Final Report to the Great Lakes Commission

FOR

# A test of the variability and usefulness of SOLEC indicators in wetlands of Lakes Huron and Michigan.

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## Introduction

Development of indicators of "ecosystem health" for Great Lakes coastal wetlands was recognized as a major need at the State-of-the-Lakes Ecosystem Conferences (SOLEC) held in Buffalo, New York in 1998 and Hamilton, Ontario in 2000. Indicators listed by the wetlands indicators task force at these conferences included indices of biotic integrity (IBI) based on macroinvertebrates, fish and plants.

We previously developed and published a macroinvertebrate based bioassessment procedure for fringing coastal wetlands of Lake Huron (e.g. Burton et al. 1999, Kashian and Burton 2000; Uzarski et al., submitted; see summary on the bioassessment wetlands working group (BAWWG) web site (http://www.epa.gov/owow/wetlands/bawwg/case.html). Wilcox et al. (2002) attempted to develop wetland IBIs for the upper Great Lakes using macrophytes, fish, and microinvertebrates. Even though some metrics showed promise, they concluded that natural water level changes from those that existed during their data collection were likely to alter communities enough to invalidate metrics in subsequent years. We were able to overcome this problem for fringing coastal wetlands by developing a method based on sampling any or all of four plant zones depending on the number inundated in any particular year (Burton et al. 1999, Uzarski et al., submitted). The IBI scores for a particular year were calculated by summing scores from each zone across the number of zones that were inundated in that year. As water levels decreased and zones were no longer inundated, the IBI scores which indicated the condition of the wetland changed, but metrics for even a single inundated zone proved to be effective in establishing wetland condition (Uzarski et al., submitted). Our system worked well for fringing wetlands of Lakes Huron and Michigan as water decreased by more than one meter from 1997 through 2002. Based on these results, we are confident that our macroinvertebrate IBI is valid under a wide range of water levels (Uzarski et al. submitted). We are also working to develop similar macroinvertebrate based IBI systems for other types of Great Lake systems with particular interest in the drowned river mouth wetlands of eastern Lake Michigan.

We are working on fish and plant based metrics that can be adjusted over water level changes and believe that a viable IBI can be developed based on these taxa as well. Minns et al. (1994) applied Karr's approach of using fish as indicators of stream biotic integrity (e.g., Karr 1981, Karr et al. 1986) to marshes of Great Lakes' Areas of Concern. The metrics employed by Minns et al. (1994) were sensitive to impacts on ecosystem integrity by exotic fishes, water quality changes, physical habitat alteration, and changes in piscivore abundance related to fishing pressure and stocking. Despite the research of Minns et al. and suggestions of several other authors and the SOLEC 1998 wetlands indicators task force, no widely accepted, fish based system for evaluation of ecosystem health for Great Lakes coastal wetlands has been developed. Our previous work and the work of Brazner (1997), Brazner and Beals (1997), Minns et al. (1994) and Thoma (1999) suggest that IBI development should be relatively straight-forward. One of our objectives for this study was to develop a fish based IBI for coastal wetlands.

In addition to metrics based on fish community composition, specific metrics that relate contaminant levels to fish condition were also listed as a need by the SOLEC task force. Brown bullhead (*Ameiuris nebulosus*) have been used as an indicator species for water and sediment contamination because of their susceptibility to deformities, eroded fins, lesions, and tumors (DELTs) in areas with elevated contaminant levels (Baumann et al. 1996, Sindermann 1979, and Pyron et al. 2001). This common Great Lakes species is an excellent organism for monitoring environmental health effects because of its benthic and philopatric life history (Leadley et al. 1998). Brown bullheads are primarily benthic feeders that forage deep in soft sediments in search of food and ingest substantial quantities of organic detritus along with a wide range of prey items. This feeding behavior and their habit of remaining motionless on the bottom during periods of inactivity result in potentially high rates of uptake of contaminants through ingestion and dermal uptake (Leadley et al. 1998). Direct dermal exposure to sediment has been related to high frequency of external lesions (Sherwood and Mearns 1979). While specific metrics have not been developed to correlate the number of DELTs observed in a population with contaminant concentrations in sediments, the presence/absence of DELT abnormalities can be a useful indicator of ecosystem health. The collection of paired sediment chemistry and DELT frequency can provide important insight as to the significance of anthropogenic contaminants in a system.

IBIs based on aquatic macrophytes have not been widely developed for wetlands. The only detailed sets of aquatic macrophyte metrics are those presently being developed for depressional wetlands in Minnesota (Gernes 1998) and Ohio (Mack et al. 2000). The aquatic-macrophyte metrics developed for depressional wetlands in Minnesota were evaluated for Great Lakes coastal wetlands by Albert (1999). Most proposed metrics were not appropriate for Great Lakes coastal wetlands, probably because of the very different physical environment of these large coastal systems and the small depressional wetlands included in the systems developed for Ohio and Minnesota. The task force on Great Lakes coastal wetlands from SOLEC 98 identified two potential metrics based on aquatic macrophytes, 1) area of wetland by type and 2) presence and percent coverage of invasive or exotic plants.

In the RFP and follow up meetings to establish protocols for this research project, we agreed to test potential metrics that had been developed previously for fringing wetlands of the Great Lakes in 20 wetlands in Lakes Huron and Michigan. Our specific task was to test the effectiveness of existing metrics and to potentially develop new metrics based on invertebrates, plants, and fish. In addition, we agreed to work with other investigators to obtain as much information as possible on potential bird and amphibian metrics for these wetlands.

## Objectives

**The objectives of this study were to test and develop biotic indicators of wetland ecological health that could be employed in a monitoring program by federal, state and local agencies to detect effects of anthropogenic disturbance on the biotic integrity of Great Lakes coastal wetlands.** Several indicators based on fish, macroinvertebrates, and plants were recommended for use in Great Lakes Coastal Wetlands at the 2000 SOLEC. Our objective was to fully develop these and other indicators of ecosystem health for coastal wetlands.

**A second objective was to evaluate the usefulness of plant, invertebrate and fish metrics in a monitoring program based on the seven criteria set forth in the original RFP from the Great Lakes Commission.** These criteria were:

1) Cost,
2) Measurability,
3) Basin-wide applicability or sampling by wetland type,
4) Availability of complementary existing research or data,
5) Indicator sensitivity to wetland condition changes,
6) Ability to set endpoint or attainment levels, and
7) Statistical Approach.

