Tolerant Indicator Genera, Sensitive Indicator Genera, and Florida Index. Community balance metrics included Mollusca (phylum taxonomic level) and Noteridae (family taxonomic level). One functional groups metric was included, percent Scrapers. The percent of Tolerant Indicator Genera, Mollusca, and Scrapers increased with increasing development intensity, whereas Sensitive Indicator Genera, Florida Index, and Noteridae decreased with increasing development intensity. Table 3-33 shows that scores

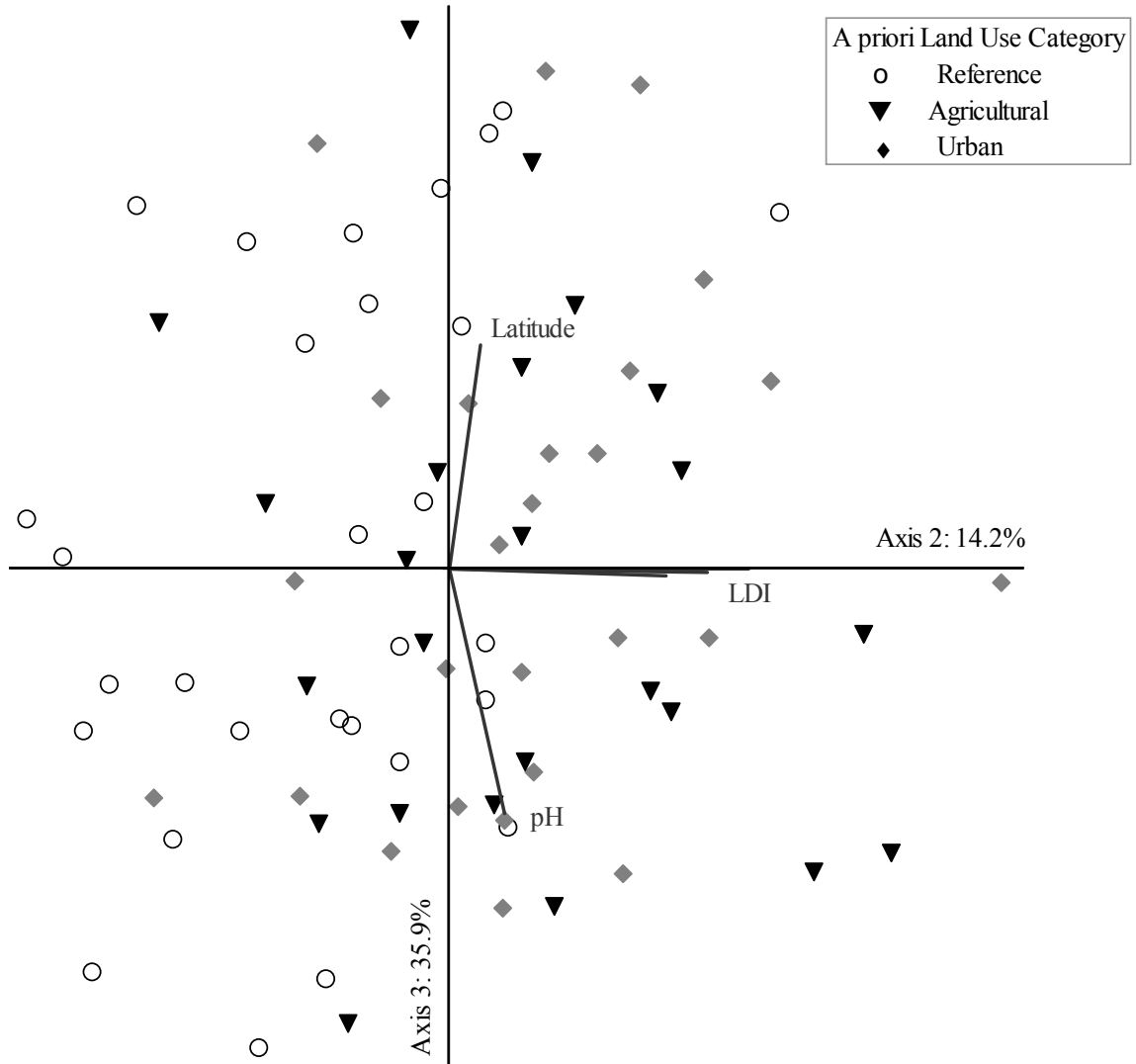


Figure 3-21. NMS ordination bi-plot for 79 wetlands in macroinvertebrate genus space with an overlay of environmental parameters. Latitude, LDI, and water column pH (shown as radiating vectors), were significantly correlated with the NMS axes based on macroinvertebrate community composition. Vector length represents the strength of the correlation, and the angle represents the direction of maximum change. Axis 2 explained 14.2% variance, axis 3 explained 35.9% variance, and axis 1 (not shown) represented an additional 18.9% variance.



Table 3-31. Pearsons r-squared correlations between environmental variables and NMDS ordination axes based on macroinvertebrate community composition.

	Axis 1	Axis 2	Axis 3
Incremental $r^2$	18.9%	14.2%	35.9%
Cumulative $r^2$	18.9%	33.0%	68.9%
Latitude	0.17	0.03	0.22
Longitude	0.05	0.02	0.09
LDI	0.01	0.25	0.01
DO (log)	0.01	0.13	0.00
Temperature (log)	0.03	0.05	0.07
Color (log)	0.02	0.02	0.10
Turbidity (log)	0.00	0.09	0.02
pH	0.00	0.06	0.24
Water ammonia-N (log)	0.04	0.08	0.01
Water nitrate/nitrite-N (log)	0.01	0.02	0.05
Water TKN (log)	0.05	0.08	0.00
Water TP (log)	0.02	0.18	0.01
Soil Moisture (arcsine sqrt)	0.02	0.04	0.09
Soil TP (log)	0.10	0.04	0.01

Table 3-32. Spearman correlations between macroinvertebrate metrics and the macroinvertebrate FWCI with LDI, pH, DO (log), and TP (log).

Macroinvertebrate Metrics	LDI	pH	DO (log)	TP (log)
Tolerance metrics				
Tolerant Indicator Genera	0.51	0.62	-0.25	
Sensitive Indicator Genera	-0.47		0.39	-0.37
Florida Index	-0.35		0.35	-0.24
Community balance				
Mollusca	0.33	0.54	-0.28	
Noteridae	-0.34			
Functional group				
Scraper	0.30			
Macroinvertebrate FWCI	-0.62	-0.56	0.48	-0.34

All correlations shown are significant ( $p < 0.05$ )

Table 3-33. Macroinvertebrate metric and FWCI scores between LDI groups.

Metric	Low LDI	High LDI	W <sup>^</sup>	p <sup>`</sup>
Tolerant indicator species	4.4 (11.8)	14.2 (15.5)	904.0	<0.001
Sensitive indicator species	15.5 (20.3)	2.2 (4.4)	1679.0	<0.001
Florida Index	2.1 (2.3)	0.9 (1.2)	1572.0	0.008
Mollusca	2.0 (3.5)	9.7 (13.4)	1025.0	0.003
Noteridae	1.8 (3.7)	0.2 (0.7)	1518.0	0.012
Scrapers	4.2 (6.7)	10.9 (12.8)	1046.0	0.006
Macroinvertebrate FWCI	36.8 (10.0)	22.3 (8.4)	1878.5	<0.001

W<sup>^</sup> = Mann-Whitney U-Test statistic.

p<sup>`</sup> = significance value.

Values represent the mean (standard deviation).

of selected metrics and the macroinvertebrate FWCI were significantly different between low and high LDI groups ( $p < 0.05$ ).

#### *Tolerance metrics*

The statewide Tolerant Indicator Genera were established at an LDI break of 4.0, and included six genera *Goeldichironomus*, *Micromenetus*, *Microvelia*, *Physella*, *Tropisternus*, and *Tanypus* (Table 3-34). Figure 3-22 shows Tolerant Indicator Genera increased with increasing development intensity. Two irregularities were apparent in the low LDI group, including SU4 (Tolerant Indicator Genera = 61%) and SA8 (Tolerant Indicator Genera = 31%). Wetlands in the high LDI group with the highest percent Tolerant Indicator Genera included four central (CA9, CA3, CU5, CA2) and one north (NA4) wetland region wetland. All five wetlands were surrounded by different land uses, including citrus crops (CA9), pullet farm spray field (CA3), residential and commercial (CU5), pasture (CA2), and row crops (NA4).

The 14 sensitive indicator genera (Table 3-35) were calculated at an LDI break of 1.75. The five Sensitive Indicator Genera with the highest Indicator Values included *Bersous*, *Hydrocanthus*, *Larsia*, *Pristina*, and *Pristinella*. Sensitive Indicator Genera included macroinvertebrates in two phyla, Annelida and Arthropoda. The phylum Annelida was represented by two genera of aquatic worms, *Pristina* and *Pristinella*, both in the family Naididae, order Haplotaxida, class Oligochaeta. The 12 remaining Sensitive Indicator Genera fell within the phylum Arthropoda, representing two classes Arachnida (including a water mite); and Insecta, aquatic insects in three orders including Coleoptera (five genera of beetles), Diptera (four genera of true flies), and Trichoptera (two genera of caddis flies).

Figure 3-23 shows the percent Sensitive Indicator Genera decreased with increasing development intensity in the landscape. Two wetlands hosted an unexpected percent of Sensitive Indicator Genera including one in each LDI group. All of the macroinvertebrates identified at PR4 (in the low LDI group) were categorized as Sensitive Indicator Genera (100%). Wetlands hosting the next highest percent macroinvertebrate Sensitive Indicator Genera were NR3 with only 42% sensitive

Table 3-34. Macroinvertebrate tolerant indicator genera. Tolerant indicator genera calculated at an LDI break of 4.0 were significant ( $p < 0.10$ ).

Phylum	Class	Order	Family	Genera	Indicator Value	p-value
Arthropoda	Insecta	Coleoptera	Hydrophilidae	<i>Tropisternus</i>	17.2	0.053
			Orthoclaudiinae	<i>Goeldichironomus</i>	56.9	0.001
		Diptera	Chironomidae	<i>Tanytus</i>	8.1	0.098
-----						
		Heteroptera	Velidae	<i>Microvelia</i>	12.9	0.033
-----						
Mollusca	Gastropoda	Basommatophora	Planorbidae	<i>Micromenetus</i>	22.5	0.084
			Physidae	<i>Physella</i>	21.5	0.002

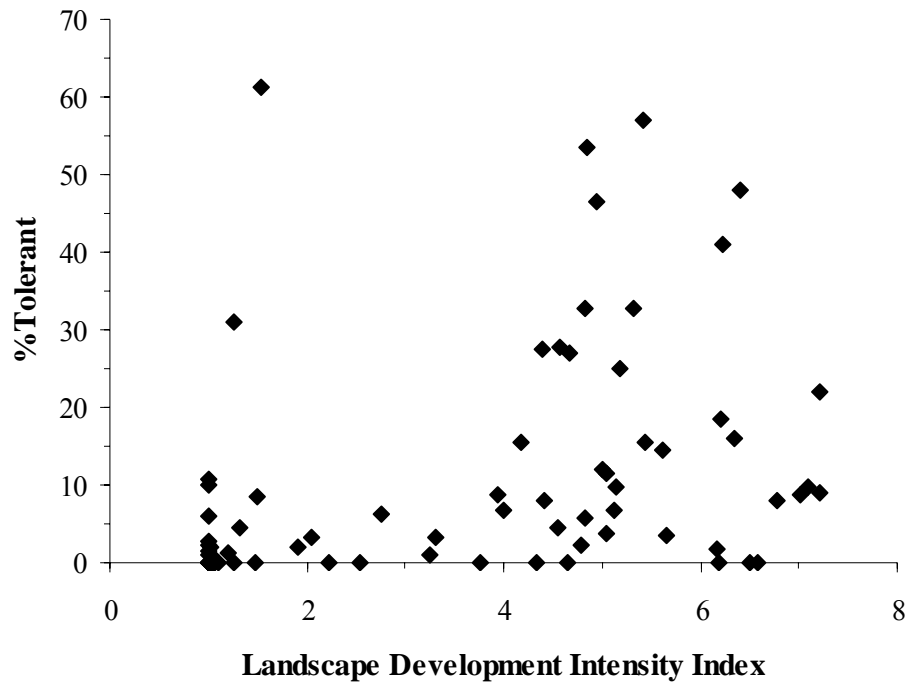


Figure 3-22. Macroinvertebrate Tolerant Indicator Genera increased with increasing development intensity (LDI).

Table 3-35. Sensitive macroinvertebrate indicator genera. Sensitive indicator genera calculated at an LDI break of 1.75 were significant ( $p < 0.10$ ).

Phylum	Class	Order	Family	Genera	Indicator Value	p-value
Annelida	Oligochaeta	Haplotaenida	Naididae	<i>Pristina</i>	35.5	0.008
				<i>Pristinella</i>	25.9	0.094
Arthropoda	Arachnida	Acariformes	Hydrachnidae	<i>Hydrachna</i>	9.4	0.049
				<i>Laccophilus</i>	8.9	0.061
Insecta	Coleoptera		Dytiscidae	<i>Halpius</i>	8.8	0.055
				<i>Berosus</i>	28.8	0.003
			Noteridae	<i>Hydrocanthus</i>	28.5	0.004
				<i>Suphis</i>	12.5	0.048
		Diptera	Chironomidae	<i>Larsia</i>	24.8	0.011
				<i>Paramerina</i>	14	0.062
			Orthocladinae	<i>Zavreliella</i>	17.3	0.008
				<i>Dicrotendipes</i>	14.8	0.065
	Trichoptera		Leptoceridae	<i>Oecetis</i>	12.5	0.023
				<i>Oxyethira</i>	12.5	0.032

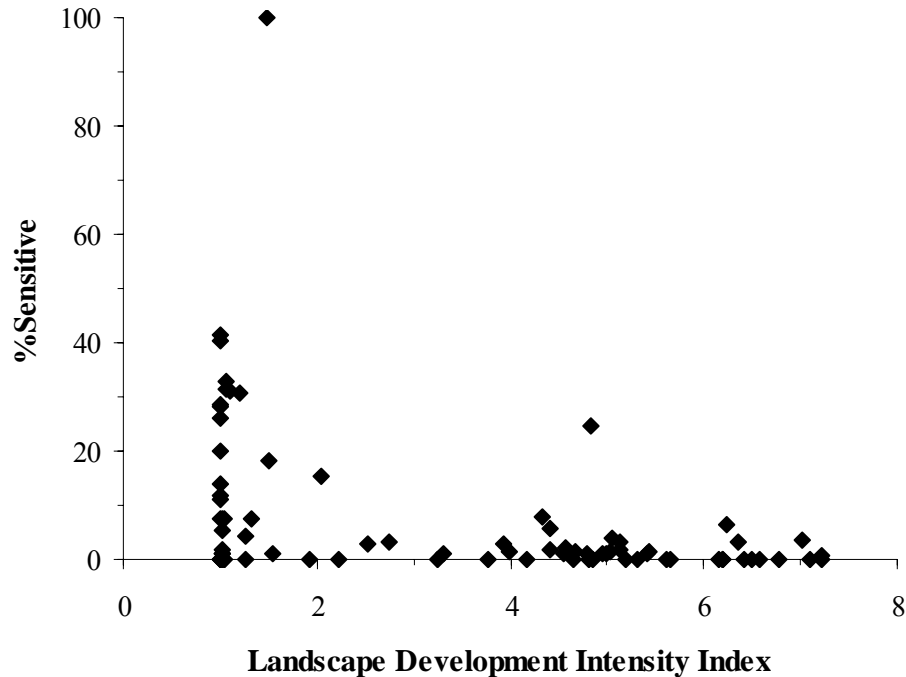


Figure 3-23. Sensitive macroinvertebrate indicator genera decreased with increasing development intensity (LDI).

Indicator Genera and CR11 (surrounded by pine flatwoods) with 41%. The inconsistent score in the high LDI group with a low presence of Sensitive Indicator Genera was SU2 (situated on the edge of a public school campus and adjacent to a high-intensity roadway with urban marsh on more than half of the wetland boundary; Sensitive Indicator Genera = 25%).

The third tolerance metric was the Florida Index, an index based on the relative pollution tolerance of macroinvertebrates identified in a water body (Beck 1954; USEPA 2002c). Calculations for the Florida Index included scoring Class I organisms, which were considered least tolerant, and Class II organisms, which were considered intolerant of pollution. Mixed taxonomic levels were included in the Florida Index from species (example: *Polypedilum halterale*) to genus (example: all species of *Elimia*) to family (example: all species of Gammaridae) to order (example: all species of Plecoptera; Beck 1954; USEPA 2002c). The Florida Index value was expected to decrease with increasing development intensity in the surrounding landscape (Barbour et al. 1996a). Figure 3-24 shows that the Florida Index score generally decreased with increasing development intensity in the surrounding landscape, with six wetlands showing an inconsistent trend including four urban and two agricultural wetlands. The five highest scoring wetlands in the low LDI group included wetlands in each wetland region including CR4 (Florida Index = 8), SR8 (Florida Index = 7), NR3 (Florida Index = 7), CR11 (Florida Index = 6), and PR8 (Florida Index = 5).

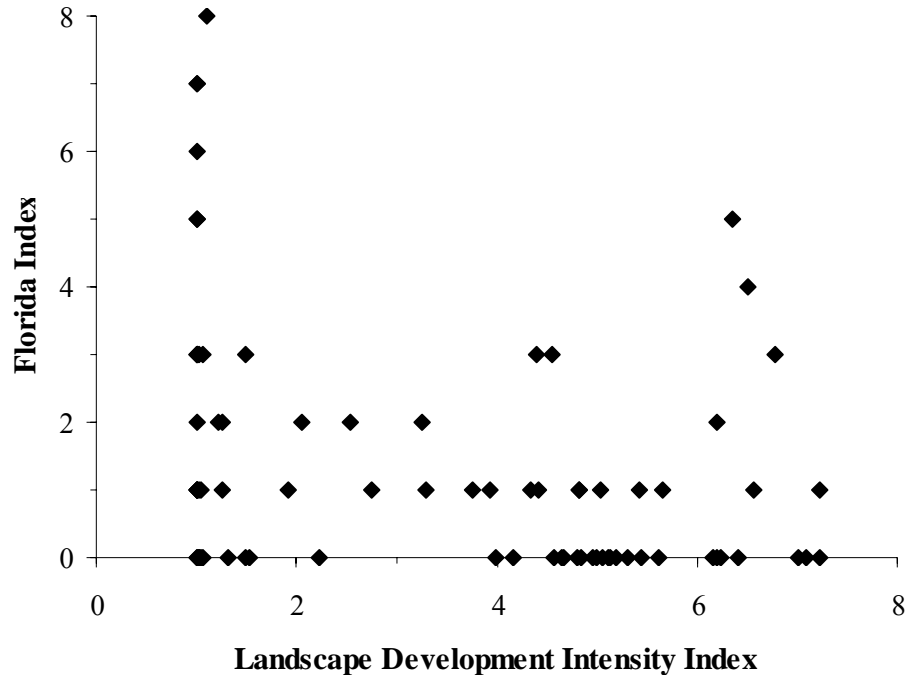


Figure 3-24. Florida Index scores decreased with increasing development intensity (LDI).