## Methods

Twenty Lake Huron and Michigan wetlands were selected for study of all flora and fauna indicators listed in Table 1 following procedures developed and/or agreed on with the Project Management Team of The Great Lakes Wetlands Consortium.

### *Site Selection*

We tested and developed indicators at open lacustrine and protected embayment wetlands selected from the U.S.A. shoreline of Lake Huron and the northern shoreline of Lake Michigan. We listed all potential sites using lists compiled in Chow-Fraser and Albert (1998) and/or open lacustrine and protected embayment sites listed by Herdendorf et al. (1981a-f) that were easily accessible (wetlands too far from an access point were eliminated from consideration). Since we suspected that many small lacustrine wetlands were no longer inundated, we made site visits to all potential sites during June 2002 to determine which sites still had inundated wetlands present. We sampled all of the inundated, accessible sites during July and August of 2002. We expected to have a list of more than 30 sites to choose from, but struggled to find 20 with access. We collaborated with Joel Ingram of Environment Canada and Steve Timmermans of Bird Studies Canada to provide 25 more sites on Lakes Erie and Ontario. Only fish data from those sites are included in this report. A no-cost extension was granted to accommodate those data yet to be processed. Invertebrate samples from Lakes Erie and Ontario are still being processed and will be provided to the GLC and cooperating investigators in May, 2003.

### *Sampling Procedures*

### *Description of Our IBI Development Methodologies Used from 1997-Present*

***Wetland Classification*** - Wetlands of the Great Lakes were classified into geomorphological classes that reflected their location in the landscape and exposure to waves, storm surges and lake level changes. For this study, we agreed to study fringing (lacustrine) open and protected wetlands. However, we continue to strive to develop metrics that extend across classes, lakes, and ecoregions since our ultimate goal is to keep the number of IBI's required to cover all five lakes to a minimum.

For invertebrates, open (lacustrine) wetlands were subdivided or analyzed along a continuum of exposure to wind and waves (Burton et al. 2002; Uzarski et al. submitted). These wetlands tend to form along bays and coves and leeward of islands or peninsulas. The more open the shoreline, the more energy the wetland is exposed to from waves and storm surges until a threshold is reached where wetlands can no longer persist. Our initial faunal research in Lake Huron suggests that a system can be developed that applies to all lacustrine wetlands despite the natural exposure gradient (Burton et al. 1999, Uzarski et al. submitted). However, the variation due to the exposure gradient must be accounted for when applying the sampling protocol. The location of the shoreline with respect to long shore current and wind fetch determines the type of wetland found along the shoreline (Burton et al. 2002), and there are marked differences in the preponderance of wetland types from Great Lake to Great Lake that have to be considered during development of indicators.

Great-Lakes wide studies of aquatic macrophytes indicate that similar geomorphic wetland types support distinctively different plant assemblages in geographically distinct ecoregions (Minc 1997: Minc and Albert 1998 and in press, Chow-Fraser and Albert 1998). Since our macroinvertebrate IBI is based on sampling all existing plant zones, we may eventually need to refine or adjust our IBI based on plant community distribution. Further resolution of classification is defined within our IBIs by including metrics to be used only under specific circumstances. For example, a suite of metrics are developed for use in wave swept bulrush zones of unprotected coastal wetlands, but these metrics may or may not vary from those to be used where dense vegetation or a peninsula dampens waves in the same class of wetlands.

***Chemical and Physical Measurements*** - Basic chemical/physical parameters were sampled each time biological samples were collected. Analytical procedures followed procedures recommended in Standard Methods for the Examination of Water and Wastewater (APHA 1985). Measurements included soluble reactive phosphorus (SRP), nitrate-N, ammonium-N, turbidity, alkalinity, temperature, DO, chlorophyll *a*, redox potential, and specific conductance. Quality assurance/quality control procedures followed protocols recommended by U.S. EPA and discussed in detail in the QAPP for this project.

***Sediment Sampling (Contaminants) and DELTs*** - Unconsolidated sediments were sampled with a 5 cm core and transferred to 4 L glass jars. Up to five samples per vegetative zone were collected from each wetland. Discrete samples were composited in the laboratory before analysis. Our intent was to analyze individual samples to determine spatial distribution of contaminants if composite samples exceeded Threshold Effect Levels (MacDonald et al 2000).

Sediments were analyzed for arsenic, cadmium, lead, chromium, mercury, copper, polychlorinated biphenyls, DDT homologs, PAH compounds, total organic carbon, grain size distribution, and ammonia by USEPA (1996) methods. DELTs were noted as fish were collected.

***Determination of Anthropogenic Disturbance*** - Wetlands that experience a wide range of anthropogenic stressors were chosen from each class or subclass. The extent of disturbance was determined using surrounding land use data in conjunction with water quality data and site-specific observations of dredging, point-source pollution, etc. Land use was determined from existing digitized maps (MIRIS 1978), topographic maps and personal observations. These data included parameters such as percent urban and agricultural area, number of adjacent dwellings, percent impervious surface, and number of connecting drainage ditches.

***Macroinvertebrates sampling*** - Macroinvertebrate samples were collected with standard 0.5 mm mesh, D-frame dip nets from late July through August. In previous studies, we have demonstrated that samples taken from ice-out through mid-July generally contain less diversity and a greater proportion of early instars of aquatic insects. The July-August time period is when emergent plant communities achieve maximum annual biomass and most insects are in late instar stages. Late instars are easier to identify than are early instars.

Macroinvertebrates were sampled from all inundated plant zones at each site including all major emergent and wet meadow zones. If more than one dominant plant association occurred at a particular depth, invertebrates were sampled from each.

Dip nets were systematically used to sweep through the water at the surface, through the middle of the water column and just above the sediment surface to ensure that an array of microhabitats were included. In the field, samples were placed in white pans, and 50, 100, or 150 invertebrates were collected by picking all specimens from one area of the pan before moving on to the next area until 150 invertebrates had been collected or one-half-person-hour of effort had been spent on picking. If 150 specimens had not been collected at the end of one-half-person-hour of effort, picking continued to the next multiple of 50 (50, 100 or 150). Special efforts were made to ensure that smaller organisms were not missed to compensate for a natural bias towards picking the easier to detect, larger, more mobile individuals. Plant detritus was sorted for a few additional minutes after the target number of specimens had been collected to ensure that sessile species were included in the sample. Three replicate samples were collected from each plant zone to obtain a measure of spatial variance within each plant zone.

Specimens were sorted to lowest operational taxonomic unit; this was most often genus or species but for some difficult to identify groups it was Family, Tribe, etc. Taxonomic keys such as Thorp and Covich (1991) and Merritt and Cummins (1996) were used for identification along with mainstream literature for species level. Accuracy was confirmed by expert taxonomists whenever possible.

***Fish sampling*** - Fish sampling was conducted with six fyke nets with square or rectangular openings either 0.5 m high x 1.0 m wide or 1.0 m high x 1.0 m wide leading into the series of hoops forming a funnel to the trap end. These nets were constructed of 12.5 mm or smaller mesh nets. Smaller nets were set in water approximately 0.25 m to 0.75 m deep, the larger nets were set in water depths greater than 75 cm. Nets were set adjacent to vegetation zones of interest with leads extending into the vegetation. Six 'minnow' or smaller fish traps were also placed in the vegetation itself in each vegetation zone for one net-night. Fish were identified and enumerated before being released. The occurrence of DELTs was noted.

***Plant Sampling*** – Past plant sampling was conducted along transects across wetlands, with several samples taken systematically in each vegetation zone (Albert et al. 1987, 1988, 1989, Minc 1997, Minc and Albert 1998). Water depth and type of substrate were recorded for each sampling point. The plant data from these earlier studies were analyzed to develop a regional classification of wetland plant communities (Minc 1997, Minc and Albert 1998, Chow-Fraser and Albert 1998). Plant data and a review of aquatic macrophyte literature were also utilized to develop proposed IBIs for aquatic macrophytes (Albert 1999).

During macrophyte sampling for this project, the focus was on evaluation of the proposed aquatic macrophyte IBIs (Albert 1999). Sampling was conducted along transects located perpendicular to the hydrological gradient. Five randomly located 0.5 $m^{-2}$ quadrats were sampled in each vegetation zone along each transect. The starting point for each transect was randomly located, beginning within 25 meters of the upland edge of the wet meadow zone, with sampling points located 25 meters apart. The location of each sampling quadrat around a sampling point was selected using randomly selected compass bearings and distances from 1 to 9 meters. Percent cover was estimated for each plant species in the sample quadrat; coverage was estimated for all emergent, floating, and submergent species. Substrate, organic depth, water depth, and water clarity (using secchi disk) were recorded. For most wetlands, sampling was restricted to the wet meadow and emergent/submergent zones. Where there was a wide submergent zone without emergent vegetation, five additional sampling points were included.

Aquatic-macrophyte data were summarized and proposed IBIs were evaluated for effectiveness in identifying gradients of human disturbance. The environmental gradients evaluated included amounts of urban and industrial development, nutrient enrichment, and water clarity. New metrics included turbidity, nutrient enrichment, fine sediment deposition, and invasive plants (including coverage and number of species).

Since overall species diversity is viewed by many as a good indicator of wetland quality or health, plant species diversity was evaluated in this study. Plant species diversity was evaluated by conducting a fifteen-minute or longer random observation in each plant zone. Many extensive coastal wetlands required a longer random walk to adequately assess habitat and species diversity. This was especially the case for wet meadow zones, where sampling was often slow. For most wetlands, joint sampling of submergent and emergent zones during the fifteen-minute random walk was adequate. In the emergent/submergent zone, the random walk required use of a rake to guarantee an adequate sampling of submergent species. Floristic Quality Indices (FQI) were carefully evaluated as another measure of native species diversity and site quality.

We evaluated invasive and exotic plants by (1) identification of dense monoculture stands with aerial photos followed by ground verification and (2) determination of presence of invasive plants in the 5 random quadrats sampled per transect and (3) noting the number of invasive species along each transect sampled.

***Identify and combine metrics into an IBI*** - Initially, correspondence analyses of invertebrate and fish community composition were used to determine if reference sites could be separated from impacted sites. When they could, individual taxa containing the most inertia responsible for separation were deemed potential metrics. Mann-Whitney U tests were used to determine whether values for these potential metrics at reference sites were significantly different from values at impacted sites. If they were, these metrics were included in the IBI. Pearson Correlation analyses was also used to link state with stressor by relating potential metrics to specific anthropogenic disturbances. Finally, stressor-land use relationships were explored to aid in management decisions.

We used medians in place of means for measuring assemblages of invertebrates. Occurrence, distribution and population size of invertebrates are highly variable in time and space. Highly variable data increases the chance that an area sampled may be unusually depleted or concentrated in constituents of a metric. If this occurs, it may be that the area is: (1) more or less isolated from anthropogenic disturbance than is the rest of the wetland, (2) receiving more or less disturbance than is typical for the entire wetland or plant zone, or (3) characterized by some unique "natural" chemical/physical component of the environment not found in the rest of the wetland. Regardless of cause, data from such unique areas are outliers and not representative of the entire wetland. Using the median in place of mean as a measure of central tendency dampens the influence of these outliers.

***Continued Testing and Validation of IBI*** - After developing the preliminary IBI (Burton et al. 1999) based on data from 1996 and 1997, we have continued to collect data from a subset of sites of known anthropogenic disturbance in order to test the ability of the IBI to separate impacted from reference wetlands over time and to check the calibration of the preliminary IBI. We have also tested the original IBI by collecting data from additional wetlands experiencing a range of anthropogenic disturbance and using these data to test whether the IBI could successfully separate new sites into impacted and reference sites. Data from new sites and repeated sampling of sites have also been used to search for new potential metrics. We continued this process with data collected from the 20 fringing wetland sites sampled in this study in 2002 and began analyses to develop a fish based IBI based on data from this and other projects.

## Results

### 1. Monitoring Costs:

Based on our 2002 costs, a monitoring program utilizing biological indicators exclusively or in conjunction with chemical/physical analyses would be cost-effective. The following are estimates of costs incurred during start up and operation of a biological and chemical/physical monitoring program. Since costs for equipment and some items only occur during program start-up, we list ‘initial’ and ‘operating’ costs separately. Operating cost estimates are based on our costs of collecting data from 20 wetlands in 2002 with a mean of 2.2 plant zones sampled per wetland. The number of plant zones sampled per wetland in 2002 varied from one to five. Sampling costs would change if number of zones sampled per wetland changed.