#### *Community balance metrics*

Two community balance metrics were incorporated into the macroinvertebrate FWCI including percent Mollusca and percent Noteridae. The percent of individuals in the phylum Mollusca was significantly correlated with LDI (Table 3-32) and significantly differentiated between low and high LDI groups (Table 3-33). Figure 3-25 shows that the percent of macroinvertebrates in the phylum Mollusca increased with increasing development intensity. Macroinvertebrates were identified in three classes within the order Mollusca, including Bivalva, Gastropoda, and Plecypoda. Nearly two-thirds of the wetlands hosted macroinvertebrates in the phylum Mollusca (n=51). In five wetlands over one-third of the macroinvertebrates that were identified belonged to the phylum Mollusca, including SU2 (situated on the edge of a public school campus and adjacent to a high-intensity roadway with urban marsh on more than half of the wetland boundary; 44.7%), CU8 (surrounded by industrial and transportation land use; 44.4%), SU7 (surrounded most recently by a high-intensity shopping mall, previously in row crops; 44.1%), SA4 (surrounded by row crops; 37.5%), and CA5 (surrounded by pasture; 34.9%). In the low LDI group, four wetlands had greater than 5% of the identified macroinvertebrates in the phylum Mollusca, including SA8 (surrounded by a suburban park and receiving run-off from animal husbandry; 16.7%), PR7 (a large, deep wetland

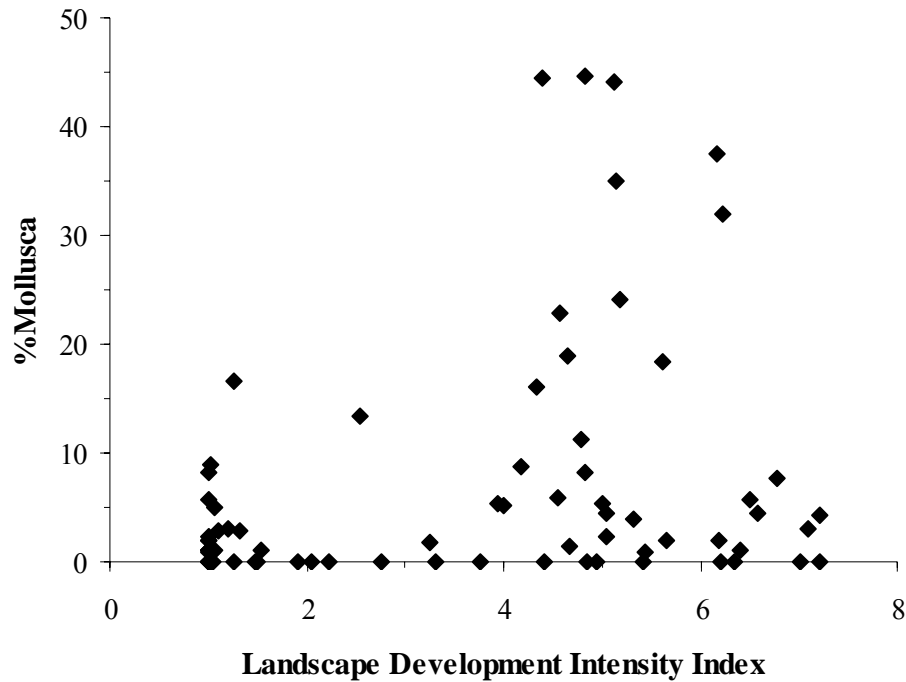


Figure 3-25. Macroinvertebrates in the phylum Mollusca increased with increasing development intensity (LDI).

on a private conservation tract ; 9.0%), SR4 (nested in the Everglades; 8.1%), and CR3 (surrounded by uplands undergoing restoration; 5.8%).

Figure 3-26 shows that the percent of macroinvertebrates in the family Noteridae decreased with increasing landscape development intensity as expected (Barbour et al 1996b). Macroinvertebrates in the family Noteridae never made up more than 15% of the individuals identified to the family taxonomic level or lower at any of the sample wetlands. The family Noteridae falls within order Coleoptera, class Insecta, phylum Arthropoda. Macroinvertebrates in the family Noteridae (burrowing water beetles) typically inhabit the shallow margins of standing or slow flowing streams, or in lentic habitats, act as climbers associated with vascular macrophytes or as burrowers (Williams and Feltmate 1992; White and Brigham 1996). Both the larvae and adults of macroinvertebrates in the family Noteridae are aquatic (Peckarsky et al. 1993), one of only five families within Coleoptera with both life stages being aquatic. As a general rule, the percent of macroinvertebrates in the order Coleoptera was found to decrease with increasing development surrounding Florida streams (Barbour et al. 1996b). One wetland not following the expected trend in the high LDI group was CA4 (surrounded by pasture; 4.1%).



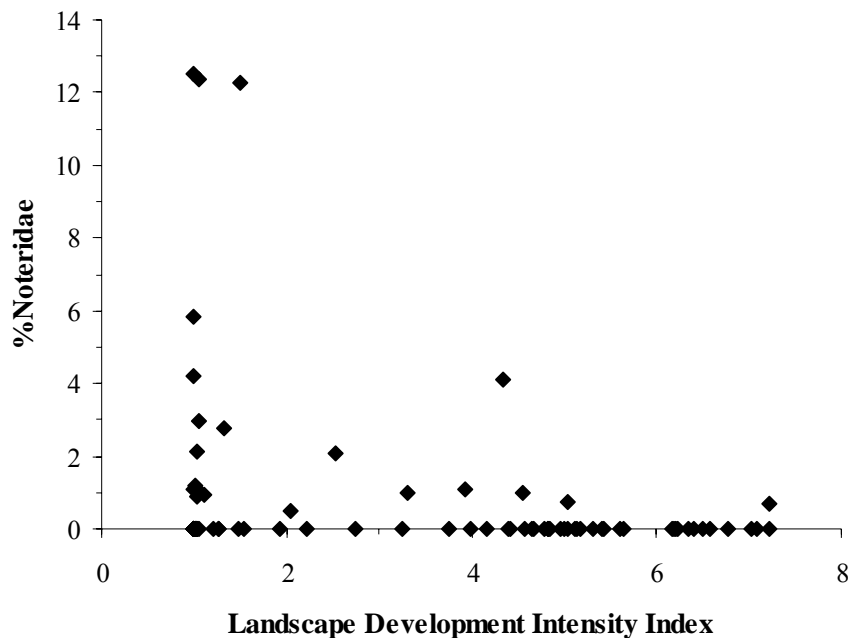


Figure 3-26. Macroinvertebrates in the family Noteridae decreased with increasing development intensity (LDI).

#### *Functional group metrics*

One functional group metric was selected for inclusion in the macroinvertebrate FWCI, the percent scrapers functional feeding group. Figure 3-27 shows the percent macroinvertebrates in the scraper functional feeding group increased with increasing LDI. The scraper functional feeding group included macroinvertebrates that scrape periphyton from mineral and organic surfaces and those that browse or graze algal materials. Five wetlands with the highest percent scrapers were found in among all four wetland regions and represented wetlands embedded in a mix of urban and agricultural land uses, including SU7 (surrounded most recently by a high-intensity shopping mall, previously in row crops; 51%), SA4 (surrounded by row crops; 40%), CA5 (surrounded by pasture; 40%), PA6 (surrounded by pasture; 38%), and CU8 (surrounded by industrial and transportation land use; 31%). Nearly one-quarter of the sample wetlands (n = 19) did not have scrapers identified in the macroinvertebrate samples.

#### *Macroinvertebrate Florida Wetland Condition Index*

The six metrics described above were included in the macroinvertebrate FWCI. Appendix F provides detailed instructions on metric scoring. Figure 3-28 shows the relationship between the macroinvertebrate FWCI and LDI. Potential scores for the macroinvertebrate FWCI range from 0-60, with higher values representing reference wetlands. Actual scores ranged from 5.2 at SU5 (a deeply flooded swamp surrounded by industrial land use, LDI = 5.2), to 57.0 at SR3 (surrounded by pine flatwoods, LDI = 1.0).

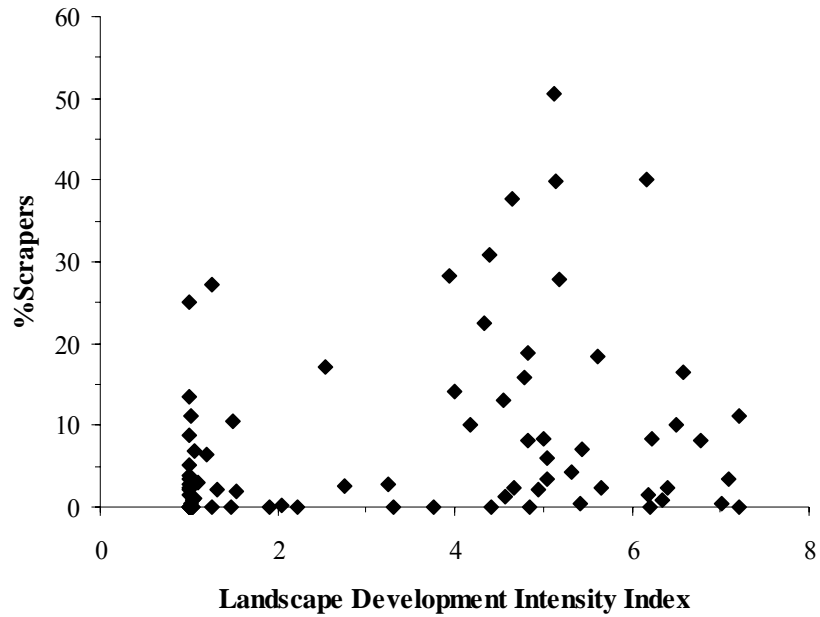


Figure 3-27. The percent of macroinvertebrates that belong to the scraper functional feeding group increased with increasing development intensity (LDI).

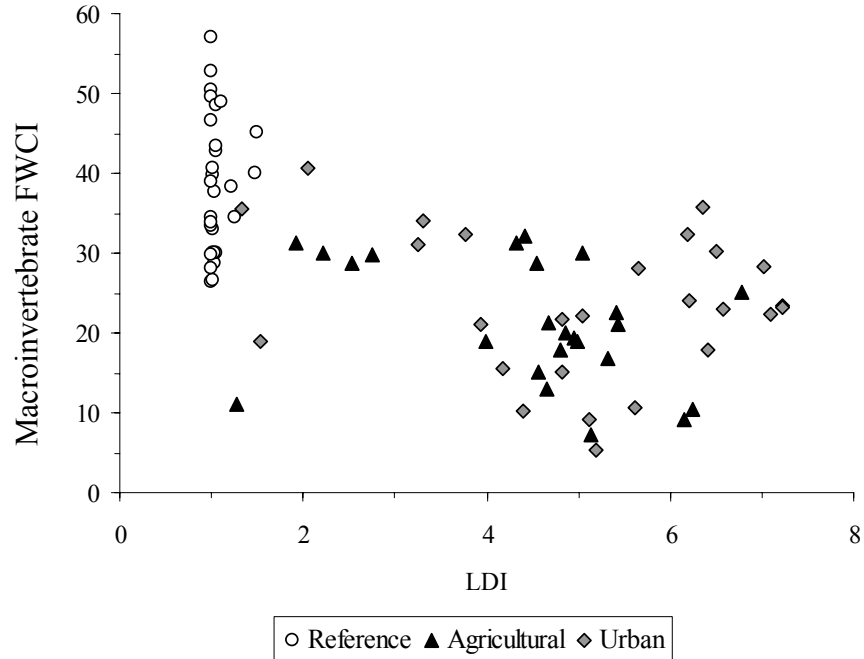


Figure 3-28. Macroinvertebrate FWCI scores decreased with increasing landscape development intensity index (LDI).

Ranges varied regionally, though the regional scores were not significantly different for either low or high LDI groups. The highest scores in each wetland region included four reference wetland, PR8 (40.7), NR3 (52.8), CR6 (50.4), and SR8 (57.0), in the panhandle, north, central, and south wetland regions, respectively. The lowest scoring wetlands in both the panhandle and central wetland regions were embedded in pasture, including PA6 (12.9) and CA5 (7.2). In the north wetland region NA4 (surrounded by row crops) scored 10.4. In the south SU5 (surrounded by commercial land use) received the lowest score overall of 5.3. The macroinvertebrate FWCI was significantly correlated with the LDI index (Spearman's  $|r| = 0.62$ ,  $p < 0.001$ ). A Kruskal-Wallis test suggested a significant difference ( $H = 36.0$ ,  $p < 0.001$ ) among median macroinvertebrate FWCI scores for study wetlands in *a priori* land use categories.

### ***Cluster Analysis***

Cluster analysis determined five categories based on macroinvertebrate community composition. Clusters were explained by regions and *a priori* land use categories including: 1: south to central low development intensity; 2: mixed region low development intensity; 3: north central to panhandle middle development intensity; 4: northern to panhandle middle development intensity; and 5: high development intensity and southern Everglades. Figure 3-29 shows that based on macroinvertebrate FWCI scores, clusters 1 and 2 were not significantly different from one another, but were significantly different from cluster 5. Clusters 3 and 4 were significantly different from cluster 1 and cluster 5. Table 3-36 provides means and standard deviations for macroinvertebrate FWCI and LDI scores of the five clusters.

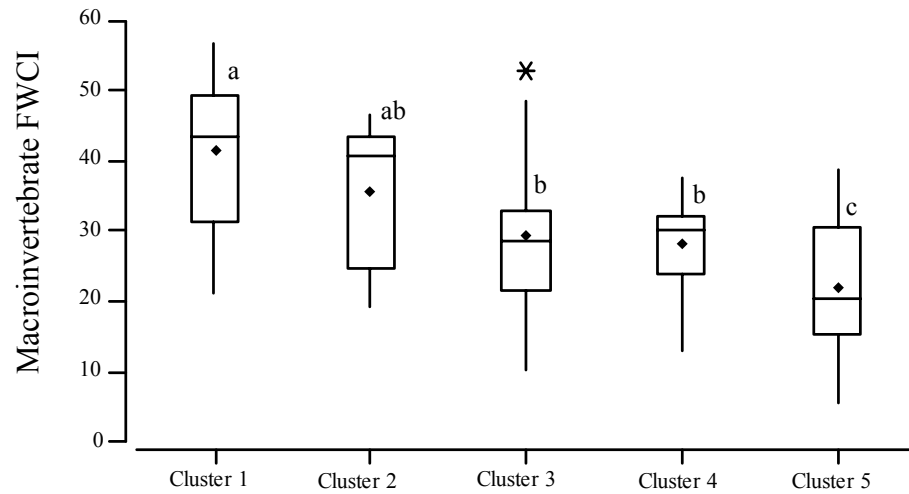


Figure 3-29. Macroinvertebrate FWCI scores for five wetland clusters based on macroinvertebrate community composition. Clusters with similar letters were not significantly different (Fisher's LSD,  $p < 0.05$ ).

Table 3-38. Macroinvertebrate FWCI scores and LDI values for wetland clusters based on macroinvertebrate community composition.

	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 5
FWCI	41.6 (10.7) <sup>a</sup>	35.5 (10.8) <sup>ab</sup>	29.3 (11.8) <sup>b</sup>	28.3 (6.2) <sup>b</sup>	21.9 (9.7) <sup>c</sup>
LDI	2.0 (1.5) <sup>a</sup>	3.1 (2.5) <sup>ab</sup>	3.4 (2.3) <sup>ab</sup>	3.4 (2.3) <sup>ab</sup>	4.0 (2.1) <sup>b</sup>

Clusters with similar letters were not significantly different ( $p < 0.05$ ).  
 Values represent the mean (standard deviation).

### Florida Wetland Condition Index

In total 19 metrics were used to construct the FWCI, including seven metrics based on the diatom assemblage, six metrics based on the macrophyte assemblage, and six metrics based on the macroinvertebrate assemblage. Table 3-37 lists the FWCI scores for 118 isolated forested wetlands for each assemblage. Scores ranged from 0-70 for the diatom FWCI and from 0-60 for both the macrophyte and macroinvertebrate FWCI. Different wetlands received the highest and lowest scores for each FWCI. The highest scores for each FWCI were found at wetlands in the south and central wetland regions, including SR2 (diatom FWCI = 68.9), CR11 (macrophyte FWCI = 59.0), and SR8 (macroinvertebrate FWCI = 57.0).

Minimum FWCI scores were found among three different wetland regions, including CA3 (receiving wastewaters from pullet farm operations; diatom FWCI = 7.9), NA1 (surrounded by pasture at an experimental research facility; macrophyte FWCI = 0.0), and SU2 (situated on the edge of a public school campus and adjacent to a high-intensity roadway with urban marsh on more than half of the wetland boundary; macroinvertebrate FWCI = 5.3). Within the north and central wetland regions some wetlands received the wetland region maximum scores for multiple assemblages, including NR3 (surrounded by pine flatwoods; diatom FWCI = 66.8; macroinvertebrate FWCI = 52.8) and CR6 (surrounded by pine flatwoods; diatom FWCI = 65.5; macroinvertebrate FWCI = 50.4). In the panhandle wetland region, PU4 (surrounded by residential and commercial land uses) received minimum FWCI scores for both the diatom and macrophyte assemblages (diatom FWCI = 10.5; macrophyte FWCI = 4.0). Figure 3-30 shows a three dimensional scatter plot for the 50 wetlands receiving scores for all three assemblages. The maximum diatom FWCI for the 50 wetlands graphed was 68.9 (of 70) at SR2. The maximum macrophyte FWCI was 58.4 (of 60) at PR6 (LDI = 1.3), and the maximum macroinvertebrate FWCI was 52.8 (of 60) at NR3 (LDI = 1.0). The 1:1:1 line is shown for convenience in interpretation.

Figure 3-31 shows two dimensional comparisons of FWCI scores for wetlands with multiple assemblages sampled, including (A) 50 wetlands with diatom and macrophyte FWCI scores, (B) 50 wetlands with diatom and macroinvertebrate FWCI scores, and (C) 79 wetlands with macrophyte and macroinvertebrate FWCI scores. The most obvious irregularity between the diatom and macrophyte FWCI scores (Figure 3-31 A) was at CU6, a wetland surrounded by residential (both single and multifamily) and a golf course that had been developed within the past five years. CU6 had low scores for the diatom (15.1 of 70) and macroinvertebrate (23.4 of 60) but a considerably higher score for the macrophyte (41.5 of 60) FWCI.

The 19 metrics incorporated into the here assemblage specific FWCI were compared across different assemblages (Table 3-38). Of the 120 potential metric comparisons (42 among diatom and macrophyte metrics, 42 among diatom and macroinvertebrate metrics, and 36 among macrophyte and macroinvertebrate metrics), 53% of the comparisons were significantly correlated at the  $p < 0.01$  level. An additional 20% of the potential comparisons were significantly correlated at the more flexible  $p < 0.05$  level; and an additional 6% at the more flexible  $p < 0.10$  level. Less than one-quarter of the comparisons among metrics of different assemblages were not significantly correlated (22%). The strongest correlation among metrics of different assemblages was

Table 3-37. Florida Wetland Condition Index scores for 118 wetlands based on three assemblages including diatoms, macrophytes, and macroinvertebrates.

Site Code	Diatom FWCI	Macrophyte FWCI	MacroinvertebrateFWCI
PA1	-	30.4	-
PA2	38.1	11.9	30.1
PA3	34.9	8.3	25.1
PA4	-	12.6	-
PA5	51.1	6.5	21.2
PA6	28.2	7.7	12.9
PA7	-	17.7	-
PA8	-	50.6	-
PA9	-	12.1	-
PA10	-	41.7	-
PR1	61.1	55.9	37.6
PR2	-	50.5	-
PR3	-	49.5	-
PR4	64.5	51.2	40.0
PR5	58.0	53.6	30.0
PR6	63.9	58.4	34.4
PR7	-	34.8	26.5
PR8	-	53.6	40.7
PU1	-	6.2	-
PU2	-	31.5	-
PU3	33.1	31.0	35.7
PU4	10.5	4.0	21.6
PU5	-	22.1	-
PU6	-	16.5	-
PU7	-	24.1	-
PU8	-	33.6	-
PU9	-	48.8	-
PU10	-	9.2	30.2
NA1	-	0.0	-
NA2	-	3.0	-
NA3	-	56.4	-
NA4	33.8	16.3	10.4

Table 3-37. Continued.