***Initial Costs to Purchase Needed Equipment and Supplies***

*Sediment Core Sampling for Contaminants*

| Item | Number | Cost (ea) | Total Cost |
|---|---|---|---|
| Piston Core Device | 2 | $90.00 | $180.00 |
| Large SS Mixing Bowls | 2 | $25.00 | $50.00 |
| SS Spoons | 2 | $8.00 | $16.00 |
| Sample Bottles Plastic (cs of 90) | 1 | $98.80 | $98.80 |
| Sample Bottles Glass (cs of 12) | 5 | $50.44 | $252.20 |
| Total Initial Cost for Field Supplies | | | $597.00 |

*Sediment Laboratory Analysis*

The costs below represent an estimate of initial investments needed for equipment to perform required analyses assuming availability of basic laboratory facilities.

| Item | Number | Cost (ea) | Total Cost |
|---|---|---|---|
| Gas Chromatograph | 1 | $30,000.00 | $30,000.00 |
| Gas Chromatograph/Mass Spectrometer | 1 | $125,000.00 | $125,000.00 |
| Mercury Analyzer | 1 | $35,000.00 | $35,000.00 |
| ICP | 1 | $75,000.00 | $75,000.00 |
| TOC Analyzer | 1 | $30,000.00 | $30,000.00 |
| Ammonia Electrode and ion meter | 1 | $8,000.00 | $8,000.00 |
| Soxhlet extraction system | 1 | $15,000.00 | $15,000.00 |
| Sample Concentrator | 1 | $15,000.00 | $15,000.00 |
| USGS Seives | 10 | $95.00 | $950.00 |
| Total Initial Cost for Field Supplies | 1 | | $333,950.00 |

Set up costs may be prohibitive for small sampling programs. An alternative would be to have samples analyzed commercially. Cost estimates below are based on typical commercial fees. Fees charged by universities or governmental agencies are often 30%- 50% less.

| Parameter | Cost per Test |
|---|---|
| % Solids | $10.00 |
| Grain Size | $45.00 |
| % TOC | $25.00 |
| PAH Compounds | $120.00 |
| DDT Compounds and PCBs | $150.00 |
| Ammonia | $20.00 |
| Chromium | $10.00 |
| Lead | $10.00 |
| Cadmium | $10.00 |
| Mercury | $25.00 |
| Total | $425.00 |

*Invertebrate Sampling*

Equipment/Consumables (number in parentheses is the suggested number of items to outfit a crew of three):

| | |
|---|---|
| Dip nets: | $47.20 (2) |
| Plastic pans: | $7.95 (3) |
| Forceps: | $2.65 (4) |
| Jars (per dozen): | $5.95 (1) |
| Ethanol (per 5 gallons): | $49.50 (1) |
| Total initial costs for macroinvertebrate sampling: | **$137.10** |

*Invertebrate Processing*

Equipment/Consumables:

| | |
|---|---|
| Microscope | $500.-$900.(1 or 2) |
| Vials (per dozen): | $3.70(20) |
| Taxonomy books: | |
| *Merritt and Cummins* | $77.95(1) |
| *McCafferty* | $71.95(1) |
| Total initial costs for macroinvertebrate processing: | **$723.90-$2023.90** |

*Fish Sampling*

Equipment/Consumables:

| | |
|---|---|
| Fyke nets | |
| Large | $498.(3) |
| Small | $351.(3) |
| Fish identification book | $11.95 |
| Total initial costs for fish sampling: | **$2558.95** |

*Plant Sampling (based on 33 wetland sites)*

Equipment:

| | |
|---|---|
| Partial costs for underwater camera and GPS unit: | $236.00 |
| SPSS Software license for analyses: | $103.50 |
| Film, plant bags and tags, and waterproof paper: | $ 95.94 |
| Total | $435.44 |

Other non-travel and non-salary charges:

Conference calls for methodology, protocol discussions: $ 38.23

Much of the equipment used is already owned by MNFI. Incorporations of these costs could add considerably to sampling. These costs include:

- Canoe or boat (Not needed at most sites) (estimated $500)
- Motor (Not needed at most sites) (estimated $500)
- Rake (for submergent plant sampling) (estimate $15.)
- PVC sampling frame (estimate $10)
- 50 meter polytape (estimate $25 to $75)
- Probe for sediment and water depth ($10)
- Compass ($50)
- Waders ($80)
- Clipboard ($5-20)

- Plant press and blotters ($90)
- Taxonomic texts ($350)

*Chemical/Physical Analyses*

Turbidity, temperature, dissolved oxygen, chlorophyll *a*, redox potential and specific conductance can be measured *in situ* with a variety of commercially available instruments. For this research, we used Hydrolab Model DataSonde4a. (approx. $12,000). However, less expensive instruments could be used to measure most parameters. A number of methods are acceptable for measuring soluble reactive phosphorus (SRP), nitrate, ammonium, chloride, sulfate and alkalinity (APHA). Instrumentation requirements and associated costs vary greatly among methods as does amount of labor required. An alternative to the relatively large initial cost for instrumentation would be to have water samples analyzed by a contract laboratory. Typical charges for measuring SRP, nitrate, ammonium, chloride, sulfate and alkalinity by a university or commercial laboratory range from $20 to $100 per sample ($40-$200 per wetland).

***Operating Costs***

*Sediment Analysis*

The costs for sediment analysis listed below assume the availability of necessary equipment and facilities to perform required tests. Costs were based on a batch of 50 samples.

| | Person Hours | Rate ($/hr) | OH | Fring | Labor | Supplies | Total | Cost/Test* |
|---|---|---|---|---|---|---|---|---|
| Grain size | 180 | $7.75 | $2.71 | $2.95 | $13.41 | 55 | $2,468.35 | $49.37 |
| % Solids | 8 | $7.75 | $2.71 | $2.95 | $13.41 | 15 | $122.26 | $2.45 |
| % TOC | 32 | $7.75 | $2.71 | $2.95 | $13.41 | 25 | $273.00 | $5.46 |
| Total PAH Compounds | 40 | $20.67 | $7.23 | $7.85 | $35.76 | 1000 | $1,826.80 | $36.54 |
| DDT Homologs and PCB | 40 | $20.67 | $7.23 | $7.85 | $35.76 | 1000 | $1,826.80 | $36.54 |
| Ammonia | 56 | $7.75 | $2.71 | $2.95 | $13.41 | 25 | $459.00 | $9.18 |
| Chromium | 40 | $20.67 | $7.23 | $7.85 | $35.76 | 50 | $876.80 | $17.54 |
| Lead | 40 | $20.67 | $7.23 | $7.85 | $35.76 | 50 | $876.80 | $17.54 |
| Cadmium | 40 | $20.67 | $7.23 | $7.85 | $35.76 | 50 | $876.80 | $17.54 |
| Mercury | 32 | $7.75 | $2.71 | $2.95 | $13.41 | 50 | $298.00 | $5.96 |
| Total Cost | | | | | | | $9,904.61 | $198.09 |

* Based on 50 analyses

Costs for sampling are based on the collection of 6 core samples per vegetation zone and preparing one composite for laboratory analysis. Travel was 36 cents per mile per vehicle. Additional travel expenses included lodging for personnel.