Site Code	Diatom FWCI	Macrophyte FWCI	Macroinvertebrate FWCI
NA5	-	2.9	-
NA6	56.3	18.8	16.9
NA7	-	37.0	-
NA8	-	46.0	-
NA9	-	37.3	-
NA10	-	51.5	30.0
NA11	-	32.6	28.7
NA12	-	8.0	-
NR1	-	52.0	-
NR2	65.8	34.8	30.0
NR3	66.8	58.2	52.8
NR4	58.3	42.2	39.8
NR5	-	52.3	-
NR6	57.9	55.0	48.6
NR7	-	52.3	-
NR8	-	58.4	30.0
NR9	-	56.7	33.0
NU1	-	35.2	-
NU2	24.1	23.7	15.5
NU3	-	25.6	-
NU4	54.5	35.1	31.0
NU5	60.0	40.1	24.0
NU6	48.8	20.7	28.0
NU7	-	11.8	-
NU8	-	38.6	-
NU9	-	37.5	-
NU10	-	17.2	23.0
CA1	-	8.9	-
CA2	10.6	0.7	19.4
CA3	7.9	7.1	20.0
CA4	56.9	38.8	31.3
CA5	43.6	26.9	7.2
CA6	22.7	7.1	21.1
CA7	-	9.8	32.1

Table 3-37. Continued.

Site Code	Diatom FWCI	Macrophyte FWCI	Macroinvertebrate FWCI
CA8	-	37.7	31.3
CA9	-	11.8	22.6
CR1	-	51.0	-
CR2	-	49.9	-
CR3	57.7	47.6	33.9
CR4	57.8	51.2	48.9
CR5	43.8	43.5	29.7
CR6	65.5	54.6	50.4
CR7	-	51.7	-
CR8	-	54.3	28.8
CR9	-	49.4	34.4
CR10	-	53.5	45.0
CR11	-	59.0	49.5
CU1	61.1	42.9	40.6
CU2	-	10.0	-
CU3	28.5	13.5	22.3
CU4	-	21.4	-
CU5	21.5	22.3	17.8
CU6	15.1	41.5	23.4
CU7	-	20.7	10.6
CU8	-	21.1	10.1
CU9	-	28.3	28.3
CU10	-	38.3	32.3
CU11	-	21.3	34.1
SA1	-	0.7	-
SA2	34.1	9.4	15.0
SA3	47.9	23.1	28.6
SA4	15.8	11.3	9.1
SA5	46.3	18.9	19.0
SA6	31.9	3.7	19.0
SA7	-	30.8	29.8
SA8	-	34.5	11.0
SA9	-	29.8	17.9
SR1	66.8	54.1	33.4



Table 3-37. Continued.

Site Code	Diatom FWCI	Macrophyte FWCI	Macroinvertebrate FWCI
SR2	68.9	50.8	46.6
SR3	51.6	51.2	26.4
SR4	43.7	57.9	28.2
SR5	39.4	49.8	38.4
SR6	41.0	51.8	39.0
SR7	-	49.9	42.7
SR8	-	47.5	57.0
SR9	-	50.1	43.4
SU1	17.2	17.8	22.1
SU2	46.2	20.3	15.2
SU3	31.7	42.6	35.4
SU4	42.3	21.8	18.9
SU5	38.9	23.9	5.3
SU6	46.1	28.1	21.0
SU7	-	12.5	9.1
SU8	-	2.7	23.3
SU9	-	20.4	32.3
SU10	-	11.7	-

between the diatom Sensitive Indicator Genera and the macrophyte Sensitive Indicator Species (Pearson's  $r = 0.74$ ,  $p < 0.01$ ). Twelve of the metric comparisons between the diatom and macrophyte assemblages were strongly significant ( $|r| \geq 0.60$ ,  $p < 0.01$ ). Only two of the comparisons between the diatom and macrophyte assemblages were not significantly correlated at the most flexible significance level ( $p < 0.10$ ).

Comparisons between the diatom and macroinvertebrate metrics were not as strong, with less than 50% of the metrics significantly correlated ( $p < 0.10$ ). Only six comparisons among diatom and macroinvertebrate metrics were correlated at the more stringent significance level ( $p < 0.01$ ). The macroinvertebrate metrics Noteridae and scrapers were not significantly correlated with any of the diatom metrics. Correlations were stronger among the macrophyte and macroinvertebrate comparisons, with 94% of the comparisons significantly correlated ( $p < 0.10$ ). In fact, 20 of the metric comparisons (56%) were correlated at the strictest significance level ( $p < 0.01$ ). Two metric comparisons between the macrophyte and macroinvertebrate metrics were not significantly correlated, including the macrophyte Wetland Status Species and macroinvertebrate Mollusca metrics and between the macrophyte Exotic Species and macroinvertebrate Scrapers metrics.

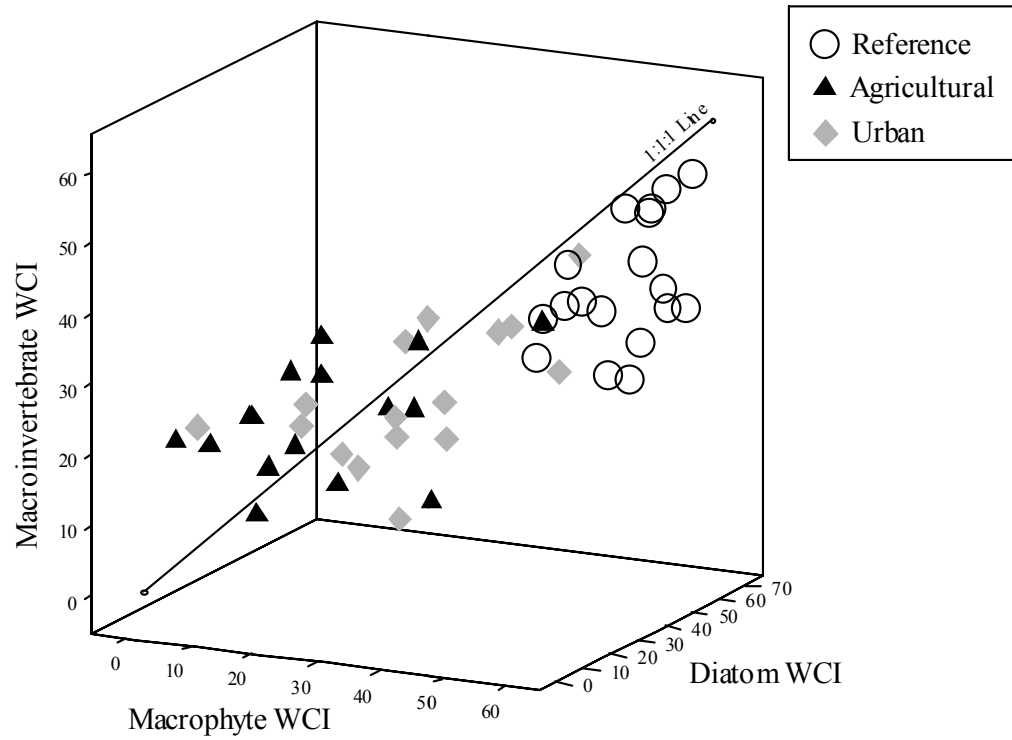


Figure 3-30. Three dimensional scatter plot of the FWCI scores based on three assemblages, including diatoms, macrophytes, and macroinvertebrates.

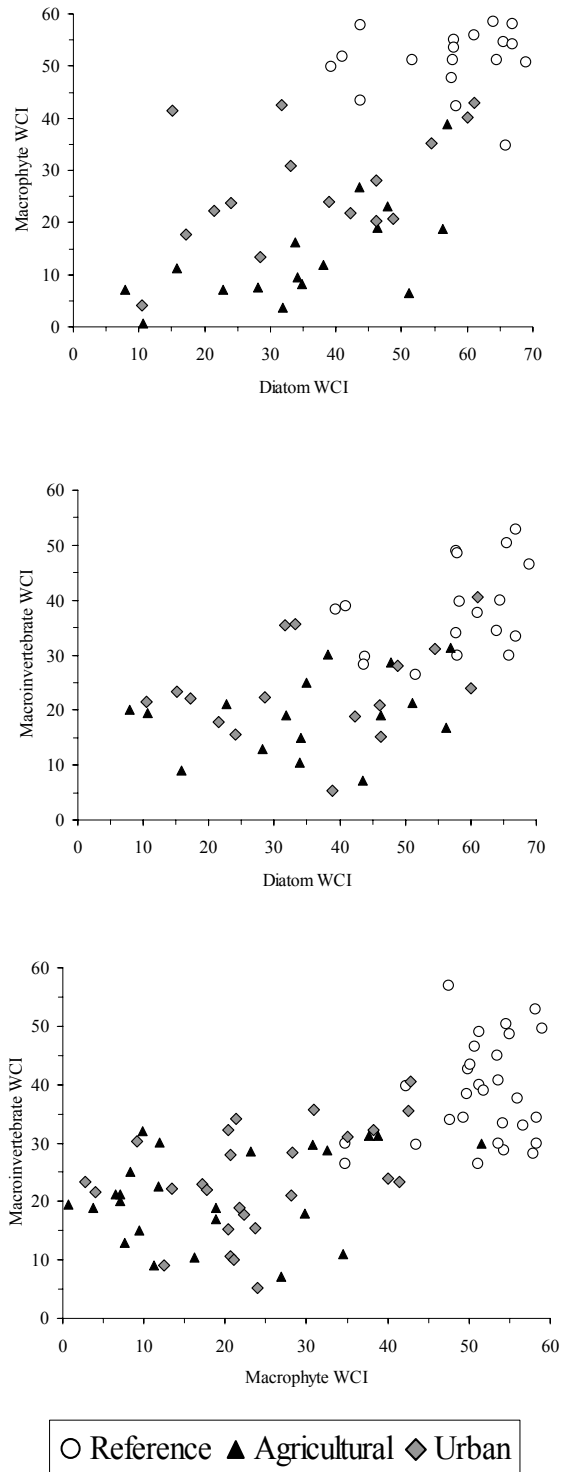


Figure 3-31. Scatterplots of FWCI scores for wetlands based on diatom, macrophyte, and macroinvertebrate assemblages. A) Diatom and macrophyte FWCI scores ( $n = 50$  wetlands). B) Diatom and macroinvertebrate FWCI scores ( $n = 50$ ). C) Macrophyte and macroinvertebrate FWCI scores ( $n = 79$ ).

Table 3-38. Pearson correlations among 19 metrics included in the three assemblage specific Florida Wetland Condition Indices.

Diatoms	Macrophytes					Wetland Status
	Tolerant	Sensitive	MFQI	Exotic	Native Perennial	
Tolerant	0.60*	-0.52*	-0.52*	0.49*	-0.60*	-0.26 <sup>#</sup>
Sensitive	-0.59*	0.74*	0.68*	-0.53*	0.57*	0.42*
Pollution Class 1	0.60*	-0.50*	-0.51*	0.59*	-0.60*	-0.36 <sup>^</sup>
Nitrogen Class 3	0.54*	-0.55*	-0.46*	0.61*	-0.58*	
Saprobity Class 4	0.51*	-0.47*	-0.44*	0.61*	-0.60*	-0.34 <sup>^</sup>
pH Class 3	0.57*	-0.60*	-0.63*	0.62*	-0.58*	-0.39*
Oxygen Class 1	-0.57*	0.68*	0.56*	-0.57*	0.60*	
Diatoms	Macroinvertebrates					Scrapers
	Tolerant	Sensitive	FL Index	Mollusca	Noteridae	
Tolerant	0.49*	0.29 <sup>^</sup>	-0.27 <sup>#</sup>			
Sensitive	-0.48*	0.38*	0.40*	-0.33 <sup>^</sup>		
Pollution Class 1	0.25 <sup>#</sup>	-0.29 <sup>^</sup>				
Nitrogen Class 3	0.33 <sup>^</sup>	-0.29 <sup>^</sup>	-0.30 <sup>^</sup>			
Saprobity Class 4		-0.30 <sup>^</sup>				
pH Class 3	0.50*	-0.30 <sup>^</sup>	-0.25 <sup>#</sup>			
Oxygen Class 1	-0.45*	0.32 <sup>^</sup>	0.35 <sup>^</sup>	-0.33 <sup>^</sup>		
Macrophytes	Macroinvertebrates					Scrapers
	Tolerant	Sensitive	FL Index	Mollusca	Noteridae	
Tolerant	0.47*	-0.41*	-0.30*	0.27 <sup>^</sup>	-0.25 <sup>^</sup>	0.23 <sup>^</sup>
Sensitive	-0.44*	0.54*	0.31*	-0.30*	0.31*	-0.27 <sup>^</sup>
MFQI	-0.47*	0.34*	0.28 <sup>^</sup>	-0.33*	0.21 <sup>#</sup>	-0.30*
Exotic	0.48*	-0.32*	0.28 <sup>^</sup>	0.27 <sup>^</sup>	-0.23 <sup>^</sup>	
Native Perennial	-0.45*	0.33*	0.29*	-0.30*	0.27 <sup>^</sup>	-0.22 <sup>#</sup>
Wetland Status	-0.35*	0.37*	0.28 <sup>^</sup>		0.26 <sup>^</sup>	-0.22 <sup>#</sup>

p\* &lt; 0.01

p<sup>^</sup> < 0.05p<sup>#</sup> < 0.10

## CHAPTER 4 DISCUSSION

The three assemblage Florida Wetland Condition Indices (FWCIs) for isolated depression forested wetlands of Florida are comprised of 19 metrics, based on the community composition of three separate species assemblages including seven diatom, six macrophyte, and six macroinvertebrate based metrics (Table 4-1). Metrics were selected for inclusion in the FWCIs based on three criteria:

- Strength and significance (Spearman's correlation coefficient) of each metric in correlation with a quantitative gradient of Landscape Development Intensity (LDI);
- Ability of a metric to visually distinguish correlations with LDI in scatter plots;
- Calculated significant difference (Mann-Whitney U-test) between low and high LDI groups.

The index for each assemblage was composed of individual metrics specific to the assemblage, which were scaled and added together.

While previous research has identified responses of wetland ecosystems to individual changes (such as increased nutrients or altered hydrology), few have combined multiple biotic components, environmental parameters, and landscape development intensity in an attempt to quantify ecological integrity. The contribution of this research to our understanding of changes in the community composition of isolated forested wetlands (based on the diatom, macrophyte, and macroinvertebrate assemblages) in relation to different development intensities in the surrounding landscape can be summarized in seven main points.

- Biological indicators along with physical and chemical parameters were useful in defining biological integrity;
- The variable turnover times and sensitivities of the three assemblages (diatoms, macrophytes, macroinvertebrates) suggest that multiple assemblage specific multi-metric FWCIs have more merit than an FWCI based on a single assemblage;
- Regionalization may strengthen the FWCI;
- A FWCI independent of wetland type may be feasible, given the strong likeness of the forested FWCI to the marsh Wetland Condition Index (Lane et al. 2003);
- The LDI index was a useful tool correlating with the measured biological condition for isolated forested wetlands;
- Urban wetlands exhibit a different vector of change than do agricultural wetlands, and while the FWCI suggests low biological integrity of both agricultural and urban wetlands, these wetlands do provide services and do work in the environment;
- Richness, evenness, and diversity of each assemblage were not sensitive to different land uses or development intensities in the surrounding landscape.

Table 4-1. The 19 metrics of the Florida Wetland Condition Indices for isolated depressional forested wetlands, including seven diatom, six macrophyte, and six macroinvertebrate metrics.

Diatom Metrics	Macrophyte Metrics	Macroinvertebrate Metrics
% Tolerant	% Tolerant	% Tolerant
% Sensitive	% Sensitive	% Sensitive
% in Pollution Class 1	Modified FQI Score	FL Index Score
% in Nitrogen Class 3	% Exotic	% Mollusca
% in Saprobity Class 4	% Native Perennial	% Noterida
% in pH Class 3	% Wetland Status	% Scraper
% in Oxygen Class 1		

### Describing Biological Integrity

Biological indicators along with chemical and physical parameters were useful in determining the biological integrity of isolated depressional forested wetlands. For the purposes of this study, biological integrity has been defined quantitatively with the FWCI. The FWCI incorporated 19 metrics from three different species assemblages (diatoms, macrophytes, and macroinvertebrates). Correlations between the diatom, macrophyte, and macroinvertebrate FWCI and the intensity of development in the surrounding landscape (based on the use of nonrenewable energy and calculated with the Landscape Development Intensity (LDI) index advocate that changes in community composition were captured by the FWCI. It has been suggested that organisms respond to environmental gradients by colonizing a range of feasible conditions beyond which the organisms fail to persist (ter Braak 1987). By selecting species that occur throughout the range of measurable environmental parameters, the FWCI defined and detected deviations from the condition of reference wetlands based on community composition. Each of the 19 FWCI metrics addressed some disparity from the assumed range of feasible conditions.

For all three assemblages, the Tolerant Indicator Species metric demonstrated the strongest correlation with LDI, suggesting that the presence of a suite of taxa characteristic of wetlands with low biological integrity may be the single most effective means of identifying changes in community composition. The isolated depressional forested wetlands sampled were influenced by various anthropogenic activities (from direct herbivory and trampling by domestic cattle, to increased nutrients from agricultural or stormwater run-off, to hydrological impoundments or drainage), yet despite the vast differences in surrounding land uses the community composition of these wetlands was similar enough to detect a universal suite of tolerant indicator species.

Clustering the isolated forested wetlands based on the three assemblages separately suggested that differences in some agricultural and urban development intensities may be too subtle to detect with compositional data. Furthermore, greater variability in the macroinvertebrate assemblage of reference wetlands as compared to that of agricultural and urban wetlands suggested that perturbations to the driving energies in isolated wetlands may result in a convergence of the taxa present. Indeed, the natural

compositional variability inherent among reference wetlands may be lost with increased development intensity in the surrounding landscape.

While the FWCI can not be used to predict changes in the physical and chemical parameters of a wetland, their strength lies in providing an overview of biological integrity through the integration of changes in community composition from cumulative effects. Among *a priori* land use categories, differences in water and soil parameters were apparent (including dissolved oxygen, color, turbidity, water column pH, specific conductivity, water ammonia-nitrogen, water TKN, water TP, soil moisture, soil organic matter, and soil TP). When soil and water parameters were used to explain variation in the community composition of each assemblage, water column pH was universally identified. Additionally, total phosphorus concentrations explained some of the variance in both the diatom and macrophyte assemblages. Perhaps preservation and restoration strategies could focus on limiting activities (in the surrounding landscape) that influence changes to water column pH and total phosphorus loading to wetlands in order to promote biological integrity.

### **Merits of a Multi-Metric FWCI**

The variable turnover times and sensitivities of the three different assemblages (diatoms, macrophytes, macroinvertebrates) suggest that species specific multi-metric FWCI have more merit than an FWCI based on a single assemblage. The FWCI can be used to infer influences in temporal and spatial changes to which a particular wetland has been exposed. For example, diatoms have rapid turnover times and may react immediately to shifts in driving energies. On the other hand, perennial macrophytes may respond to changes over a longer period of time, particularly in the case of the woody mid- and over-story species. While the macrophyte FWCI score may remain relatively high in a recently enriched wetland, the diatom FWCI score may reflect lower biological integrity. Whereas macrophytes assimilate nutrients for growth, this process has a longer time frame than the rapid growth rate typical of the algal assemblage. An explosion of algal growth may in turn alter the available food resources within a wetland affecting other assemblages, for example there may be an increase in macroinvertebrate that scrape algae (scrapers), and therefore a decline in the macroinvertebrate FWCI score.

While agreement in the ranking of the biological condition of study wetlands using the FWCI was anticipated, discrepancies among the ranking from the different assemblages may provide great insight into wetland condition as different species assemblages respond to changes in driving energies over different time scales. There was variation among the ranking of wetlands for the diatom, macrophyte, and macroinvertebrate FWCI; though there were no obvious outliers when the three assemblages were compared. While the *a priori* reference wetlands were generally differentiated from the agricultural and urban wetlands, differences between the agricultural and urban land uses were not as apparent.