*Core Sampling*

| | |
|---|---|
| Labor (per wetland): | |
| 1 vegetation zone | 2 person-hours |
| 2 vegetation zones | 4 person-hours |
| 3 vegetation zones | <u>6 person-hours</u> |
| Total for average wetland at $10.00/person-hour: | $50.00 |

*Macroinvertebrate Sampling*

| | |
|---|---|
| Labor (per wetland): | |
| 1 vegetation zone | 3 person-hours |
| 2 vegetation zones | 4½ person-hours |
| 3 vegetation zones | <u>6 person-hours</u> |
| Total for average wetland at $10.00/person-hour: | **$50.00** |

*Macroinvertebrate Processing*

| | |
|---|---|
| Labor (in the laboratory with trained personnel): | |
| per wetland | 24 ¾ person-hours |
| Total for average wetland at $10.00/person-hour: | **$247.50** |

*Fish Sampling*

| | |
|---|---|
| Labor (for typical wetland site, with 6 fyke nets): | 9 person-hours |
| Total for average wetland at $10.00/person-hour: | **$90.00** |

*Plant Sampling*

| | |
|---|---|
| Field sampling (per 2 zone wetland): | |
| 99 hrs. X $40.88 | $4047.12 |
| 16 hrs. X $12.96 | $ 207.36 |
| Plant identification (evenings) | |
| 41 hrs. X $40.88 | $1675.88 |
| 20 hrs. X $12.96 | $ 259.20 |
| Travel time | |
| 43 hrs. X $40.88 | $1757.84 |
| 10 hrs. X $12.96 | $ 129.60 |
| Training calls, etc. | |
| 12 hrs. X $40.88 | $ 490.56 |
| Data Input | |
| 80 hrs. X $12.96 | $1036.80 |
| Data analysis | |
| 124 hrs X $12.96 | $1607.04 |
| *Data Review* | |
| 20 hrs X $40.88 | $ 817.60 |
| *Administration* | |
| 13 hrs X $40.88 | $ 531.44 |
| **Total labor** | **$12,560.44** |
| (Total labor cost per sample site: | $380.62) |
| Presentations | |
| 24 hrs X $40.88 | $ 981.12 |
| Travel costs: | |
| 3571 miles X $.36 | $1285.56 |
| bridge tolls | $ 6.00 |
| **Total mileage costs** | **$1291.56** |
| (Total mileage/toll costs per sample site: | $39.14) |
| Room and board | |
| **Total room and board** | **$ 175.68** |
| (Total room and board per sample site: $5.32*) | |
| **Approximate total cost for collection and analysis of plant data** | **$15,444.24** |
| Approximate total cost per sample site (33 sites) | $ 468.00 |

* Room and board is low because it was shared with other projects sampled during the same trips.  Typically the room and board cost would be at least $12-15. per site.

*Chemical/Physical*

| | |
|---|---|
| Labor (for typical wetland site) | 1 ¾ person-hours |
| Supplies: | $21.00 |
| Total for average wetland at $10.00/person-hour: | **$38.50** |

*Travel*

Travel was 36 cents per mile per vehicle. Additional travel expenses included meals and lodging for personnel.

Equipment and supplies required for macroinvertebrate and fish sampling/processing cost significantly less than equipment and instrumentation required for chemical/physical analysis. However, labor expenses for processing macroinvertebrate samples is higher than labor expenses for chemical/physical analysis. It is likely that agencies that would utilize a biological monitoring program would already possess most of the equipment required for macroinvertebrate and fish sampling/processing (i.e. dip nets, fyke nets, microscopes and taxonomic keys), but probably not have the instruments required for extensive chemical/physical sampling.

Instrumentation requirements and the associated expenses for chemical/physical analysis could be prohibitive for agencies that do not currently possess the necessary instruments. For this research alkalinity was measured by titration with acid, SRP and ammonium were measured with a continuous flow auto-analyzer (Bran-Leubbe model: AutoAnalyzer3). Nitrate, chloride and sulfate were measured with an ion chromatograph (Dionex Corp.). Other protocols, especially those using a single sample spectrophotometer, may involve higher labor expenses than those that we incurred while using semi-automated instruments. In a monitoring program, costs associated with chemical/physical water quality could vary greatly and selection of protocols would depend largely on available instrumentation and specific expertise of personnel.

Travel distances and associated expenses would vary considerably among sites and crews. However, because travel expenses would be incurred regardless of monitoring protocol, we suggest that travel expense not be a significant factor in determining the feasibility of implementing an IBI for monitoring wetland integrity. Furthermore, because the biota integrate ambient conditions over time, a biological-based monitoring program would require a less frequent sampling regimen than other types of monitoring.

**2. Measurability:**

*Biological Samping*

*Invertebrates and fish:* In most cases, field and laboratory staff acquired the expertise necessary to carry out biological sampling at the beginning of the field season. However, each sampling crew had either a principal investigator or a previously trained technician to supervise sampling and invertebrate processing. This is likely to be similar to the situation that other agencies will experience when using the monitoring protocol. In very few cases, errors were made by field staff in regards to sampling protocol. Potential deviations from protocol may include error in differentiating between vegetation zones (i.e. ‘inner’ and ‘outer’ Scirpus zones), bias towards one particular type of macroinvertebrate while picking (i.e. more mobile or larger organisms) and misidentification of fish. We recommend in-the-field training of field staff to ensure proper understanding of biological sampling protocol and feel that minimal pre-sampling training would be necessary when implementing such a monitoring program.

Identification of macroinvertebrates to lowest operational taxonomic unit was conducted in the laboratory by technicians who had either taken an undergraduate course in aquatic entomology or received on-the-job training. Staff members who had previous training and/or experience were much more efficient at processing macroinvertebrate samples than staff that had no previous training. Agencies implementing this monitoring protocol would benefit from having staff acquire some taxonomic training, although, formal instruction on macroinvertebrate taxonomy should not be considered a prerequisite to using the protocol.