Many of the metrics of the different assemblages were significantly correlated. Perhaps the value of each of the 19 selected metrics is inherent in the differentiation between categories of landscape development intensity. Diatom and macrophyte metrics were strongly correlated with one another, and yet diatom and macroinvertebrate metrics were not, reinforcing the value of including various species assemblages in an assessment

of biological integrity. Perhaps, with regular biological monitoring it may be possible to further explore the temporal effects of changing development intensity on the biological integrity of the community composition of different species assemblages and the entire wetland community.

### **Regionalization of the Florida Wetland Condition Index**

The climate of Florida is considered humid subtropical, though pronounced differences occur in the local climate across Florida, such as differences in the amount of annual rainfall, seasonal maximum temperatures, and number of freeze days (Fernald and Purdum 1992; Lane 2000). Despite the broad latitudinal and longitudinal ranges of sample wetlands throughout Florida (26.0°N -31.0°N latitude, 80.1°W-87.5°W longitude), statewide significant difference in water and soil parameters among *a priori* land use categories were detected, suggesting the statewide scale may be appropriate for a physical and chemical assessment of wetland condition. Nevertheless, the influence of latitude and longitude was reflected in the compositional difference of all three species assemblages found among the Florida wetland regions (Lane 2000). Latitude and longitude were significantly correlated with macrophyte community composition, and latitude explained partial variance in macroinvertebrate community composition. In addition, wetlands in the Florida Everglades were outliers in many of the diatom metrics, and the southern Everglades wetlands formed distinguished clusters based on diatom and macroinvertebrate community composition. Most of the human development in Florida has occurred along the east and west coastal areas of peninsular Florida (Fernald and Purdum 1992), suggesting that while the reference wetlands selected in the south and central ecoregions were selected as the best possible examples of reference type conditions, they may be more affected by development in the surrounding landscape (such as compounded secondary effects) than their panhandle and far north wetland region counterparts.

Regionalization was explored for the macrophyte assemblage because of the sufficient number of wetlands sampled within each wetland region. There were clear regional differences in the statewide macrophyte FWCI scores for wetlands in the low LDI group, which down scored the reference wetlands of the south and central ecoregion. This led to the use of regionalized scoring of the macrophyte metrics. While the ease and utility of statewide species lists for metrics such as Indicator Species would seemingly prevail over four regional species lists, the necessity of scoring each wetland region based on the best possible reference conditions (Karr and Chu 1999) cannot be overlooked. Regionalization of biological indices has been suggested throughout the literature, as the main premise behind indices of biological integrity is based on a comparison of “like to like” (Gerristen et al. 2000), that is, to reduce the noise in background variability in biological data (which could be accomplished through regional based indices). For instance, differences in the macroinvertebrate community composition among wetland regions may be of importance in improving the macroinvertebrate FWCI. For example, none of the sample wetlands in the panhandle wetland region hosted macroinvertebrates in the order Trichoptera (caddis flies), whereas no wetland in the north wetland region hosted macroinvertebrates in the order Ephemeroptera (mayflies). While both of these



orders are characteristic of lotic environments (Edmunds and Waltz 1996), the absence of an entire order from wetland regions suggests the value of regionalization of the FWCI.

### **Florida Wetland Condition Index Independent of Wetland Type**

Recent work by Lane et al. (2003) presents a 24 metric herbaceous Wetland Condition Index (FWCI) for isolated depressional herbaceous wetlands in Florida based on the community composition of the diatom, macrophyte, and macroinvertebrate assemblages. The herbaceous FWCI was created based on 75 freshwater marshes surrounded by reference (n=34) and agricultural (n=40) land uses throughout peninsular Florida. Of the 14 metrics for the diatom assemblage, the forested and marsh FWCI share five metrics, including all of the forested diatom FWCI metrics except for species lists for the Sensitive and Tolerant Indicator Species. Though both the forested and herbaceous FWCI include Sensitive and Tolerant Indicator Species metrics, shared species were limited between wetland types, as the Tolerant Indicator Species list had only two mutual species (*Navicula confervacea* and *N. minima*), of 12 species for the forested FWCI and 21 species for the herbaceous FWCI. Similarly, the sensitive indicator species lists shared only five species (*Eunotia flexuosa*, *E. naegeli*, *E. rhomboidea*, *Frustulia rhomboids*, and *F. rhomboids crassinervia*), of 18 species for the forested FWCI and 22 species for the herbaceous FWCI. The five remaining diatom metrics were based on autecological relationships, including Pollution Class 1 (Bahls 1993), Nitrogen Uptake Metabolism Class 3 (van Dam et al. 1994), Saprobity Class 3 (van Dan et al. 1994), pH Class 3 (van Dan et al. 1994), and Dissolved Oxygen Class 1 (van Dan et al. 1994). Of the remaining seven marsh FWCI diatom metrics, five were considered too similar to selected forested FWCI metrics and were excluded to avoid redundancy. The final two marsh FWCI diatom metrics were based on Salinity Class (van Dam et al. 1994), and were not significantly correlated with LDI for isolated depressional forested wetlands.

Five macrophyte metrics were incorporated into the depressional herbaceous FWCI, and variants of these were included in the metrics of the isolated depressional forested FWCI. Tolerant and Sensitive Indicator Species lists were constructed separately for each wetland type. Of the 46 statewide tolerant macrophyte indicator species for the herbaceous FWCI, 28 also occur on the statewide macrophyte Tolerant Indicator Species list for the forested FWCI (of 61 species). Similarly, 20 statewide macrophyte Sensitive Indicator Species were shared for the herbaceous FWCI (of 36) and the forested FWCI (of 69). The three additional metrics included in the herbaceous FWCI were percent Exotic Species, Annual to Perennial Ratio, and a metric based on scores from a Floristic Quality Assessment Index (similar to the one conducted in this study, but with scores specific to Florida depressional herbaceous wetlands). In the isolated depressional forested FWCI, a variant of the Annual to Perennial Ratio was used, the percent Native Perennial Species (to account for anticipated conditions at urban wetlands). The sixth forested FWCI metric was the percent Wetland Status Species.

There was less similarity between the five macroinvertebrate depressional herbaceous FWCI metrics and the six isolated depressional forested macroinvertebrate FWCI metrics. Tolerant and Sensitive Indicator metrics were constructed separately for each wetland type and were included in both indices. Three Tolerant Indicator Genera

occurred on both lists (*Goeldichironomus*, *Micromenetus*, and *Physella*), and only one Sensitive Indicator Genera was shared (*Larisa*). The herbaceous FWCI included three additional metrics: %Predators, %Odonata, and %Orthocladinae. The forested FWCI included four different metrics: Florida Index, %Mollusca, %Noteridae, and %Scrapers.

Overall, the herbaceous and forested FWCI were similar, with many shared metrics. Some additional variability between selected metrics was expected as the isolated depression forested FWCI included two additional sources of variability (wetlands in the panhandle wetland region and urban land uses, which were not included in the sample wetlands for the herbaceous FWCI). Perhaps the strong similarity of metrics suggests that a universal assessment index could be constructed regardless of wetland type. However, it would likely be necessary to maintain independent indicator species lists specific and metric scoring criteria for different wetland types.

### **Landscape Development Intensity Index**

The Landscape Development Intensity index (LDI) is a useful tool in approximating ecological integrity. Its primary power is the reproducible, objective, and quantitative methods employed to obtain a score based on the use on non-renewable energy in the surrounding landscape, which in turn corresponds strongly and significantly with the FWCI for diatoms, macrophytes, and macroinvertebrates. By correlating the community composition based FWCI and the landscape based LDI, we are better able to understand driving influences to ecological integrity, while also identifying areas where landscape scale changes in surrounding land use may not be manifest as apparent changes in species assemblage level community composition.

Some urban wetlands received somewhat contradictory LDI and FWCI scores, which were concurrent to expected trends. However, many of these urban wetlands had been exposed to an assortment of additional land uses in the recent past. For example, in areas of Florida just inland of the extensive coastal development and that were once highly agrarian, development has spread, and so wetlands that would have been considered agricultural within the past decade are now categorized as urban. Many of these previous agricultural fields and pasture lands have only recently been transformed for residential and commercial uses, suggesting that there may be limitations to the predictive power of LDI for wetlands situated in rapidly changing landscapes. Further modifiers for “time-since-disturbance” factors, or perhaps weighted averages based on previous land use may be appropriate means of modifying LDI, though further work is needed. Species assemblage specific response time appears extremely important, particularly for longer-lived species, where reproduction may be inhibited by current conditions, and so ecosystem organization would currently reflect a certain higher level of ecological integrity despite impeded possibilities for system perpetuity.

There are some additional deviations from expected LDI and FWCI correlations. For example, agricultural wetlands generally receive lower LDI scores than urban wetlands, however in the case of wetlands with active cattle grazing within the wetland ecosystem FWCI scores can be lower for grazed wetlands than for urban wetlands. However, as a whole, the LDI is a surrogate for the FWCI, taking into account landscape scale impacts that would alter the community composition of a target species assemblage. While the sites grazed by cattle reflected low macrophyte FWCI scores, sample wetlands

that were actively grazed by cattle were not clustered into separate groups based on the diatom or macroinvertebrate assemblages.

### **Agricultural and Urban Wetlands**

Urban wetlands appear to exhibit a different vector of change than do agricultural wetlands; however the FWCI did not significantly differentiate between agricultural and urban wetlands. The LDI also did not specifically differentiate between these land use categories, as LDI coefficients for agricultural land uses range from 2.6 (unimproved pasture) to 6.6 (high intensity agriculture). Urban LDI coefficients overlap that range with variants of Open Space/Recreational land uses ranging from 2.1 (low intensity) to 4.8 (middle intensity) to 6.9 (high intensity). Similarly, other measures of anthropogenic influence like the Wetland Rapid Assessment Procedure and the Minnesota disturbance index did not clearly differentiate between agricultural and urban land uses.

The main land use specific conclusion we can draw from the FWCI is that both agricultural and urban wetlands have lowered biological integrity. However, this statement is not meant to imply that these wetlands lack value, as they provide important services and do considerable work in the environment. Wetlands embedded in a developed landscape matrix provide an abundance of potential services. For example, they may store and purify stormwater, process nutrients and toxins (perhaps acting as a sink and protecting hydrologically connected systems), provide habitat for local wildlife and perhaps migratory species, produce oxygen, filter the air, provide noise abatement, and act as refugia for urban ecologists. Specifically in the case of urban wetlands, there is a debate as to the value of small remnant wetlands embedded within highly developed landscape matrices. While wetlands do exist in highly urbanized areas, they do not appear to closely resemble wetlands in undeveloped landscapes. This leaves us to question just how much of the inherent function of the wetland has been lost.

Under current Florida law, mitigation ratios for urban wetlands will be small, and some people may dispute the idea of keeping urban wetlands of reduced biological integrity on expensive real estate parcels. Perhaps mitigating off-site into near-by areas with low development intensity would improve the chances of creating or restoring a wetland with the possibility of successfully meeting mitigation criteria. However, off-site mitigation undervalues the services provided by urban wetlands. Urban wetlands clearly provide some function, and perchance they are doing more work processing nutrients, storing urban stormwater run-off, and storing toxins, than wetlands in undeveloped landscapes. The continued existence of urban wetlands is crucial (for the maintenance of biotic diversity, buffering pollution and contamination to protect nearby environments, increasing oxygen production in an urban center, etc.). While the FWCI scores for urban wetlands reflect lowered biological integrity, perhaps maintaining 30-70% on average of the biological integrity of reference wetland is more important than having no wetland and therefore no services or free work. Wetlands with the lowest biological integrity could have scored a 0 for the FWCI, and yet only three heavily grazed wetlands did and only based on one of three the species assemblages assessed. There is no doubt that the intensity of human land use across the landscape plays a role in the loss of biological integrity of wetlands, however we should reconsider our willingness to

remove all of the biological integrity of a wetland by otherwise erasing its existence by filling.

### **Richness, Evenness, and Diversity**

Measures of richness, evenness, and diversity of the diatom, macrophyte, and macroinvertebrate assemblage were not sensitive to difference in land use or development intensity in the surrounding landscape. For both the diatom and macroinvertebrate assemblages neither *a priori* land use classification nor categories of landscape development intensity showed significant differences in richness, evenness, or diversity calculations. Differences in macrophyte evenness and diversity between reference and agricultural wetlands may be attributable to both direct (for example grazing by domestic cattle) or more indirect (increased nutrients from fertilizer carried in run-off from surrounding agricultural fields) activities in the surrounding landscape. However, macrophyte evenness and diversity were higher for wetlands surrounded by more developed land uses, contrasting earlier findings on decreases in plant diversity from grazing pressures (Blanch and Brock 1994; Grace and Jutila 1999) and nutrient enrichment (Bedford et al. 1999). Mitsch and Gosselink (1993) report that freshwater forested wetlands have low species diversity, so perhaps macrophyte species that enter wetlands in developed landscape are merely taking advantage of available habitat and are in fact increasing the overall species diversity.

The increased incidence of exotic species have long been associated with disturbed ecosystems (Cronk and Fennessy 2001; Galatowitsch 1999b), suggesting more specifically that as anthropogenic development intensity increases, the incidence of exotic species may escalate. An increase in the frequency of exotic species has been attributed to drainage and hydrologic alterations (Hobbs and Heunneke 1992; David 1999; Galatowitsch et al. 1999b), increased human development (Cronk and Fennessy 2001), and ecosystem scale alterations such as clear-cut harvests (Devine 1998). Within the study wetlands, the percent of exotic macrophyte species increased with increasing development intensity in the surrounding landscape. The influx of exotic species added to, rather than diminished, the species evenness and diversity within the isolated forested wetlands sampled.

### **Limitations and Further Research**

Several limitations to this study should be noted, including sampling methods and drought conditions. One water sample was collected to represent the water environment of the entire sample wetland, and water samples were taken at a range of times throughout the day. While water samples were always taken when a crew initially arrived at a sample wetland, the time of day the crew arrived fluctuated. Additionally, while an attempt was made to avoid taking water samples immediately following extreme rain event, there is the possibility that the sample was taken during a period of time without rain when the wetland was drying down. Therefore, there was no consistency as to recent weather conditions when water samples were taken. There were also strict requirements of preservation, temperature control, and shipping protocols associated with water samples. When these requirements were breached the sample had to be discarded.

Similarly, one composite soil sample was taken for each sample wetland, and bulk density was not measured, which complicates the use of soil nutrient data. As well, generally wetlands were visited only once, with a complete sample effort lasting just one day. This provided a mere snapshot of wetland condition. Revisits were conducted at some wetlands to collect water, soil, algae, or macroinvertebrates in the case of dry conditions on the initial visit or a discarded sample (generally for quality control reasons). Visiting these wetlands only once or twice did not allow insight into seasonal or yearly variations in the assemblages. As an additional confounding factor, Florida experienced drought conditions in 2001, and the macrophyte assemblage at many wetlands was sampled without standing water, which allowed many flood intolerant species to encroach into the sample wetlands.

While the FWCI has satisfactorily distinguished between wetlands embedded in an array of land uses with varying development intensities, much needs to be done to insure accuracy and usability. First, seasonal and yearly variation should be identified for the study wetlands. Wetlands are pulsing systems, and as such wetland organisms must adapt to wide fluctuations in hydrology, temperature, salinity, and dissolved oxygen (Evans et al. 1999; Leslie et al. 1999; Sharitz and Batzer 1999). A new set of wetlands should be sampled and scored based on the FWCI to test the reliability of these indices. The FWCI was limited to 19 metrics due to the redundant nature of many of the candidate metrics, as well as the high variability of community composition within the dataset. A larger sample size could improve the significance of the FWCI based on ecoregions for metrics such as indicator species analysis. Regionalization may be an important step in refining the FWCI, as this study was somewhat limited to a statewide approach due to small sample sizes within each ecoregion.

### **Conclusions**

The use of three separate species assemblages for a biological assessment of isolated depression forested wetland provided a useful tool for detecting changes in biological integrity associated with changes in the driving energies of a wetland measured through landscape development intensity. While richness, evenness, and diversity measures were not particularly sensitive to changes in landscape development intensity, biological indicators along with physical and chemical parameters were useful in defining biological integrity. In the future multiple assemblage specific multi-metric FWCI could be constructed for all freshwater wetlands throughout the state of Florida, with specific Indicator Species and metric scoring criteria based on Florida wetland regions. While the FWCI suggests low biological integrity of both agricultural and urban wetlands, these wetlands provide services and do work in the environment. Therefore, the quantitative score of biological integrity established through the FWCI should not be used as a surrogate for wetland value, but an objective, quantitative means of comparing changes in community composition along gradients of development intensity.

## APPENDIX A STANDARD OPERATING PROCEDURES

Standard Operating Procedures (SOPs) have been included for sampling methods employed for the entire project, which included more data collection than that included in this report. These additional methods were included in Appendix A to provide readers with a complete picture of field methodology, and to understand the order of events during field sampling. Data omitted from this report include tree basal area along transects, fisheye canopy photography, algae analysis of epiphyton, metaphyton, and phytoplankton, and dry benthic algae analysis. Canopy photo analysis was explored in a non-thesis project by Lisa Spurrier at the University of Florida in 2000. Laboratory identification of additional algae samples was not completed at the time of publication.

While vegetation zone descriptions are provided for soil sampling, these procedures were initially created for use in freshwater isolated depressional herbaceous wetlands (Lane et al. 2003). Zonation for soil samples was only employed at three of the 118 forested wetlands included in this study. These three wetlands were characterized by open centers with no canopy trees occurred in the deep pooled center area of the wetland. In these instances soil samples were taken in both the outer forested zone and the inner marsh zone and analyzed separately. As suggested in the soil SOPs, the soil data values were weighted for each vegetation zone based on the approximate area occupied by each vegetation zone.

### ORDER OF FIELD EVENTS FOR ISOLATED DEPRESSIONAL FORESTED WETLANDS:

1. Water quality is ALWAYS taken first. Two field crew members take the water samples. One records data while the other takes the sample. Data are recorded on BOTH the FDEP lab submittal form and on the wetland characterization form.
2. While two crewmembers are collecting water, the other(s) unloads the vehicle and prepare(s) field equipment.
3. After the water samples are obtained (follow SOPs for water quality), complete the Site/Habitat Characterization Data Sheet & WRAP assessment.
4. When completed, establish vegetation transects. This includes delineating the upland/wetland boundary and running all four transects (follow SOPs for vegetation).
5. The remaining field crew should:
  - Collect algae samples (follow SOPs for algae)
  - Collect macroinvertebrates (follow SOPs for macroinvertebrates)
  - Collect soil samples (follow SOP for soil)
6. Take site photographs and GPS point

**CHECKLIST OF MATERIALS/FIELD EQUIPMENT:**

- Miscellaneous
  - SOPs
  - Large cooler with frozen ice bottles for soils and vegetation
  - Digital camera
  - Waders (knee, hip, etc.)
  - Garmin III - GPS unit
  - Aerial photo & FLUCCS codes of site
  - Clipboard, pencils, sharpies, masking tape, plastic bags, index cards, hand lens
  - Data Sheets:
    - Water Quality: FDEP Central Lab submittal form
    - Site Characterization & WRAP sheets – 1 per person per site
    - Bioassessment macrophyte data sheets – a minimum of 8 per site
  
- Water Quality
  - Small cooler with ice (can purchase for shipping)
  - YSI meter (DO/temp)
  - 2 500-mL water sample bottles – turbidity/color/conductivity/pH & NH<sub>3</sub>/NO<sub>x</sub>/TKN/TP (or other sizes as supplied by FDEP)
  - Pipette for H<sub>2</sub>SO<sub>4</sub>
  - Bottle of 1:1 H<sub>2</sub>SO<sub>4</sub>
  - FedEx or DHL air bills
  
- Transects & Macrophytes
  - 2 100m transect tapes
  - 1 m PVC
  - 2-3 compasses
  - Plant press, newspaper, and cardboard
  - Field ID manuals
  - Prism for basal area
  
- Macroinvertebrates
  - US Std 30 mesh sweep net
  - Large 1 gallon jar for sample
  - Bottle of formalin for preserving sample
  
- Algae
  - Collecting jars – 3 100-mL pea cups & 1 1-L sample bottle
  - Collection jar with bottom missing for benthic algae
  - Large pipette – aka turkey baste
  - Knife
  - Falcon phytoplankton sampler – aka 50-mL centrifuge tube
  - Bottle brush & scraper
  - M3 preservative
  - Pipette for M3 preservative
  - 1-L deionized water for dry sites

- Canopy Photos
  - Digital camera
  - Spare batteries
  - Film disks
  - Tripod
  - Compass
  - Height pole
- Soils
  - 3-inch diameter PVC pipe
  - Knife
  - Piece of wood
  - Dampened hammer
  - Buckets
  - Stainless steel spoon
  - De-ionized water



**SOPs for Isolated Depressional Forested Wetlands: WATER QUALITY**

1. The YSI meter must be on and calibrated for 15 minutes before using.
2. *Always take this collection first!!*
3. Water samples can ONLY be collected Monday, Tuesday, Wednesday, or Thursday. Samples are sent to FDEP Central Lab overnight.
4. One 500 mL bottle for turbidity/color/conductivity/pH and one 500 mL bottle for NH<sub>3</sub>/NO<sub>x</sub>/TKN/TP are collected per site. These have labels provided by FDEP.
5. Only take water samples if the water depth is greater than 10 cm.
6. Carefully enter the water without stirring up organic material and silt.
7. Remove cap from each 500 mL bottle without touching the lip or interior surfaces.
8. Rinse the bottle 3 times in wetland water, dumping the water away from collection area.
9. Place the sample bottle upside down in the standing wetland water.
10. Carefully tilt back end into the water and press on bottom of bottle to allow water to slowly flow inside.
11. Be deliberate, making sure that no suspended organic matter enters the sample bottle. If organic materials do enter the sample, dump the sample and begin again.
12. When all exiting air bubbles have stopped, carefully lift bottle out of water.
13. Repeat, so both 500 mL bottles are full.
14. After the water is collected, take dissolved oxygen and temperature readings using the YSI meter. Take measurements within the top 10 cm of the water column. Apply constant, gentle motion to the dissolved oxygen probe, as the meter is consuming oxygen during measurement. Measure water depth.
15. Preserve only the bottle for NH<sub>3</sub>/NO<sub>x</sub>/TKN/TP, using 2 mL of 1:1 H<sub>2</sub>SO<sub>4</sub> per 500 mL sample.
16. Place both sample bottles on ice in a six-pack cooler. For transport reasons, the ice should be in a bag atop the samples.
17. Fill out the FDEP Central Lab Sample Submittal Form. Place in a zip-lock bag in cooler.
18. If 2 sites are sampled in 1 day, place all 4 sample bottles in the cooler along with the forms (one form is sufficient if properly filled out).
19. Tape the cooler shut and make sure the air bill is filled out properly. Call 1-800-GO-FEDEX to find a nearby office.
20. If you cannot get to FedEx in time, dump the samples taken. Repeat procedure another day.

**SOPs for Isolated Depressional Forested Wetlands: VEGETATION**

1. Using a compass, locate the 4 cardinal point directions (north, south, east, and west). The 4 transects will begin at each cardinal point running from the edge of the wetland into the interior/middle of the wetland. These 4 transects will intersect in the middle and divide the wetland into 4 approximately equal sections.
2. At the beginning of each transect, delineate the edge of the wetland using a combination of wetland plants and hydric soils. Be conservative on the side of the wetland.
3. Establish the transect using the meter tapes. Start with 0 meters at the wetland edge, and increase distance towards the wetland interior.
4. Use a separate field data sheet for each cardinal direction. If the number of species located on a transect exceeds the number of columns on the data sheet, start a new data sheet.
5. Creating quadrats that are 0.5 m on either side of the transect (1 m wide) and 5 m long, record all species present within these elongated quadrats.
6. Plant species names are recorded on the data sheets using the full genus and species names. Each unknown species is given a unique ID code using the transect location (ex. N-1).
7. Voucher specimens for all unknown species are collected, being sure to get plant inflorescence and roots, tagged with properly labeled masking tape, and put into a labeled collection bag. Note the color of the inflorescence on the label, as the flowers often do not preserve well. Index cards can be used to protect especially sensitive parts.
8. All collected plants are identified in the field on the day of sampling and placed in a plant press for further clarification and identification. Plant nomenclature follows FDEP's *Florida Wetland Plant Identification Manual* (Tobe et al. 1998).
9. At each 10 m along each transect, starting at 10 m, 20 m, 30 m, etc., tree basal area will be recorded. Use the data sheet for basal area, and record basal area per species using variable area plots and a 10 factor prism. Hold the prism at eye level, with a bent elbow and count the number of trees per species that fall within the variable area plot. The prism shall be centered over the sampling point at all times, with the field person rotating around the prism so that the entire circular area (360°) around the point of sampling is included.
10. As the sun lowers on the horizon, take canopy photos at 1 point along each transect. Placement of tripod will be 10 paces out from the center of the wetland along each transect. In those instances when the cypress dome is a "hole in the doughnut" and there are no cypress trees in the center of the dome, tripod placement will be 10 paces along the transect out from the ring of trees. Follow directions according to *A Manual for the Analysis of Hemispherical Photography* (Rich 1989).
11. At each photograph spot, insert a wooden stake so that photo sites can be revisited in the future.
12. Center the tripod over the stake with the top of the lens cap at the height of the provided height pole [which is at breast height 1.3 m]. The top of the camera should face south, so that the photographer's back is to the north.

13. Level the tripod, so that the bubble on top of the lens cap is centered within the circle.
14. Turn the camera on to automatic. Set the camera to XGA Fine, using the dial at the front right and the button on the back of the camera.
15. Zoom out the camera all of the way so that the back display shows the canopy as a circle, surrounded by a dark/black border.
16. Record the photo number for the position (ex. north, south, east, or west).
17. Complete the canopy photo data sheet, noting time of day, cloud condition, surrounding vegetation, etc.

## SOPs for Isolated Depressional Forested Wetlands: ALGAE

### AT WET SITES:

- Separate samples by substrates of the site you are working on (i.e. epiphyton, benthic algae, metaphyton, and phytoplankton).
- For each substrate, collect 10 aliquots, and keep each substrate type separate in their own collection jars. At the end of the collection there should be between 100-120 mL of wetland algae-water mix in the cups, except for phytoplankton which should have approximately 1000 mL.
- Rinse all sample equipment in wetland water prior to sampling.

- EPIPHYTON – divide appropriately among herbaceous and woody debris based of the proportion of the area of wetland of each:

1. For herbaceous vegetation:

Cut plant stems under water and place in zip-lock bag with wetland water; shake and knead vigorously in zip-loc bag; use turkey baste to extract 10 mL of algal suspension and place in labeled pea cup; distribute the aliquots appropriately throughout the different vegetation/habitat zones.

2. For woody debris (roots, snags):

Using a brush, brush the wood for algae. Place brush in bag with water and shake algae off of the brush. Pipette the algae into the collection jar;  
-or-

Use bottomless pea cup to isolate a spot on the debris. Use turkey baste to stir algae from surface of debris; extract 10 mL of algal suspension and place in pea cup; since woody and herbaceous are within the same 10 aliquots, they must be divided appropriately between the two.

- BENTHIC ALGAE:

1. Use bottomless pea cup to isolate a spot on the sediment;
2. Use turkey baste to gently stir algae from the surface of the sediment;
3. Extract 10 mL of algal suspension and place in pea cup.

- METAPHYTON:

1. Collect approximately 100 mL of wetland water in a pea cup;
2. Using your fingers, collect a thumbnail size portion of the algal mat;
3. Obtain aliquots from 10 different areas of the wetland.

### PHYTOPLANKTON (1 L is collected):

1. In total use the 50 mL centrifuge tube (x2) to collect ten 100 mL aliquots;
2. Divide aliquots proportionately between the major vegetation zones;
3. Rinse tube with wetland water. With the cap on the tube, lower into the water column and then remove the cap to allow the tube to fill with water;
4. Cap the tube under the water and then bring tube out of water;
5. Carefully pour contents into the dark algae collection bottle;
6. Since the tube is only 50 mL, you will need to do this twice in each of the 10 aliquots.

## ALL SAMPLES:

- Preserve the samples.
  - Add 5 mL of  $M^3$  per 100 mL of algal suspension in pea cups.
  - Add 20 mL of  $M^3$  per 1 L of algal suspension in column algae bottle.
- Properly label collection jars, identifying site, date, collector, and sample type.
- Carefully clean equipment with deionized water to avoid cross-contamination at future sites.
- When at the Center for Wetlands, clean all equipment with Clorox/water solution.
- Return full collection jars to room 8 at the Center for Wetlands to await laboratory analysis.

## AT DRY SITES ONLY BENTHIC ALGAE IS TO BE COLLECTED:

1. Use bottomless pea cup to isolate a spot on the sediment.
2. Extract the upper 0.5 cm of soil into an additional pea cup, this depth is marked on the collection pea cup.
3. Add 100 mL deionized water and stir well with turkey baste.
4. Extract 10 mL of algal suspension and place in sample pea cup.
5. Repeat, so that you have collected 10 aliquots representative of the vegetation/habitat zones in the wetland.
6. Preserve the sample with 5 mL of  $M^3$  per 100 mL of algal suspension.
7. Properly label collection jar, identifying site, date, collector, and sample type.
8. Carefully clean equipment with DI water to avoid cross-contamination at future sites.  
When at the Center for Wetlands, clean all equipment with Clorox/water solution.
9. Return full collection jars to room 8 at the Center for Wetlands.

DIRECTIONS FOR  $M^3$  FIXATIVE PREPARATION:Materials

10 g Iodine  
 5 g Potassium iodide (KI)  
 50 mL Glacial acetic acid  
 250 mL Formalin (37% W/W formaldehyde)  
 1 L Deionized water

Methods

1. Dissolve 10 g iodine in a small quantity of deionized water to aid in solution of iodine.
  2. Dissolve 5 g potassium iodide, 10 g dissolved iodine (from step 1), 50 mL glacial acetic acid and 250 mL formalin (37% W/W formaldehyde) in 1 L deionized water.
  3. Store in the dark.
- (p.10-8, Standard Methods for the Examination of Water and Wastewater, 17<sup>th</sup> edition)

## **SOPs for Isolated Depressional Forested Wetlands: MACROINVERTEBRATES**

1. There are to be 20 sweeps (evenly divided into the vegetation/habitat zones) to send to FDEP unpicked for identification.
2. Always do your sweeps in undisturbed areas where you have not walked through yet.
3. A single sweep is one net width and two net lengths to equal 0.5 m<sup>2</sup>.
4. Using a U.S. Standard 30 mesh net, sweep from the bottom of the substrate up the plant stalks. Use your hands to strip the plant of all material into the net. If you are in a forested site, use a brush to clean any snags and roots of material.
5. Vigorously sample the area repeatedly (3 times) to ensure good coverage.
6. Dip net into water repeatedly, without letting the sample out, to try and sift the muck and silt through the net.
7. Do not sample in the muck!
8. Place the contents of each sweep into the 3.8 L jar. When all 20 samples are complete, preserve the sample by adding Formalin at a rate of 10% of the sample volume. Seal the jar. Shake to ensure thorough mixing.
9. Place masking tape over the lid to prevent leakage during travel & shipment. Properly label the jar with the site name, date, and collector.
10. Thoroughly clean all equipment off with water.
11. Return samples to room 8 at the Center for Wetlands for later shipment to the FDEP.

### **DIRECTIONS FOR BUFFERED FORMALIN PREPARATION:**

#### Materials

Sodium bicarbonate (sodium borate may also be used)

Formalin (37% W/W formaldehyde)

pH meter

#### Methods

Note that formalin is the common name for 37% W/W formaldehyde. “Formalin” and “Buffered Formalin” are 2 separate things in this recipe.

1. Calibrate the pH meter (directions follow). The pH electrode and temperature probe should rest in a beaker of deionized water between measurements. Rinse the electrode and probe off with a spray bottle of deionized water before submerging in other solutions.
2. Select a container for preparing the buffered formalin. Usually this is simply the plastic container that the formalin was shipped in.
3. Fill the container with formalin to just below (1-2 cm) where the top of the desired buffered formalin solution level.
4. Scoop a small amount of sodium bicarbonate into the container, close, and shake vigorously (at least 1 minute to ensure proper mixing). All of the sodium bicarbonate may not dissolve into the formalin, as this is a supersaturated solution.
5. Measure the pH of the resulting solution.
6. Repeat steps 4 and 5 until the pH is at least 7.5 but not higher than 8.0.
7. If desired, transfer the buffered formalin to smaller containers for field use.
8. Make sure that all containers are clearly labeled “Buffered Formalin pH 7.5-8.0.”

9. When disposing of used buffered formalin, deposit it in an appropriately labeled waste container. The waste container should have a yellow Hazardous Materials sticker, call the Environmental Health and Safety department at 392-1591.

Original recipe from the Florida Department of Environmental Protection Biology Section, Standard Operating Procedure (SOP) #IZ-10, "Preparation of Buffered Formalin." Expanded upon by Melissa Yontek on May 2, 2001.

#### CALIBRATION OF pH METER (Hanna Instruments HI 9025C)

##### Materials

pH meter  
pH 4.00 buffer solution  
pH 7.00 buffer solution  
3 beakers  
deionized water bottle

##### Methods

\*When not being used, rest the electrode and probe in a beaker of deionized water. Rinse the electrode and probe off with a spray bottle of deionized water before submerging in other solutions.

1. Pour small quantities of the pH 4.00 and pH 7.00 buffer solutions into each of 2 clean beakers.
2. Immerse the pH electrode and temperature probe into the pH 4.0 buffer solution, stir briefly. The electrode and probe should be close together, and they should be submerged approximately 4 cm (1½ inch) into the solution.
3. Press the CAL key. The "CAL" and buffer indicators will be displayed. The secondary LCD display should read "4.01." If not, adjust it using the "□□c" key.
4. After the pH reading becomes stable, the "READY" and "CON" indicators will blink. Once this happens, press the CON key to confirm the calibration.
5. After rinsing with deionized water, immerse the pH electrode and temperature probe into the pH 7.00 solution, and stir briefly.
6. Select the second buffer value ("7.01") on the secondary display using the "□□c" key.
7. After the pH reading becomes stable, the "READY" and "CON" indicators will blink. Once this happens, press the CON key to confirm the calibration.
8. Press "CAL" key to end calibration process and begin measuring.
9. When finished using the pH meter, pat the electrode and probe dry with a KimWipe. Place the pH electrode in the yellow/orange cap with a small amount of deionized water.

The pH meter should be recalibrates:

- whenever the pH electrode or temperature probe is replaced
  - at least once a month
  - after testing aggressive chemicals
  - if greatest accuracy is required
- whenever the batteries have been replaced.

**SOPs for Isolated Depressional Forested Wetlands: SOIL**

1. The wetland will be visually divided into vegetation zones. Cores will be taken within each zone and combined, so that each vegetation zone has one cumulative soil sample. The number of cores taken per zone is generally 4, 1 along each transect. In some cases there will be fewer than 4 cores per vegetation zone as the zones may not all fall along the established transects.
2. To sample soils:
  - a. Clean off the detritus from the site that will be sampled. This means removal of plant material that appears less than 6 months old, or the recognizable fallen plant material.
  - b. Place the 7.9 cm diameter PVC pipe on the soil surface at the sample location.
  - c. Using the knife carefully cut a circular shape around the sampling pipe, so that the pipe will easily slide through the soil and roots. This reduces soil compaction.
  - d. Using the dampened hammer, gently pound the sampling pipe into the soil. Hammer the core 10 cm into the soil. There is a black line indicating this depth on the soil core.
  - e. With the core in place, dig down to the bottom of the core and extract the core into a bucket that has been marked with the name of the vegetation zone.
3. Repeat along each transect for each vegetation zone, making sure that the 10 cm soil sample is placed into the properly marked bucket (to assure vegetation zones are not mixed).
4. Thoroughly mix each bucket of soil with the large stainless steel spoon. Clean and rinse the spoon with de-ionized water between buckets.
5. Gather several quart-sized freezer zip-loc bags and a permanent marker. Label each small zip-loc bag with the site name, vegetation zone, number of cores, the bag number (i.e. 4 of 7), date, and name of collector.
6. Using a clean stainless steel spoon, take enough randomly selected spoonfuls of soil to fill the labeled quart size Zip-loc bag.
7. Seal the Zip-loc bag and place in a larger zip-loc freezer bag labeled with the site name, date, and number of smaller bags contained. Place in cooler and ice down.
8. Since the resultant nutrient and % organic matter will be weighted based on the % area of each vegetation zone, it is imperative that the vegetation zones marked on the soil bags are also marked on the vegetation zone map that is part of the wetland characterization sheet. Do not forget to include the approximate % of each area in the wetland.
9. Rinse field equipment with deionized water.
10. Return the samples to the Center for Wetlands, and store in the refrigerator in the back lab pending laboratory analysis.



APPENDIX B  
COEFFICIENT OF CONSERVATISM SCORES

Table B-1. Coefficient of Conservatism (CC) scores for 561 macrophytes identified in isolated depressional freshwater forested wetlands in Florida.

Species	CC	Species	CC
<i>Acalypha gracilens</i>	3.3	<i>Begonia cucullata</i>	1.5
<i>Acer rubrum</i>	5.2	<i>Berchemia scandens</i>	5.1
<i>Acrostichum danaeifolium</i>	6.2	<i>Betula nigra</i>	4.8
<i>Agalinis filifolia</i>	6.7	<i>Bidens alba</i>	1.0
<i>Agrostis hyemalis</i>	5.4	<i>Bidens discoidea</i>	4.8
<i>Albizia julibrissin</i>	0.0	<i>Bidens mitis</i>	3.8
<i>Aloe vera</i>	0.0	<i>Bignonia capreolata</i>	4.8
<i>Alternanthera philoxeroides</i>	0.0	<i>Bischofia javanica</i>	0.0
<i>Alternanthera sessilis</i>	0.7	<i>Blechnum serrulatum</i>	5.5
<i>Amaranthus australis</i>	2.6	<i>Blechnum pyramidatum</i>	0.0
<i>Amaranthus blitum</i>	0.0	<i>Boehmeria cylindrica</i>	4.5
<i>Amaranthus spinosus</i>	0.0	<i>Boltonia diffusa</i>	3.8
<i>Ambrosia artemisiifolia</i>	0.7	<i>Bromus catharticus</i>	0.0
<i>Ampelopsis arborea</i>	3.3	<i>Bulbostylis stenophylla</i>	4.4
<i>Amphicarpum muhlenbergianum</i>	5.0	<i>Callicarpa americana</i>	2.4
<i>Andropogon glomeratus</i>	3.1	<i>Callisia repens</i>	0.0
<i>Andropogon virginicus</i>	2.6	<i>Campsis radicans</i>	3.3
<i>Annona glabra</i>	6.8	<i>Canna flaccida</i>	5.7
<i>Anthraenantia villosa</i>	7.1	<i>Caperonia castaneifolia</i>	2.4
<i>Apios americana</i>	3.1	<i>Carex debilis</i>	6.5
<i>Ardisia crenata</i>	1.0	<i>Carex frankii</i>	6.0
<i>Ardisia escallonioides</i>	0.0	<i>Carex gigantea</i>	6.4
<i>Aristida beyrichiana</i>	9.8	<i>Carex glaucescens</i>	7.1
<i>Aristida patula</i>	6.3	<i>Carex longii</i>	3.6
<i>Aristida purpurascens</i>	6.0	<i>Carex striata</i>	5.7
<i>Aristida spiciformis</i>	6.4	<i>Carex verrucosa</i>	7.1
<i>Asplenium platyneuron</i>	4.8	<i>Carphephorus odoratissimus</i>	7.6
<i>Aster carolinianus</i>	6.9	<i>Carphephorus paniculatus</i>	6.0
<i>Aster dumosus</i>	3.6	<i>Celtis laevigata</i>	5.0
<i>Aster elliotii</i>	4.2	<i>Centella asiatica</i>	1.9
<i>Aster pilosus</i>	5.4	<i>Cephalanthus occidentalis</i>	6.0
<i>Aster subulatus</i>	4.5	<i>Cercis canadensis</i>	4.0
<i>Aster tenuifolius</i>	7.1	<i>Chamaecrista fasciculata</i>	0.0
<i>Axonopus fissifolius</i>	2.8	<i>Chamaecrista nictitans</i>	2.9
<i>Axonopus furcatus</i>	2.4	<i>Chamaesyce hypericifolia</i>	0.0
<i>Azolla caroliniana</i>	2.6	<i>Chaptalia tomentosa</i>	7.9
<i>Baccharis halimifolia</i>	2.1	<i>Chenopodium album</i>	0.0
<i>Bacopa caroliniana</i>	6.0	<i>Chiococca alba</i>	5.6
<i>Bacopa monnieri</i>	4.3	<i>Chrysobalanus icaco</i>	6.3

Table B-1. Continued.