*Plants:* The Michigan plant sampling was done partially by a team and partially by D. Albert working alone. The final plant identifications were done by D. Albert, with Dr. Reznicek checking a few difficult specimens. Plant sampling on Lake Ontario was done by Joel Ingram of Environment Canada and Greg Gravas, Wildlife Conservation Biologist for the Canadian Wildlife Service. Long Point sampling was done by Dr. Jane M. Bowles. Both sets of Canadian researchers were responsible for their own plant identification.

Plant sampling in the coastal wetlands requires a familiarity with a relatively diverse monocot flora of sedges, rushes, spike-rushes, bulrushes, grasses, pond weeds, and many other genera, often in sterile condition. Typically, some specimens require further examination with a microscope for final identification. Coastal wetland sampling will require someone who has worked extensively with aquatic plants.

One question we hoped to answer was related to the effectiveness of collecting plant data along transects versus timed random walks. For computations based on only the species identification and number of species, both approaches seem to be relatively similar in effectiveness. This conclusion is based on computing Coefficients of Conservation and FQIs at each site, from data collected during a timed random walk and from transect sampling. The FQIs from these two approaches were typically quite similar (See Appendix II.1 (Figure 1)). However, any analyses based on quantification of plant abundance or spatial distribution require some form of plot sampling. Given a choice of using only one type of sampling, plot sampling along transects provides added analytic opportunities.

The identification of dense monocultures prior to sampling was not carried out, as funding was not in place to allow for this sampling prior to the field season. Sampling sites were visited without prior photo interpretation, using the random walks and reconnaissance visits to identify areas for the transect sampling. The specific location of transects was then randomly located. Subsequent photo interpretation with the most recent black and white photography available from the Michigan DNR, 1998 and 1999 photography, demonstrated that at most sites differentiation of specific exotic or invasive native species on the basis of the photography could not be done consistently by the author. The reason that the black and white photography was not effective for identification of monocultures of exotics is two-fold. First, the major exotics and invasive native species respond to water level fluctuations, creating different photo signatures. Cattail beds are dense and uniform in texture during high water periods, but in low water periods the cattails die back and are mixed with a diversity of asters, goldenrods, shrubs, and other species, creating a very different texture to the stand, no longer exhibiting the uniformity that most of us consider characteristic of cattail beds. Second, color infrared photography (MI DNR 1978) appears to be more effective for identifying beds of exotic species based on different color signatures (characteristics).

While state-wide black and white aerial photography is not necessarily adequate for identification of exotics, low elevation photography taken specifically for the purpose of identifying aquatic vegetation would likely be more adequate for this purpose. The constraining issue for low level photography is high cost.

Measurability of human disturbance was probably the weakest part of this study. One problem area is that many of the variables that were identified could not be readily investigated in the field without much more time consuming preliminary field sampling. Culverts, number of out buildings and dwellings, small ditches, and other landscape variables are not accurately quantifiable from the site. On some sites, access is partially limited by private property, as well as the logistics related to viewing the entire site. Determination of types of road can be time consuming for large sites. For many variables, this problem was solved by utilizing aerial photography or imagery, which allowed for a more accurate evaluation of area as well. The aerial photography presently provides more accurate evaluation of land use categories, but it requires more time to accurately quantify the variables. Some of the most recent satellite imagery appears to have potential for this evaluation, while older imagery is inadequately ground (water) checked and falsely classifies many coastal meadows as agricultural lands.

Probably the greatest challenge related to land use is evaluating the relative significance of various types of disturbance on vegetation and other biotic variables. Which is more detrimental to the health of a wetland, twenty houses, a coastal highway, or an agricultural drain?

*Chemical/Physical Sampling*

Turbidity, temperature, dissolved oxygen, chlorophyll *a*, redox potential and specific conductance) were measured *in situ* with a multi-probe instrument (Hydrolab Corp. DataSonde4a). Field staff members that were inexperienced with the instrument were able to use it with minimal instruction. However, maintenance and calibration of the multi-probe was left to either a principle investigator or the supervising technician.

Chemical/physical parameters that were measured in the laboratory (alkalinity, SRP, ammonium, nitrate, chloride and sulfate) required the most analytical expertise and equipment. If a monitoring program were to include chemical/physical sampling, specific training and equipment requirements would vary with protocols.

**3. Basin-wide applicability:**

*Faunal sampling:* No modifications to sampling were required to implement our protocols at any of our sites in northern Lake Huron, northern Lake Michigan or Saginaw Bay. We did not anticipate problems of applicability associated with any of our monitoring protocols since we had used them previously in every geomorphic wetland type delineated by the Great Lakes Coastal Wetlands Consortium PMT.

The analytical methods used for this research have basin wide acceptance for routine monitoring and environmental research. This ensured both acceptance and applicability of the data and the methods.

*Plant sampling:* For plant sampling, it is not clear that a single IBI is appropriate for the entire basin. One reason for this is that fringing open wetlands are characterized by much lower plant diversity than protected wetlands. Placed in terms of Floristic Quality Indices (FQIs), a high FQI for a fringing wetland is in the range of 25, while that of a protected embayment is in the mid to high 30s. Fringing wetlands typically have only an emergent zone with a very depauperate wet meadow zone, while a protected wetland contains a well developed wet meadow, emergent, and sometimes submergent zone.

Geographic differences account for major differences in plant species dominance. For example, on Lake Huron, the dominant emergent species in northern protected wetlands is hardstem bulrush (*Scirpus acutus*), in many fringing northern wetlands (northern fens), spike-rush (*Eleocharis rostellata*), and in fringing wetlands along Saginaw Bay, three-square (*Schoenoplectus pungens*). Associate species in each of these geographic types are also different, making it difficult to assume the changes to each of these type resulting from human land use will be similar enough to allow them to be lumped.

One of the differences associated with different wetland types is difference in zone width. Typically, our transects of coastal wetlands have been conducted perpendicular to the shoreline to increase the diversity of species encountered in the zones (wet meadow, emergent, and sometimes submergent). In some of the open fringing wetlands, the zones were too narrow to allow for this, and transects were placed in zones parallel to the shoreline. This change in sampling protocol did not appear to alter the results of sampling significantly.

**4. Availability of Complementary Existing Research or Data:**

To the best of our knowledge, Uzarski and Burton have accumulated and maintain the largest macroinvertebrate data set ever collected for Great Lakes Coastal Wetlands and Albert has collected and maintains the largest data set on macrophytes. With collaborators from Ontario, we collected a single year of fish data in 2002 from 61 sites as part of this study. We have been sampling fish from a subset of these systems in Lakes Michigan and Huron since 1999 and have data on fish for a few sites dating back to 1994. We are also aware of and share information with other leading coastal wetlands researchers such as Doug Wilcox, Pat Chow-Fraser, Gerry Niemi, Tom Simon, and Mary Moffet. For aquatic plants, we also utilized the data sets collected by Jane Bowles, Greg Grabas, and Joel Ingram. Additional data sets are available from Mary Moffet and her research collaborators at the Duluth EPA Lab.