Species	CC	Species	CC
<i>Cicuta maculata</i>	5.0	<i>Diodia teres</i>	1.9
<i>Cinnamomum camphora</i>	0.2	<i>Diodia virginiana</i>	2.4
<i>Cirsium nuttallii</i>	4.8	<i>Dioscorea bulbifera</i>	0.0
<i>Cissus trifoliata</i>	4.2	<i>Diospyros virginiana</i>	4.0
<i>Citrus Xaurantium</i>	0.0	<i>Drosera brevifolia</i>	6.7
<i>Cladium jamaicense</i>	5.5	<i>Drosera capillaris</i>	6.7
<i>Cleistes bifaria</i>	7.1	<i>Drymaria cordata</i>	1.2
<i>Clethra alnifolia</i>	5.2	<i>Duchesnea indica</i>	3.6
<i>Cliftonia monophylla</i>	5.0	<i>Dulichium arundinaceum</i>	6.8
<i>Coelorachis cylindrica</i>	5.6	<i>Echinochloa colona</i>	0.7
<i>Coelorachis rugosa</i>	6.3	<i>Echinochloa crusgalli</i>	0.0
<i>Coelorachis tuberculosa</i>	6.5	<i>Echinochloa walteri</i>	3.1
<i>Colocasia esculenta</i>	0.0	<i>Eclipta prostrata</i>	1.7
<i>Commelina diffusa</i>	1.7	<i>Eleocharis baldwinii</i>	2.1
<i>Commelina erecta</i>	4.8	<i>Eleocharis flavescens</i>	3.6
<i>Commelina virginica</i>	4.8	<i>Eleocharis interstincta</i>	5.5
<i>Conoclinium coelestinum</i>	4.3	<i>Eleocharis microcarpa</i>	3.0
<i>Conyza canadensis</i>	0.3	<i>Eleocharis vivipara</i>	2.4
<i>Cornus foemina</i>	4.8	<i>Elephantopus nudatus</i>	4.0
<i>Crataegus viridis</i>	8.6	<i>Eleusine indica</i>	0.0
<i>Crinum americanum</i>	7.6	<i>Elymus virginicus</i>	4.0
<i>Ctenium aromaticum</i>	10.0	<i>Eragrostis atrovirens</i>	1.8
<i>Cuphea carthagenensis</i>	1.4	<i>Erechtites hieracifolia</i>	2.1
<i>Cyclospermum leptophyllum</i>	1.2	<i>Erianthus giganteus</i>	6.0
<i>Cynanchum scoparium</i>	4.8	<i>Erigeron quercifolius</i>	2.9
<i>Cynodon dactylon</i>	0.0	<i>Erigeron strigosus</i>	2.4
<i>Cyperus croceus</i>	1.8	<i>Erigeron vernus</i>	4.3
<i>Cyperus distinctus</i>	3.8	<i>Eriocaulon compressum</i>	6.7
<i>Cyperus erythrorhizos</i>	4.2	<i>Eriocaulon decangulare</i>	6.7
<i>Cyperus haspan</i>	2.6	<i>Eriocaulon ravenelii</i>	4.8
<i>Cyperus iria</i>	1.2	<i>Eryngium prostratum</i>	4.0
<i>Cyperus lanceolatus</i>	2.4	<i>Eugenia uniflora</i>	0.0
<i>Cyperus odoratus</i>	3.6	<i>Eupatorium capillifolium</i>	0.5
<i>Cyperus polystachyos</i>	2.4	<i>Eupatorium leptophyllum</i>	3.6
<i>Cyperus retrorsus</i>	1.7	<i>Eupatorium mohrii</i>	5.5
<i>Cyperus surinamensis</i>	1.9	<i>Eupatorium rotundifolium</i>	6.2
<i>Cyperus virens</i>	3.9	<i>Eupatorium serotinum</i>	4.8
<i>Cyrilla racemiflora</i>	4.5	<i>Eustachys glauca</i>	2.4
<i>Desmodium incanum</i>	0.0	<i>Eustachys petraea</i>	0.0
<i>Desmodium lineatum</i>	6.0	<i>Euthamia caroliniana</i>	2.6
<i>Desmodium paniculatum</i>	3.6	<i>Euthamia minor</i>	3.6
<i>Dichondra caroliniensis</i>	1.9	<i>Ficus aurea</i>	5.7
<i>Digitaria bicornis</i>	0.0	<i>Fimbristylis dichotoma</i>	4.0
<i>Digitaria ciliaris</i>	0.3	<i>Fraxinus caroliniana</i>	7.1
<i>Digitaria serotina</i>	1.8	<i>Fuirena scirpoidea</i>	3.8

Table B-1. Continued.

Species	CC	Species	CC
<i>Galactia elliotii</i>	3.8	<i>Ixora chinensis</i>	0.0
<i>Galactia volubilis</i>	3.6	<i>Jacquemontia tamnifolia</i>	0.0
<i>Galium hispidulum</i>	3.3	<i>Juncus coriaceus</i>	5.1
<i>Galium tinctorium</i>	3.1	<i>Juncus dichotomus</i>	2.9
<i>Galium uniflorum</i>	5.1	<i>Juncus effusus</i>	1.9
<i>Gaylussacia frondosa</i>	6.7	<i>Juncus marginatus</i>	2.4
<i>Gaylussacia mosieri</i>	7.4	<i>Juncus megacephalus</i>	3.3
<i>Gelsemium sempervirens</i>	4.0	<i>Juncus polycephalus</i>	3.3
<i>Gnaphalium falcatum</i>	1.9	<i>Juncus repens</i>	5.2
<i>Gnaphalium obtusifolium</i>	2.4	<i>Juncus tenuis</i>	2.4
<i>Gordonia lasianthus</i>	6.7	<i>Juniperus virginiana</i>	5.2
<i>Gratiola ramosa</i>	5.0	<i>Justicia angusta</i>	6.0
<i>Gratiola virginiana</i>	7.1	<i>Justicia ovata</i>	5.5
<i>Habenaria repens</i>	4.8	<i>Kummerowia striata</i>	0.0
<i>Hedychium coronarium</i>	0.0	<i>Kyllinga brevifolia</i>	0.3
<i>Hedyotis corymbosa</i>	2.0	<i>Kyllinga pumila</i>	3.3
<i>Hedyotis uniflora</i>	3.6	<i>Lachnanthes caroliniana</i>	3.1
<i>Hemarthria altissima</i>	0.0	<i>Lachnocaulon anceps</i>	5.5
<i>Hydrocotyle bonariensis</i>	3.3	<i>Lachnocaulon engleri</i>	4.8
<i>Hydrocotyle ranunculoides</i>	3.1	<i>Lachnocaulon minus</i>	6.0
<i>Hydrocotyle umbellata</i>	2.9	<i>Lactuca graminifolia</i>	2.7
<i>Hydrocotyle verticillata</i>	3.1	<i>Lantana camara</i>	0.0
<i>Hymenachne amplexicaulis</i>	0.0	<i>Leersia hexandra</i>	4.8
<i>Hypericum brachyphyllum</i>	6.8	<i>Lemna minor</i>	1.0
<i>Hypericum chapmanii</i>	7.1	<i>Lepidium virginicum</i>	0.2
<i>Hypericum cistifolium</i>	5.0	<i>Leptochloa uninervia</i>	3.0
<i>Hypericum fasciculatum</i>	5.7	<i>Leucothoe axillaris</i>	6.0
<i>Hypericum galioides</i>	6.0	<i>Leucothoe racemosa</i>	6.2
<i>Hypericum hypericoides</i>	4.0	<i>Ligustrum japonicum</i>	0.0
<i>Hypericum mutilum</i>	3.6	<i>Ligustrum lucidum</i>	0.0
<i>Hypericum myrtifolium</i>	5.5	<i>Ligustrum sinense</i>	0.0
<i>Hypoxis curtissii</i>	6.0	<i>Limnobiium spongia</i>	4.8
<i>Hyptis alata</i>	4.3	<i>Linaria canadensis</i>	0.3
<i>Hyptis mutabilis</i>	0.0	<i>Lindernia crustacea</i>	0.6
<i>Ilex cassine</i>	8.1	<i>Lindernia grandiflora</i>	3.6
<i>Ilex coriacea</i>	6.0	<i>Liquidambar styraciflua</i>	3.3
<i>Ilex glabra</i>	4.3	<i>Litsea aestivalis</i>	9.8
<i>Ilex myrtifolia</i>	8.3	<i>Lobelia floridana</i>	6.5
<i>Ilex opaca</i>	6.0	<i>Lolium perenne</i>	0.0
<i>Ilex vomitoria</i>	4.8	<i>Lonicera japonica</i>	0.0
<i>Ilex x attenuata</i>	7.1	<i>Lophiola aurea</i>	6.5
<i>Ipomoea indica</i>	0.6	<i>Ludwigia alata</i>	4.5
<i>Ipomoea sagittata</i>	5.4	<i>Ludwigia curtissii</i>	4.4
<i>Iris hexagona</i>	7.1	<i>Ludwigia hirtella</i>	6.0
<i>Itea virginica</i>	7.9	<i>Ludwigia linifolia</i>	4.5

Table B-1. Continued.

Species	CC	Species	CC
<i>Ludwigia maritima</i>	3.3	<i>Nymphaea odorata</i>	5.5
<i>Ludwigia microcarpa</i>	3.1	<i>Nymphoides aquatica</i>	5.7
<i>Ludwigia octovalvis</i>	2.4	<i>Nyssa aquatica</i>	3.6
<i>Ludwigia palustris</i>	4.0	<i>Nyssa biflora</i>	7.4
<i>Ludwigia peruviana</i>	1.2	<i>Oeceoclades maculata</i>	0.4
<i>Ludwigia repens</i>	2.9	<i>Oplismenus hirtellus</i>	3.3
<i>Ludwigia virgata</i>	3.9	<i>Osmunda cinnamomea</i>	5.5
<i>Luziola fluitans</i>	4.8	<i>Osmunda regalis</i>	6.9
<i>Lycopodiella alopecuroides</i>	6.7	<i>Oxalis corniculata</i>	1.2
<i>Lycopodiella prostrata</i>	7.1	<i>Oxalis debilis</i>	0.0
<i>Lycopus rubellus</i>	5.2	<i>Oxypolis filiformis</i>	6.7
<i>Lycopus virginicus</i>	5.2	<i>Paederia foetida</i>	0.0
<i>Lygodium japonicum</i>	0.0	<i>Panicum aciculare</i>	4.8
<i>Lygodium microphyllum</i>	0.0	<i>Panicum chamaelonche</i>	4.8
<i>Lyonia ligustrina</i>	6.9	<i>Panicum ciliatum</i>	4.5
<i>Lyonia lucida</i>	6.0	<i>Panicum commutatum</i>	4.5
<i>Lythrum alatum</i>	3.0	<i>Panicum dichotomum</i>	4.0
<i>Magnolia grandiflora</i>	3.6	<i>Panicum ensifolium</i>	5.0
<i>Magnolia virginiana</i>	8.1	<i>Panicum erectifolium</i>	5.7
<i>Malus angustifolia</i>	6.0	<i>Panicum hemitomom</i>	5.0
<i>Matelea floridana</i>	6.7	<i>Panicum repens</i>	0.0
<i>Mecardonia acuminata</i>	3.9	<i>Panicum rigidulum</i>	4.5
<i>Melaleuca quinquenervia</i>	0.0	<i>Panicum scabriusculum</i>	5.0
<i>Melia azedarach</i>	0.0	<i>Panicum sphaerocarpon</i>	5.1
<i>Melochia corchorifolia</i>	1.5	<i>Panicum spretum</i>	5.4
<i>Melothria pendula</i>	1.8	<i>Panicum tenerum</i>	5.0
<i>Micranthemum glomeratum</i>	3.6	<i>Panicum tenue</i>	4.2
<i>Micranthemum umbrosum</i>	4.3	<i>Panicum verrucosum</i>	4.3
<i>Micromeria brownei</i>	4.8	<i>Parietaria floridana</i>	1.8
<i>Mikania scandens</i>	2.4	<i>Parthenocissus quinquefolia</i>	3.0
<i>Mitchella repens</i>	6.7	<i>Paspalidium geminatum</i>	3.6
<i>Mitreola petiolata</i>	5.4	<i>Paspalum acuminatum</i>	2.0
<i>Mitreola sessilifolia</i>	5.4	<i>Paspalum conjugatum</i>	3.1
<i>Modiola caroliniana</i>	3.2	<i>Paspalum laeve</i>	3.8
<i>Momordica charantia</i>	0.0	<i>Paspalum monostachyum</i>	9.1
<i>Morrenia odorata</i>	0.0	<i>Paspalum notatum</i>	0.0
<i>Morus alba</i>	1.2	<i>Paspalum plicatulum</i>	2.4
<i>Morus rubra</i>	3.6	<i>Paspalum repens</i>	4.0
<i>Myrica cerifera</i>	3.1	<i>Paspalum setaceum</i>	2.1
<i>Myrica heterophylla</i>	7.9	<i>Paspalum urvillei</i>	1.2
<i>Myrica inodora</i>	9.0	<i>Passiflora incarnata</i>	3.0
<i>Nandina domestica</i>	0.0	<i>Passiflora suberosa</i>	3.0
<i>Nephrolepis biserrata</i>	5.2	<i>Peltandra virginica</i>	3.6
<i>Nephrolepis exaltata</i>	4.8	<i>Pentodon pentandrus</i>	6.0
<i>Nuphar luteum</i>	5.2	<i>Persea borbonia</i>	6.3

Table B-1. Continued.

Species	CC	Species	CC
<i>Persea palustris</i>	7.4	<i>Quercus phellos</i>	7.4
<i>Phalaris angusta</i>	0.0	<i>Quercus virginiana</i>	4.2
<i>Phanopyrum gymnocarpon</i>	6.0	<i>Rapanea punctata</i>	5.2
<i>Phlebodium aureum</i>	6.8	<i>Rhexia alifanus</i>	6.9
<i>Photinia pyrifolia</i>	5.7	<i>Rhexia lutea</i>	6.5
<i>Phyla nodiflora</i>	1.4	<i>Rhexia mariana</i>	3.8
<i>Phyllanthus tenellus</i>	0.0	<i>Rhexia nashii</i>	6.2
<i>Phyllanthus urinaria</i>	0.0	<i>Rhexia petiolata</i>	6.2
<i>Physalis angulata</i>	1.2	<i>Rhexia virginica</i>	5.0
<i>Phytolacca americana</i>	1.2	<i>Rhododendron viscosum</i>	7.6
<i>Pieris phillyreifolia</i>	9.5	<i>Rhodomyrtus tomentosa</i>	0.0
<i>Pinus clausa</i>	5.6	<i>Rhoeo discolor</i>	0.0
<i>Pinus elliotii</i>	4.0	<i>Rhus copallinum</i>	2.4
<i>Pinus palustris</i>	7.1	<i>Rhynchospora capitellata</i>	6.0
<i>Pinus serotina</i>	7.1	<i>Rhynchospora cephalantha</i>	4.3
<i>Pinus taeda</i>	3.3	<i>Rhynchospora chalarocephala</i>	4.8
<i>Plantago lanceolata</i>	1.2	<i>Rhynchospora chapmanii</i>	6.0
<i>Pluchea camphorata</i>	4.3	<i>Rhynchospora colorata</i>	5.5
<i>Pluchea carolinensis</i>	3.6	<i>Rhynchospora corniculata</i>	6.0
<i>Pluchea foetida</i>	3.8	<i>Rhynchospora decurrens</i>	6.3
<i>Pluchea longifolia</i>	2.8	<i>Rhynchospora fascicularis</i>	4.5
<i>Pluchea odorata</i>	3.8	<i>Rhynchospora filifolia</i>	6.0
<i>Pluchea rosea</i>	3.6	<i>Rhynchospora gracilentia</i>	6.0
<i>Polygala cymosa</i>	9.0	<i>Rhynchospora inundata</i>	6.0
<i>Polygala lutea</i>	3.6	<i>Rhynchospora latifolia</i>	6.9
<i>Polygonum hydropiperoides</i>	2.6	<i>Rhynchospora microcarpa</i>	4.5
<i>Polygonum punctatum</i>	2.6	<i>Rhynchospora microcephala</i>	4.8
<i>Polygonum sagittatum</i>	4.8	<i>Rhynchospora miliacea</i>	7.1
<i>Polypremum procumbens</i>	1.2	<i>Rhynchospora odorata</i>	6.7
<i>Pontederia cordata</i>	5.0	<i>Rhynchospora plumosa</i>	6.4
<i>Populus deltoides</i>	1.2	<i>Rhynchospora pusilla</i>	6.7
<i>Pouzolzia zeylanica</i>	0.4	<i>Rhynchospora tracyi</i>	8.3
<i>Proserpinaca palustris</i>	3.8	<i>Rhynchospora wrightiana</i>	7.1
<i>Proserpinaca pectinata</i>	3.8	<i>Richardia brasiliensis</i>	0.0
<i>Prunus caroliniana</i>	3.0	<i>Rivina humilis</i>	1.2
<i>Prunus serotina</i>	3.6	<i>Rosa carolina</i>	7.1
<i>Psilotum nudum</i>	3.6	<i>Rosa palustris</i>	6.9
<i>Psychotria nervosa</i>	3.6	<i>Rubus argutus</i>	2.1
<i>Psychotria sulzneri</i>	3.6	<i>Rubus cuneifolius</i>	1.9
<i>Pteridium aquilinum</i>	3.6	<i>Rubus trivialis</i>	1.9
<i>Ptilimnium capillaceum</i>	3.1	<i>Ruellia caroliniensis</i>	4.3
<i>Pueraria montana</i>	0.0	<i>Rumex crispus</i>	0.2
<i>Quercus geminata</i>	5.2	<i>Rumex obtusifolius</i>	0.7
<i>Quercus laurifolia</i>	3.6	<i>Rumex pulcher</i>	0.6
<i>Quercus nigra</i>	2.1	<i>Sabal palmetto</i>	4.5

Table B-1. Continued.

Species	CC	Species	CC
<i>Sabatia bartramii</i>	6.8	<i>Solanum americanum</i>	1.4
<i>Sacciolepis indica</i>	1.9	<i>Solanum capsicoides</i>	1.4
<i>Sacciolepis striata</i>	3.6	<i>Solanum carolinense</i>	1.2
<i>Sageretia minutiflora</i>	7.9	<i>Solanum nigrum</i>	0.0
<i>Sagittaria graminea</i>	5.5	<i>Solanum tampicense</i>	0.7
<i>Sagittaria lancifolia</i>	4.5	<i>Solanum viarum</i>	0.0
<i>Sagittaria latifolia</i>	5.0	<i>Solidago canadensis</i>	3.0
<i>Salix caroliniana</i>	2.1	<i>Solidago fistulosa</i>	3.6
<i>Salix nigra</i>	3.3	<i>Solidago gigantea</i>	3.2
<i>Salvia lyrata</i>	0.0	<i>Solidago latissimifolia</i>	1.8
<i>Sambucus canadensis</i>	1.7	<i>Solidago sempervirens</i>	5.0
<i>Samolus ebracteatus</i>	5.7	<i>Sonchus asper</i>	1.5
<i>Sapium sebiferum</i>	0.0	<i>Sorghum bicolor</i>	0.0
<i>Sarcostemma clausum</i>	2.4	<i>Spartina bakeri</i>	5.5
<i>Sarracenia flava</i>	9.3	<i>Spermacoce assurgens</i>	1.2
<i>Sarracenia minor</i>	4.8	<i>Spermacoce verticillata</i>	0.0
<i>Saururus cernuus</i>	5.5	<i>Sporobolus floridanus</i>	7.1
<i>Schinus terebinthifolius</i>	0.0	<i>Sporobolus indicus</i>	0.2
<i>Scirpus cyperinus</i>	4.5	<i>Stachys floridana</i>	1.4
<i>Scleria baldwinii</i>	6.7	<i>Stenotaphrum secundatum</i>	0.8
<i>Scleria georgiana</i>	6.2	<i>Stillingia aquatica</i>	7.4
<i>Scleria reticularis</i>	5.1	<i>Styrax americanus</i>	6.9
<i>Scleria triglomerata</i>	4.8	<i>Syngonanthus flavidulus</i>	5.2
<i>Scoparia dulcis</i>	2.4	<i>Taxodium ascendens</i>	8.8
<i>Scutellaria integrifolia</i>	5.7	<i>Thalia geniculata</i>	6.2
<i>Senecio glabellus</i>	4.0	<i>Thelypteris dentata</i>	6.0
<i>Senna obtusifolia</i>	0.0	<i>Thelypteris hispidula</i>	4.5
<i>Senna pendula</i>	0.0	<i>Thelypteris interrupta</i>	5.2
<i>Serenoa repens</i>	4.5	<i>Thelypteris kunthii</i>	5.2
<i>Sesbania herbacea</i>	1.0	<i>Thelypteris palustris</i>	3.6
<i>Sesbania vesicaria</i>	0.5	<i>Tilia americana</i>	5.5
<i>Setaria parviflora</i>	3.1	<i>Toxicodendron radicans</i>	1.9
<i>Seymeria cassioides</i>	6.0	<i>Tradescantia fluminensis</i>	0.0
<i>Sida acuta</i>	1.0	<i>Tradescantia ohiensis</i>	0.9
<i>Sida rhombifolia</i>	1.0	<i>Tradescantia zebrina</i>	0.0
<i>Sideroxylon celastrinum</i>	6.0	<i>Triadenum virginicum</i>	5.0
<i>Sideroxylon reclinatum</i>	6.0	<i>Trifolium repens</i>	0.0
<i>Smilax auriculata</i>	3.8	<i>Tripsacum dactyloides</i>	4.0
<i>Smilax bona-nox</i>	2.6	<i>Typha domingensis</i>	1.2
<i>Smilax glauca</i>	3.3	<i>Typha latifolia</i>	1.2
<i>Smilax laurifolia</i>	5.2	<i>Ulmus americana</i>	7.4
<i>Smilax rotundifolia</i>	3.2	<i>Urena lobata</i>	0.0
<i>Smilax smallii</i>	4.5	<i>Urochloa mutica</i>	0.0
<i>Smilax tamnoides</i>	3.6	<i>Utricularia gibba</i>	3.6
<i>Smilax walteri</i>	6.0	<i>Utricularia purpurea</i>	6.7

Table B-1. Continued.

Species	CC	Species	CC
<i>Vaccinium arboreum</i>	6.4	<i>Vitis shuttleworthii</i>	1.2
<i>Vaccinium corymbosum</i>	5.7	<i>Vittaria lineata</i>	1.2
<i>Vaccinium darrowii</i>	6.2	<i>Waltheria indica</i>	2.4
<i>Vaccinium elliotii</i>	6.7	<i>Wedelia trilobata</i>	0.0
<i>Vaccinium myrsinites</i>	4.8	<i>Woodwardia areolata</i>	5.7
<i>Valeriana scandens</i>	7.1	<i>Woodwardia virginica</i>	4.8
<i>Verbena bonariensis</i>	0.0	<i>Xanthosoma sagittifolium</i>	0.0
<i>Verbena brasiliensis</i>	0.0	<i>Xyris ambigua</i>	5.7
<i>Viburnum nudum</i>	3.6	<i>Xyris caroliniana</i>	5.7
<i>Viburnum obovatum</i>	1.2	<i>Xyris elliotii</i>	5.7
<i>Viburnum odoratissimum</i>	0.0	<i>Xyris fimbriata</i>	5.7
<i>Vicia sativa</i>	0.4	<i>Xyris jupicai</i>	1.7
<i>Vigna luteola</i>	3.6	<i>Xyris platylepis</i>	3.6
<i>Viola lanceolata</i>	4.8	<i>Youngia japonica</i>	0.0
<i>Vitis aestivalis</i>	2.9	<i>Yucca aloifolia</i>	1.2
<i>Vitis cinerea</i>	2.0	<i>Zea mays</i>	0.0
<i>Vitis rotundifolia</i>	2.1		

APPENDIX C  
**CANDIDATE METRICS**

Table C-1. Candidate metrics based on the diatom assemblage.

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Tolerance Metrics
Indicator Species Sensitive Taxa - Abundance
Indicator Species Sensitive Taxa - Presence/Absence
Indicator Species Tolerant Taxa - Abundance
Indicator Species Tolerant Taxa - Presence/Absence
Community Composition Metrics
Richness
Evenness
Shannon Diversity
Simpson's Index
Autecological Metrics
Morphological Guild - Erect
Morphological Guild - Stalked
Morphological Guild - Unattached
Morphological Guild - Prostrate/Adnate
Morphological Guild - Variable
Motility - Highly Motile
Motility - Moderately Motile
Motility - Highly & Moderately Motile
Motility - Not Motile
Motility - Variable
Pollution Tolerance - Very Tolerant
Pollution Tolerance - Moderately Tolerant
Pollution Tolerance - Very & Moderately Tolerant
Pollution Tolerance - Sensitive / Intolerant
Dissolved Oxygen Class 1 (Bahls 1993)
Dissolved Oxygen Class 2 (Bahls 1993)
Dissolved Oxygen Class 3 (Bahls 1993)
Dissolved Oxygen Class 4 (Bahls 1993)
Dissolved Oxygen Class 5 (Bahls 1993)
Wet/Dry Preference Class 1 (van Dam et al. 1994)
Wet/Dry Preference Class 2 (van Dam et al. 1994)

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Table C-1. Continued.

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*Autecological Metrics - continued*

Wet/Dry Preference Class 3 (van Dam et al. 1994)  
Wet/Dry Preference Class 4 (van Dam et al. 1994)  
Wet/Dry Preference Class 5 (van Dam et al. 1994)  
Nitrogen Metabolism Class 1 (van Dam et al. 1994)  
Nitrogen Metabolism Class 2 (van Dam et al. 1994)  
Nitrogen Metabolism Class 3 (van Dam et al. 1994)  
Nitrogen Metabolism Class 4 (van Dam et al. 1994)  
pH Class 1 (van Dam et al. 1994)  
pH Class 2 (van Dam et al. 1994)  
pH Class 3 (van Dam et al. 1994)  
pH Class 4 (van Dam et al. 1994)  
pH Class 5 (van Dam et al. 1994)  
pH Class 6 (van Dam et al. 1994)  
Salinity Class 1 (van Dam et al. 1994)  
Salinity Class 2 (van Dam et al. 1994)  
Salinity Class 3 (van Dam et al. 1994)  
Salinity Class 4 (van Dam et al. 1994)  
Saprobity Class 1 (van Dam et al. 1994)  
Saprobity Class 2 (van Dam et al. 1994)  
Saprobity Class 3 (van Dam et al. 1994)  
Saprobity Class 4 (van Dam et al. 1994)  
Saprobity Class 5 (van Dam et al. 1994)  
Trophic Class 1 (van Dam et al. 1994)  
Trophic Class 2 (van Dam et al. 1994)  
Trophic Class 1 & 2 (van Dam et al. 1994)  
Trophic Class 3 (van Dam et al. 1994)  
Trophic Class 4 (van Dam et al. 1994)  
Trophic Class 5 (van Dam et al. 1994)  
Trophic Class 6 (van Dam et al. 1994)  
Trophic Class 7 (van Dam et al. 1994)

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Table C-2. Candidate metrics based on the macrophyte assemblage.

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Wetland Plant Status
Obligate Species
Facultative Wetland Species
Facultative Species
Facultative Upland Species
Upland Species
Obligate + Facultative Wetland Species
Obligate + Facultative Wetland + Facultative Species
Facultative Upland + Upland Species
Upland + Facultative Upland + Facultative Species
Plant Growth Form & Taxa Metrics
Graminoid Species
<i>Carex</i> sp.
Herbaceous Species
Species in Asteraceae
<i>Polygonum</i> sp.
Graminoids to Herbaceous
Vine Species
Vines that are Woody
Shrub Species
Tree Species
Tree and Shrub Species
<i>Salix</i> sp.
Hardwoods
Trees as Hardwoods
<i>Nyssa</i> sp.
Trees as <i>Nyssa</i> sp.
<i>Acer rubrum</i>
Trees as <i>Acer rubrum</i>
Trees as Conifers
Trees as <i>Taxodium</i> sp.
Native Evergreen Shrubs
Native Ferns
Native Perennial Graminoids
Native Perennial Herbs

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Table C-2. Continued.

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Indicator Species
Sensitive (R:I Ratio, where R equals the number of Reference Sites and I equals number of agricultural and urban sites a species was found at)
Tolerants (R:I Ratio, where R equals the number of Reference Sites and I equals number of agricultural and urban sites a species was found at)
TWINSpan Sensitive Species
TWINSpan Sensitive Species by Presence Absence
TWINSpan Tolerant Species
TWINSpan Tolerant Species by Presence Absence
TWINSpan Sensitive Species, Excluding Exotic Species
TWINSpan Sensitive Species by Presence Absence, Excluding Exotic Species
TWINSpan Tolerant Species, Excluding Exotic Species
TWINSpan Tolerant Species by Presence Absence, Excluding Exotic Species
Indicator Species Sensitive Taxa – Occurrence
Indicator Species Sensitive Taxa - Presence/Absence
Indicator Species Tolerant Taxa – Occurrence
Indicator Species Tolerant Taxa - Presence/Absence
Indicator Species Sensitive Taxa, Excluding Exotic Species – Occurrence
Indicator Species Sensitive Taxa, Excluding Exotic Species - Presence/Absence
Indicator Species Tolerant Taxa, Excluding Exotic Species – Occurrence
Indicator Species Tolerant Taxa, Excluding Exotic Species - Presence/Absence
Modified FQI Score
Exotic Species Metric
Longevity Metrics
Annuals
Native Annuals
Perennials
Native Perennials

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Table C-2. Continued.

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*Longevity Metrics - continued*

Annual to Perennial Ratio

Native Annual to Native Perennial Ratio

## Richness Metrics

Species Richness by Site

Species Richness by Quadrat

Species Richness by Occurrence

Species Richness by Transect

Mean Site Evenness

Dominant Species

Log (Proportion of Dominant Species)

Vascular Genera

Nonvascular Genera

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Table C-3. Candidate metrics based on the macroinvertebrate assemblage.


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Tolerance Metrics
Indicator Species Analysis
Sensitive Taxa - Abundance
Sensitive Taxa - Presence/Absence
Tolerant Taxa - Abundance
Tolerant Taxa - Presence/Absence
Florida Index
Lake Condition Index
Community Structure & Balance Metrics
Mixed Taxonomic Levels
Crustacea + Mollusca
Dominant Taxa
Exotic Richness
Taxa Richness
Tubificida/Insecta
Phylum
Phylum Richness
Annelida
Arthropoda
Mollusca
Platyhelminthes
Class
Class Richness
Arachnida
Bivalva
Crustacea
Gastropoda
Insecta
Oligochaeta
Plecypoda
Turbellaria
Order
Order Richness
Acariformes
Amphipoda
Anostraca
Basommatophora
Coleoptera
Collembola
Decapoda
Diptera

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Table C-3. Continued.

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 Community Structure & Balance Metrics - *continued*
Order - *continued*

Diptera - non-Chironomid  
 Ephemeroptera  
 Ephemeroptera + Plecoptera + Trichoptera  
 Ephemeroptera + Trichoptera + Odonata  
 Haplotaxida  
 Hemiptera  
 Heteroptera  
 Hoplonemertea  
 Isopoda  
 Lepidoptera  
 Lumbriculida  
 Megaloptera  
 Mesogastropoda  
 Odonata  
 Oribatei  
 Plecoptera  
 Trichoptera  
 Tricladida  
 Trombidiformes  
 Tubificida  
 Veneroida  
 Zygoptera

## Family

Family Richness  
 Aeshnidae  
 Ancylidae  
 Arrenuridae  
 Asellidae  
 Baetidae  
 Belostomatidae  
 Cambaridae  
 Ceratopogonidae  
 Chaoboridae  
 Chironomidae  
 Coenagrionidae  
 Corixidae  
 Crangonyctidae

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Table C-3. Continued.

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 Community Structure & Balance Metrics - *continued*
Family - *continued*

Culicidae  
 Curulionidae  
 Dryopidae  
 Dytiscidae  
 Enchytraeidae  
 Haliplidae  
 Helodidae  
 Hydrophilidae  
 Libellulidae  
 Lumbriculidae  
 Naididae  
 Noteridae  
 Notonectidae  
 Physidae  
 Planorbidae  
 Tabanidae  
 Tipulidae  
 Tubificidae

## Sub-Families of Chironomidae

Chironominae  
 Orthoclaadiinae  
 Tanypodinae  
 Ratio Tanypodinae/Orthoclaadiinae  
 Ratio Chironominae/Orthoclaadiinae  
 Ratio (Tanypodinae +  
 Chironominae)/Orthoclaadiinae

## Genus

Genus Richness  
*Ablabesmyia*  
*Anopheles*  
*Arrenurus*  
*Atrichopogon*  
*Beardius*  
*Belostoma*  
*Berosus*  
*Bratislavia*  
*Buenoa*  
*Caecidotea*  
*Callibaetis*  
*Chaoborus*  
*Chironomus*  
*Crangonyx*

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Table C-3. Continued.

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 Community Structure & Balance Metrics - *continued*
Genus - *continued*

*Culex*  
*Dero*  
*Desmopachria*  
*Eclipidrilus*  
*Goeldichironomus*  
*Haemonais*  
*Hydrocanthus*  
*Hydrochus*  
*Ischnura*  
*Kiefferulus*  
*Labrundinia*  
*Larsia*  
*Micromenetus*  
*Monopelopia*  
*Notonecta*  
*Ochlerotatus*  
*Pachydiplax*  
*Pachydrus*  
*Parachironomus*  
*Paramerina*  
*Pelonomus*  
*Physella*  
*Polypedilum*  
*Pristina*  
*Pristinella*  
*Scirtes*  
*Tanytarsus*  
*Tropisternus*  
*Zavreliella*

## Functional Feeding Group Metrics

Browsers and Grazers of Periphyton  
 Collector-Filterers/Suspension Feeders  
 Collector-Gatherers/Deposit Feeders  
 Macrophyte Piercers  
 Macrophyte Shredders  
 Parasites  
 Periphyton Scrapers  
 Predators & Carnivores  
 Scavenger (animals)

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**APPENDIX D**  
**QUANTIFYING ANTHROPOGENIC INFLUENCE**

Table D-1. LDI, WRAP, and Minnesota disturbance index scores for 118 wetlands.

Site Code	LDI	WRAP	Minnesota Disturbance Index	Site Code	LDI	WRAP	Minnesota Disturbance Index
SA1	6.3	6.9	16	CA1	6.1	5.8	16
SA2	4.6	4.6	14	CA2	5.0	6.3	9
SA3	4.6	5.5	9	CA3	4.9	8.1	14
SA4	6.2	7.5	17	CA4	4.3	3.4	5
SA5	4.0	4.5	11	CA5	5.1	5.5	13
SA6	5.0	6.0	9	CA6	5.4	4.8	17
SA7	2.8	2.6	2	CA7	4.4	5.7	8
SA8	1.3	3.6	5	CA8	1.9	2.7	9
SA9	4.8	3.2	6	CA9	5.4	6.3	17
SR1	1.0	1.0	0	CR1	1.0	1.1	5
SR2	1.0	1.2	0	CR2	1.0	1.8	1
SR3	1.0	1.4	3	CR3	1.0	1.5	2
SR4	1.0	1.0	1	CR4	1.1	1.4	0
SR5	1.2	1.0	1	CR5	1.0	1.0	0
SR6	1.0	1.5	2	CR6	1.0	1.3	0
SR7	1.1	1.8	3	CR7	1.1	1.8	6
SR8	1.0	1.7	2	CR8	1.0	3.0	2
SR9	1.0	3.2	3	CR9	1.0	1.3	2
SU1	5.0	6.2	14	CR10	1.5	1.8	5
SU2	4.8	6.0	8	CR11	1.0	1.5	0
SU3	1.3	5.9	15	CU1	2.1	2.4	7
SU4	1.5	4.9	8	CU2	3.9	6.0	7
SU5	5.2	6.3	17	CU3	7.1	6.4	17
SU6	3.9	5.5	15	CU4	4.5	6.0	13
SU7	5.1	7.1	13	CU5	6.4	6.1	12
SU8	7.2	8.4	17	CU6	7.2	6.0	13
SU9	6.2	4.6	16	CU7	5.6	6.4	16
SU10	3.2	6.0	8	CU8	4.4	5.1	16
				CU9	7.0	7.2	14
				CU10	3.8	5.8	10
				CU11	3.3	6.2	15

Table D-1 Continued.

Site Code	LDI	WRAP	Minnesota Disturbance Index	Site Code	LDI	WRAP	Minnesota Disturbance Index
NA1	5.1	6.9	13	PA1	3.1	4.7	9
NA2	4.9	6.3	9	PA2	5.0	6.2	18
NA3	2.1	2.9	4	PA3	6.8	6.1	19
NA4	6.2	6.8	19	PA4	6.6	6.8	18
NA5	5.1	7.5	17	PA5	4.7	6.7	19
NA6	5.3	5.5	16	PA6	4.7	6.0	17
NA7	5.0	4.3	7	PA7	4.9	6.6	17
NA8	2.0	3.1	4	PA8	2.2	4.9	10
NA9	2.2	4.2	5	PA9	5.8	5.4	7
NA10	2.2	3.0	6	PA10	2.0	4.9	6
NA11	2.5	3.9	9	PR1	1.0	1.6	0
NA12	5.5	5.1	7	PR2	1.0	1.3	0
NR1	1.1	2.1	1	PR3	1.1	1.9	0
NR2	1.0	1.5	1	PR4	1.5	1.7	2
NR3	1.0	1.2	1	PR5	1.0	1.0	0
NR4	1.0	1.2	2	PR6	1.3	1.3	0
NR5	1.0	1.4	1	PR7	1.0	1.3	0
NR6	1.1	1.1	0	PR8	1.0	1.2	0
NR7	1.8	1.8	1	PU1	5.3	8.3	17
NR8	1.0	2.5	0	PU2	5.9	5.3	9
NR9	1.0	1.9	1	PU3	6.3	5.6	12
NU1	2.8	3.9	7	PU4	4.8	7.5	17
NU2	4.2	4.4	9	PU5	4.0	6.4	13
NU3	5.3	6.6	10	PU6	4.8	5.1	8
NU4	3.2	5.4	11	PU7	3.8	5.4	10
NU5	6.2	6.2	9	PU8	5.0	5.4	9
NU6	5.6	4.8	15	PU9	3.1	3.9	5
NU7	4.2	5.3	10	PU10	6.5	8.6	17
NU8	3.8	4.8	11				
NU9	6.3	5.9	12				
NU10	6.6	6.4	17				

**APPENDIX E**  
**SUMMARY STATISTICS**

Table E-1. Summary statistics of richness (R), evenness (E), Shannon diversity (H), and Simpson's index (S) for the diatom assemblage (genus level).