The USEPA Great Lakes National Program Office maintains a large database on water quality and sediment chemistry. The use of the same reference methods in this project ensured that program data would be complementary and comparable to other locations in the basin.

**5. Indicator Sensitivity to Wetland Condition Changes:**

*Contaminated Sediments*

Sediment monitoring provided a baseline assessment of the level of anthropogenic chemicals present (Table 1). Since wetlands can function as sinks for contaminants, sediments represent an ideal media for monitoring. The analytical techniques are sensitive enough to detect small changes in concentration. Subsequent monitoring can, therefore, be used to determine if accumulation of anthropogenic chemicals is increasing or decreasing in a particular wetland. The data could also be useful to compare the status of anthropogenic enrichment in wetlands throughout the Great Lakes Basin.

*DELTs*

We examined all fish captured in this study for the presence of DELTs. They were extremely rare with only three recorded out of several hundred fish collected and examined. Even these three DELTs could have been injuries sustained while fish were in the net. At Shepard Bay, we collected a single pugnose shiner from the Outer *Scirpus* zone with a mouth deformity. A black bullhead had a lesion on its ventral side at Cedarville Bay and a Redhorse sucker had an eroded caudal fin at Portage River collected from the *Eleocharis* zone.

DELTs are not likely to serve as good indicators using our fishing techniques because the majority of fish captured were: (1) migratory (in and out of the wetland), (2) short-lived species, or (3) young of the year. These fish may not have been exposed to contaminants long enough to produce visible effects. Therefore, we do not recommend DELTs as a tool for determining wetland health.

*Invertebrate Data*

*IBI scores*- We applied our modified IBI (modified from Uzarski et al. (submitted) (Appendix 1) to enable family-level macroinvertebrate identification) to macroinvertebrate data from twenty wetland sites. When the modified IBI was calculated using family level data, sites separated along a perceived gradient of anthropogenic disturbance. IBI scores ranged from 86.1% of the total points possible at the Cedarville site to 40.9% at the Bradleyville Rd. site (Tables 2 and 3). The four sites that scored highest fell into the 'mildly impacted' category, while nine fell into the 'moderately impacted' category. The remaining seven sites were categorized as 'moderately degraded'. Three of the four sites that scored in the 'mildly impacted' range were northern Lake Michigan sites (Rapid River, Garden Bay and Ogontz Bay). The remaining four northern Lake Michigan sites were shown to be more degraded with the Big Fishdam, Ludington Park and Pt. St. Ignace sites all falling into the 'moderately impacted' category and the Escanaba site falling into the 'moderately degraded' category. All northern Lake Huron sites, with the exception of Cedarville, fell into the 'moderately impacted' category. As expected, Saginaw Bay sites had the lowest IBI scores with six of the seven sites falling into the 'moderately degraded' category (Table 2). The Jones Rd. site was among these six sites. Because Typha was the only vegetation zone found at the Jones Rd. site, and our Typha zone specific metrics are still being developed, we scored this site using the Inner Scirpus metrics. Therefore, the score for this site may not be an accurate reflection of its biotic integrity. Wigwam Bay was categorized as 'moderately impacted' being placed among the northern Lake Huron sites. This was expected *a priori* because Wigwam bay was located closest to the outer bay of Saginaw Bay where anthropogenic disturbances would be diluted. This site had a largely forested watershed and was located furthest from the mouth of the Saginaw River, a known source of pollution for Saginaw Bay. Tables 2 and 3 show IBI metric scores and site ranking based on the modified IBI.

Invertebrates from eight of these sites were identified to the generic level, thus our unmodified IBI (u-IBI) (Uzarski et al. submitted) was applied to these (Tables 4 and 5) along with the modified IBI. The ranked order of sites produced by the u-IBI with data at the higher taxonomic resolution was identical to the order produced by the modified IBI using family-level macroinvertebrate data. Once again, the Cedarville site ranked highest, scoring 86.1% of the total points possible, while the Vanderbilt Park site ranked lowest at 46.7%. Three sites, Cedarville, Mackinaw Bay and Shepard Bay fell into the 'mildly impacted' category and Pt. St. Ignace and Wildfowl Bay fell into the 'moderately impacted' category. Allen Rd. and Jones Rd. were placed into the 'moderately degraded' category while Vanderbilt Park was the only wetland to score in the 'degraded' range. Again, the Jones Rd. site was scored with Inner Scirpus metrics, and therefore, may be misrepresented.

Anthropogenic disturbance was characterized using analyses of 11 water chemical/physical parameters for each plant zone in each site (Table 6). These were used in conjunction with five land-use/cover parameters calculated from a 1 km buffer around each site (Table 7). Principal components analysis (PCA) of all 16 parameters was of little value in partitioning sites along a gradient of anthropogenic disturbance (Fig. 1). However, a PCA including just the 11 water chemical/physical parameters revealed a gradient of anthropogenic disturbance characterized by increasing Cl, SpC, $NO_3$ and $SO_4$ in PC 2 (which explained 23.6% of variability in the data) (Fig. 2). Chemical/physical parameters that could be perceived as indicators of anthropogenic disturbance did not contribute strongly to PC 1. Therefore, PC 2 scores were used to characterize water quality among wetland sites. The Jones Rd. site scored highest in PC 2 and had the highest SpC, Cl and $SO_4$ of the 20 sites. Saginaw Bay sites generally scored highest in PC 2 while sites of northern Lake Huron and northern Lake Michigan scored lowest (Fig. 2).

Since the PCA was conducted on chemical/physical data from individual plant zones, within-wetland spatial variability could be examined. In most cases, plant zones of a given site plotted near one another. Wet meadow zones of the St. Ignace, Shepards Bay and Big Fishdam sites, however, had significantly higher PC 2 scores than did their respective Inner and Outer Scirpus zones, suggesting pronounced spatial heterogeneity in water quality at those sites.