Site	R	E	H	S	Site	R	E	H	S
PR1	12	0.82	2.03	0.82	CA3	18	0.63	1.81	0.73
PR4	16	0.63	1.75	0.69	CA4	17	0.78	2.21	0.86
PR5	12	0.71	1.77	0.77	CA5	22	0.73	2.27	0.83
PR6	11	0.60	1.44	0.68	CA6	13	0.89	2.28	0.87
NR2	22	0.68	2.09	0.77	SA2	31	0.75	2.57	0.85
NR3	13	0.76	1.96	0.79	SA3	25	0.84	2.69	0.90
NR4	12	0.83	2.07	0.84	SA4	20	0.78	2.33	0.84
NR6	16	0.81	2.25	0.87	SA5	24	0.61	1.95	0.73
CR3	30	0.84	2.87	0.92	SA6	20	0.86	2.56	0.90
CR4	22	0.74	2.28	0.85	PU3	28	0.83	2.78	0.90
CR5	26	0.81	2.63	0.86	PU4	20	0.72	2.17	0.82
CR6	9	0.66	1.45	0.68	NU2	23	0.83	2.60	0.89
SR1	19	0.65	1.90	0.77	NU4	14	0.66	1.73	0.73
SR2	13	0.59	1.50	0.63	NU5	12	0.57	1.41	0.58
SR3	26	0.81	2.63	0.90	NU6	23	0.74	2.31	0.85
SR4	22	0.77	2.37	0.86	CU1	9	0.76	1.66	0.78
SR5	35	0.84	2.98	0.93	CU3	39	0.79	2.89	0.90
SR6	36	0.81	2.89	0.91	CU5	31	0.69	2.37	0.78
PA2	12	0.80	1.98	0.83	CU6	26	0.84	2.73	0.91
PA3	10	0.70	1.62	0.73	SU1	17	0.62	1.77	0.72
PA5	19	0.74	2.17	0.83	SU2	28	0.88	2.92	0.93
PA6	17	0.74	2.08	0.83	SU3	24	0.73	2.31	0.84
NA4	34	0.78	2.75	0.89	SU4	21	0.62	1.87	0.67
NA6	14	0.64	1.68	0.70	SU5	23	0.83	2.59	0.89
CA2	14	0.76	2.00	0.80	SU6	15	0.59	1.59	0.70

Table E-2. Summary statistics of richness (R), jackknife estimators of species richness (Jack<sub>1</sub>, Jack<sub>2</sub>), evenness (E), Shannon diversity (H), and Whittaker's beta diversity ( $\beta_w$ ) for the macrophyte assemblage (species level).

Site	R	Jack <sub>1</sub>	Jack <sub>2</sub>	E	H	$\beta_w$
PR1	37	43	45	0.89	3.2	3.5
PR2	31	37	41	0.87	3.0	2.6
PR3	37	50	56	0.80	2.9	5.5
PR4	24	30	30	0.79	2.5	4.8
PR5	23	28	31	0.88	2.7	4.0
PR6	27	37	41	0.83	2.7	4.1
PR7	32	43	42	0.71	2.5	8.3
PR8	34	47	54	0.83	2.9	2.0
NR1	14	15	11	0.83	2.2	2.8
NR2	40	55	62	0.84	3.1	6.8
NR3	28	37	44	0.84	2.8	5.1
NR4	32	43	49	0.85	2.9	4.8
NR5	29	38	43	0.85	2.9	6.4
NR6	42	48	49	0.89	3.3	3.6
NR7	35	44	45	0.83	3.0	3.1
NR8	31	38	40	0.86	2.9	3.8
NR9	15	17	16	0.79	2.1	1.2
CR1	31	35	36	0.91	3.1	2.3
CR2	31	40	46	0.82	2.8	6.7
CR3	53	72	84	0.86	3.4	5.0
CR4	40	54	58	0.83	3.1	3.4
CR5	31	46	56	0.89	3.1	9.4
CR6	27	31	32	0.92	3.0	1.4
CR7	49	62	69	0.88	3.4	4.9
CR8	22	28	30	0.79	2.4	1.4
CR9	53	69	76	0.87	3.5	5.0
CR10	35	42	42	0.89	3.2	3.4
CR11	46	56	58	0.91	3.5	3.2
SR1	27	36	40	0.85	2.8	4.1
SR2	25	31	31	0.86	2.8	3.3
SR3	25	31	33	0.86	2.8	4.6
SR4	20	25	28	0.85	2.6	3.1
SR5	29	38	42	0.88	3.0	3.0
SR6	16	20	21	0.91	2.5	1.5
SR7	60	77	79	0.89	3.6	3.8
SR8	40	53	61	0.84	3.1	3.1
SR9	26	34	40	0.85	2.8	1.1

Table E-2. Continued.

Site	R	Jack <sub>1</sub>	Jack <sub>2</sub>	E	H	$\beta_w$
PA1	36	45	47	0.85	3.0	7.9
PA2	29	37	39	0.88	3.0	4.9
PA3	29	38	40	0.82	2.7	7.5
PA4	25	34	39	0.87	2.8	6.7
PA5	50	68	75	0.87	3.4	6.2
PA6	34	39	42	0.89	3.1	4.5
PA7	52	62	64	0.89	3.5	5.6
PA8	22	29	33	0.87	2.7	1.6
PA9	44	56	58	0.89	3.4	3.3
PA10	35	53	68	0.88	3.1	3.8
NA1	19	24	25	0.86	2.5	2.8
NA2	36	49	57	0.82	2.9	3.6
NA3	13	17	17	0.72	1.8	5.6
NA4	53	74	85	0.90	3.6	4.7
NA5	45	60	71	0.89	3.4	6.1
NA6	41	50	55	0.86	3.2	4.6
NA7	44	55	60	0.91	3.4	4.0
NA8	21	25	27	0.90	2.7	3.4
NA9	60	73	80	0.92	3.8	3.4
NA10	36	45	46	0.88	3.2	4.1
NA11	53	73	85	0.89	3.5	5.9
NA12	77	99	114	0.90	3.9	6.6
CA1	44	56	64	0.89	3.4	2.8
CA2	18	23	26	0.91	2.6	1.4
CA3	34	45	49	0.88	3.1	4.2
CA4	43	51	53	0.89	3.3	4.8
CA5	26	33	37	0.80	2.6	2.3
CA6	26	34	39	0.80	2.6	6.2
CA7	60	81	91	0.85	3.5	4.6
CA8	47	63	69	0.85	3.3	4.4
CA9	31	41	47	0.90	3.1	6.1
SA1	21	26	29	0.87	2.7	4.3
SA2	34	43	45	0.90	3.2	5.5
SA3	38	45	46	0.91	3.3	2.1
SA4	31	40	45	0.83	2.9	5.8
SA5	27	35	36	0.83	2.7	7.0
SA6	50	65	74	0.86	3.4	4.8
SA7	20	27	31	0.91	2.7	7.2
SA8	40	52	55	0.87	3.2	7.5
SA9	36	47	51	0.87	3.1	5.9

Table E-2. Continued.

Site	R	Jack <sub>1</sub>	Jack <sub>2</sub>	E	H	$\beta_w$
PU1	37	47	54	0.89	3.2	5.9
PU2	34	42	43	0.87	3.1	5.8
PU3	42	59	68	0.86	3.2	5.4
PU4	43	57	66	0.93	3.5	6.1
PU5	42	61	76	0.88	3.3	4.4
PU6	29	39	46	0.85	2.8	5.3
PU7	24	30	34	0.91	2.9	0.8
PU8	35	50	59	0.83	2.9	1.6
PU9	16	22	26	0.79	2.2	0.2
PU10	38	55	63	0.87	3.1	7.5
NU1	37	46	50	0.89	3.2	3.8
NU2	46	51	50	0.86	3.3	5.3
NU3	48	60	62	0.88	3.4	5.4
NU4	27	34	38	0.87	2.9	4.0
NU5	34	44	49	0.86	3.0	3.9
NU6	26	39	49	0.78	2.5	7.7
NU7	41	55	65	0.89	3.3	5.1
NU8	41	50	50	0.88	3.3	4.2
NU9	35	49	58	0.86	3.1	1.7
NU10	42	55	61	0.86	3.2	5.5
CU1	46	56	61	0.85	3.2	5.5
CU2	44	54	58	0.88	3.3	3.1
CU3	33	40	43	0.86	3.0	5.4
CU4	42	58	67	0.84	3.1	7.0
CU5	34	40	37	0.85	3.0	4.5
CU6	23	27	24	0.83	2.6	4.0
CU7	45	59	64	0.87	3.3	4.3
CU8	63	92	111	0.85	3.5	4.5
CU9	32	40	45	0.86	3.0	1.7
CU10	26	36	40	0.77	2.5	2.8
CU11	38	49	56	0.90	3.3	3.0
SU1	55	73	81	0.88	3.5	6.4
SU2	38	50	59	0.89	3.2	3.3
SU3	24	32	36	0.85	2.7	4.5
SU4	38	53	63	0.85	3.1	4.8
SU5	16	20	21	0.90	2.5	2.6
SU6	21	30	33	0.83	2.5	3.8
SU7	48	59	60	0.91	3.5	3.5
SU8	26	37	45	0.86	2.8	2.8
SU9	47	59	64	0.90	3.5	5.3
SU10	39	50	56	0.87	3.2	3.9

Table E-3. Summary statistics of richness (R), evenness (E), Shannon diversity (H), and Simpson's index (S) for the macroinvertebrate assemblage (genus level).

Site	R	E	H	S	Site	R	E	H	S
PR1	11	0.58	0.62	1.40	CA5	14	0.68	0.71	1.80
PR4	1	0.00	0.00	0.00	CA6	11	0.59	0.60	1.42
PR5	5	0.97	0.78	1.56	CA7	20	0.90	0.92	2.70
PR6	10	0.87	0.84	2.01	CA8	12	0.65	0.70	1.62
PR7	18	0.84	0.89	2.41	CA9	9	0.55	0.59	1.20
PR8	15	0.69	0.74	1.86	SA2	14	0.79	0.84	2.09
NR2	8	0.63	0.65	1.30	SA3	17	0.73	0.78	2.07
NR3	24	0.86	0.91	2.72	SA4	7	0.53	0.48	1.03
NR4	21	0.82	0.89	2.50	SA5	15	0.89	0.89	2.41
NR6	19	0.88	0.90	2.59	SA6	14	0.68	0.73	1.79
NR8	4	0.15	0.08	0.21	SA7	11	0.60	0.63	1.45
NR9	11	0.56	0.59	1.34	SA8	19	0.73	0.81	2.14
CR3	11	0.76	0.80	1.81	SA9	8	0.51	0.46	1.05
CR4	17	0.82	0.87	2.33	PU3	23	0.84	0.90	2.63
CR5	16	0.76	0.84	2.12	PU4	16	0.79	0.82	2.19
CR6	20	0.86	0.90	2.57	PU10	13	0.83	0.85	2.13
CR8	8	0.29	0.23	0.60	NU2	7	0.76	0.71	1.47
CR9	7	0.37	0.29	0.72	NU4	11	0.49	0.47	1.16
CR10	25	0.87	0.92	2.79	NU5	8	0.83	0.77	1.72
CR11	21	0.84	0.89	2.55	NU6	10	0.72	0.73	1.66
SR1	17	0.67	0.72	1.90	NU10	8	0.68	0.66	1.41
SR2	10	0.87	0.84	2.01	CU1	25	0.82	0.90	2.64
SR3	18	0.76	0.82	2.21	CU3	9	0.68	0.69	1.49
SR4	11	0.78	0.79	1.87	CU5	9	0.57	0.63	1.25
SR5	21	0.84	0.89	2.56	CU6	11	0.51	0.54	1.22
SR6	15	0.68	0.71	1.84	CU7	11	0.72	0.75	1.72
SR7	11	0.61	0.60	1.46	CU8	21	0.89	0.91	2.72
SR8	22	0.85	0.89	2.62	CU9	13	0.69	0.70	1.78
SR9	14	0.45	0.44	1.19	CU10	5	0.19	0.12	0.31
PA2	24	0.66	0.77	2.09	CU11	17	0.71	0.77	2.02
PA3	26	0.78	0.87	2.54	SU1	11	0.63	0.69	1.50
PA5	18	0.79	0.85	2.28	SU2	13	0.79	0.82	2.04
PA6	8	0.63	0.63	1.32	SU3	19	0.65	0.73	1.91
NA4	13	0.83	0.84	2.13	SU4	12	0.58	0.60	1.44
NA6	9	0.73	0.77	1.60	SU5	16	0.87	0.89	2.42
NA10	5	0.38	0.32	0.61	SU6	20	0.63	0.75	1.89
NA11	18	0.81	0.87	2.35	SU7	14	0.67	0.74	1.76
CA2	20	0.73	0.78	2.20	SU8	8	0.65	0.65	1.36
CA3	8	0.67	0.65	1.40	SU9	10	0.47	0.45	1.09
CA4	18	0.76	0.84	2.20					

**APPENDIX F**  
**METRIC SCORING FOR THE FLORIDA WETLAND CONDITION INDEX**  
**FOR DEPRESSIONAL FORESTED WETLANDS**

**Scoring the Diatom Florida Wetland Condition Index**

1. Calculate values for the 7 metrics:
  - 1 - Percent tolerant indicator species
  - 2 - Percent sensitive indicator species
  - 3 – Percent pollution class 1
  - 4 – Percent nitrogen class 3
  - 5 – Percent saprobity class 4
  - 6 – Percent pH class 3
  - 7 – Percent oxygen class 1
  
2. Take the natural log of metrics to improve distribution.  
 =  $\ln(\text{metric value} + 10)$
  
3. Use the scoring equations to normalize scores between 0 and 10.  
 Metrics that increase with increasing LDI – tolerant, pollution class 1, nitrogen class 3, saprobity class 4, pH class 3 metrics:  
 =  $10 - ((\text{metric} - 5^{\text{th}} \text{ percentile}) * (10 / (95^{\text{th}} \text{ percentile} - 5^{\text{th}} \text{ percentile})))$   
  
 Metrics that decrease with increasing LDI – sensitive, oxygen class 1:  
 =  $((\text{metric} - 5^{\text{th}} \text{ percentile}) * (10 / (95^{\text{th}} \text{ percentile} - 5^{\text{th}} \text{ percentile})))$

Below are the 5<sup>th</sup> and 95<sup>th</sup> percentiles for each metric (transformed values are presented, see step 2 above):

	5 <sup>th</sup> Percentile	95 <sup>th</sup> Percentile
%Tolerant indicator species	2.30	2.35
%Sensitive indicator species	2.30	2.37
%Pollution class 1	2.30	4.11
%Nitrogen class 3	2.30	4.31
%Saprobity class 4	2.30	3.96
%pH class 3	2.36	4.48
%Oxygen class 1	3.06	4.66

4. Rescore, so that the metrics in the outer 5<sup>th</sup> percentiles receive scores of 0 or 10.  
 =  $\text{IF}(\text{score} < 0, 0, (\text{IF}(\text{score} \leq 10, \text{score}, 10)))$



**Scoring the Macrophyte Florida Wetland Condition Index**

1. Calculate values for the 6 metrics:
  - 1 - Percent tolerant indicator species
  - 2 - Percent sensitive indicator species
  - 3 - Percent exotic species
  - 4 - Modified FQI Score
  - 5 - Percent native perennial species
  - 6 - Percent wetland status species
  
2. Take the natural log of metrics to improve distribution.
 

For tolerant, sensitive, and exotics species metrics: = ln (metric value + 10)

For modified FQI, native perennial, and wetland status use the straight metric values (do not take the natural log)
  
3. Use the scoring equations to normalize scores between 0 and 10.
 

Metrics that increase with increasing LDI - tolerant and exotic metrics:  
 =  $10 - ((\text{metric} - 5^{\text{th}} \text{ percentile}) * (10 / (95^{\text{th}} \text{ percentile} - 5^{\text{th}} \text{ percentile})))$

Metrics that decrease with increasing LDI - sensitive, modified FQI, native perennial, and wetland status:  
 =  $((\text{metric} - 5^{\text{th}} \text{ percentile}) * (10 / (95^{\text{th}} \text{ percentile} - 5^{\text{th}} \text{ percentile})))$

Below are the regionalized 5th and 95th percentiles for each metric (transformed values are presented, see step 2 above):

<b>%Tolerant Indicator Species</b>		
Region	5 <sup>th</sup> Percentile	95 <sup>th</sup> Percentile
Panhandle	2.30	4.16
North	2.30	4.13
Central	2.64	4.02
South	2.42	3.91

<b>%Sensitive Indicator Species</b>		
Region	5 <sup>th</sup> Percentile	95 <sup>th</sup> Percentile
Panhandle	2.30	4.28
North	2.51	4.17
Central	2.30	4.04
South	2.30	4.10

**%Exotic Species**

Region	5 <sup>th</sup> Percentile	95 <sup>th</sup> Percentile
Panhandle	2.30	3.63
North	2.30	3.72
Central	2.30	3.58
South	2.59	3.71

**Modified FQI Score**

Region	5 <sup>th</sup> Percentile	95 <sup>th</sup> Percentile
Panhandle	2.68	6.11
North	2.55	5.70
Central	2.83	4.83
South	2.73	5.08

**%Native Perennial Species**

Region	5 <sup>th</sup> Percentile	95 <sup>th</sup> Percentile
Panhandle	67.72	100.00
North	60.00	100.00
Central	63.57	95.55
South	61.94	95.73

**%Wetland Status Species**

Region	5 <sup>th</sup> Percentile	95 <sup>th</sup> Percentile
Panhandle	38.28	77.09
North	47.66	83.01
Central	41.96	80.01
South	33.33	87.05

4. Rescore, so that the metrics in the outer 5<sup>th</sup> percentiles receive scores of 0 or 10.

$$= \text{IF} (\text{score} < 0, 0, (\text{IF} (\text{score} \leq 10, \text{score}, 10)))$$

### Scoring the Macroinvertebrate Florida Wetland Condition Index

1. Calculate values for the 6 metrics:
  - 1 – Percent tolerant indicator genera
  - 2 – Percent sensitive indicator genera
  - 3 – Florida Index
  - 4 – Percent Mollusca
  - 5 – Percent Noteridae
  - 6 – Percent scrapers
  
2. Take the natural log of metrics to improve distribution.
 
$$= \ln (\text{metric value} + 10)$$
  
3. Use the scoring equations to normalize scores between 0 and 10.
 

Metrics that increase with increasing LDI – tolerant, Mollusca, scrapers:

$$= 10 - ( (\text{metric} - 5^{\text{th}} \text{ percentile}) * ( 10 / ( 95^{\text{th}} \text{ percentile} - 5^{\text{th}} \text{ percentile})))$$

Metrics that decrease with increasing LDI –sensitive, Florida Index, Noteridae:

$$= ((\text{metric} - 5^{\text{th}} \text{ percentile}) * ( 10 / ( 95^{\text{th}} \text{ percentile} - 5^{\text{th}} \text{ percentile})))$$

Below are the 5th and 95th percentiles for each metric (transformed values are presented, see step 2 above):

	5 <sup>th</sup> Percentile	95 <sup>th</sup> Percentile
%Tolerant Indicator Genera	2.30	4.04
%Sensitive Indicator Genera	2.30	3.73
Florida Index	2.30	2.71
%Mollusca	2.30	3.73
%Noteridae	2.30	3.81
%Scraper	2.30	2.66

4. Rescore, so that the metrics in the outer 5<sup>th</sup> percentiles receive scores of 0 or 10.
 
$$= \text{IF} (\text{score} < 0, 0, (\text{IF} (\text{score} \leq 10, \text{score}, 10)))$$

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