PCA of five land-use/cover parameters separated sites in three directions based on agriculture/meadow/idle land, developed land/road density and forested land (Fig. 3). The Allen Rd. and Vanderbilt Park sites were characterized by a high proportion of surrounding agriculture while the Jones Rd. and Ludington Park sites were characterized by a high proportion of surrounding developed land and high road density. Sites that had high proportions of surrounding forested land included Big Fishdam, Ogontz Bay and Moscoe Channel. Most sites, however, could not be characterized as having a predominant land-use/cover type. Hence, anthropogenic disturbance could not be determined directly from the PCA of land-use/cover.

Pearson correlations between PC 2 scores of chemical/physical data and IBI scores (% possible) were conducted to test both IBIs. A significant correlation ($p<0.05$, $r=-0.503$) existed between PC 2 scores and IBI scores of individual vegetation zones using the modified IBI with family-level macroinvertebrate data (Fig. 4). A Pearson correlation was also conducted between IBI scores and the means of PC 2 scores for each site (integrating all vegetation zones). This correlation was also significant ($p<0.05$, $r=-0.622$) (Fig. 5).

Pearson correlations were also conducted for sites where lowest operational taxonomic unit data were available. The correlation was significant ($p<0.05$, $r=-0.599$ between u-IBI scores for individual plant zones and corresponding PC 2 scores. The best correlation ($p<0.05$, $r=-0.93$) was between mean PC 2 scores (means of all plant zones/site) and site u-IBI scores calculated using lowest operational taxonomic unit (Fig. 6). The Jones Road site was excluded from analysis.

Significant correlations between PC 2 scores and IBI scores showed that the IBI functionally ranked sites along a gradient of anthropogenic disturbance. In this case, PC 2 was composed primarily of Cl, SpC, $NO_3$ and $SO_4$. These parameters can be considered surrogates for anthropogenic disturbance related to runoff from urban or agricultural areas.

Both IBIs separated more-impacted sites of Saginaw Bay from reference sites of northern Lake Huron and northern Lake Michigan. However, Wigwam Bay, the least impacted Saginaw Bay site because of its distance from the mouth of the Saginaw River and proximity to the less polluted outer bay, scored among the northern sites. The Pinconning and Wildfowl Bay sites were also a significant distance from the outlet of the Saginaw River and near the outer bay, and their respective IBI scores also reflected better water quality. The PCA did not separate Wigwam Bay, Wildfowl Bay and Pinconning from other outer Saginaw Bay sites suggesting that chemical/physical data alone could not account for a gradient of water quality in Saginaw Bay.

The Escanaba site had the lowest IBI score of any northern Lake Huron or Lake Michigan site. This low score reflects impacts on this wetland from the Escanaba River which is dammed and has a paper mill near its mouth and the expansive urbanization and industry of Escanaba. The Ludington Park site was adjacent to an urban residential area/park and near the port facilities for Escanaba and scored among the lowest three northern sites. The IBI score of the Ludington Park site may have been confounded by the morphology of the wetland. The Scirpus at this site was designated as 'Inner Scirpus' even though the site had a substantial fetch. Despite the fetch, the Scirpus at the Ludington Park site was partially protected by a barrier sand bar and was very dense, a characteristic of an inner Scirpus zone. The Scirpus grew in dense 'islands' unlike the vegetation zonation at any other site. This relatively unique setting makes this particular vegetation zone difficult to categorize. While this site demonstrates the problem of classification of plant zones when these zones are not discrete or are unique, the IBI still ranked the Ludington Park site as predicted by the chemical/physical analyses. Furthermore, recalculation of the IBI score for the Ludington Park site with the Scirpus islands classified as an outer Scirpus zone did not change the ranked order of sites suggesting that the IBI is robust enough to handle such discrepancies.

The Jones Rd. site was the only site sampled that did not include either a Scirpus or wet meadow zone. Since our current IBI depends on these types of vegetation (our Typha zone metrics are currently being reevaluated and improved), we could not accurately describe the Jones Rd. site. However, in our research on Great Lakes fringing wetlands, we have seen very few sites that did not contain either a Scirpus or wet meadow zone. In the case of Jones Rd., we scored the Typha zone as Inner Scirpus, which placed the site among the other moderately-degraded Saginaw Bay sites. The chemical/physical nature of the Jones Rd. site also suggests that the site is one of the most degraded sites sampled.

The u-IBI for lowest operation taxonomic units, as well as the modified IBI, ranked the Cedarville site as the most pristine of the 20 wetlands. However, field observations, and studies over the past six years indicate that the Cedarville site is impacted by a number of anthropogenic inputs (e.g. Burton et al. 1999, Kashian and Burton 2000). The wetland is adjacent to the city of Cedarville, adjacent to a busy boat channel and receives sewage effluent twice per year. The sediment at the Cedarville site appeared heavily organic and the Scirpus community was mixed with dense duckweed (*Lemna sp.*) mats. Analysis of the chemical/physical nature of the Cedarville site, however, did not reflect the perceived anthropogenic disturbance and was consistent with the IBI score, which showed the site to be relatively pristine.

*Fish Data*

We were able to include fish data from 61 sites spanning all five Great Lakes in our analyses (5 Superior, 18 Michigan, 13 Huron, 13 Erie, and 12 Ontario) by including the data collected by our collaborators, Joel Ingram and Steve Timmermans from Environment Canada and Bird Studies Canada respectively and our data on Lake Superior and additional Lake Huron and Lake Michigan sites from a separate project funded by the Michigan Great Lakes Protection

Fund. All of the inundated vegetation zones were fished in each wetland providing us with 15,263 fish from seven different plant zones (104 observations after combining replicate plant zones within wetlands) with 260 total net-nights fished. Our objective for this portion of the project was to determine if fish community composition was being structured based on lake to lake differences among the Great Lakes (Superior, Michigan, Huron, Erie, and Ontario), ecoregion (eastern Lake Superior-northern Lake Huron, Saginaw Bay-Lake Huron, northern Lake Michigan, northeastern Lake Michigan, southeastern Lake Michigan, Long Point-Lake Erie, western Lake Ontario, and eastern lake Ontario), wetland type (protected embayment, open lacustrine, barrier beach, and drowned river mouth), vegetation type (bulrush, spikerush, wild rice, lily, pickerel weed-arrowhead-arrow arum, burreed, and cattail), or chemistry and land use. The ultimate goal was to determine the feasibility of developing a Great Lakes basin wide IBI using key fish taxa that could be used without regard to ecoregional, lake to lake differences, etc.

We included fish data and the accompanying SRP, $NH_4$, $NO_2/NO_3$, SO4, Cl, DO, temperature, turbidity, sp. conductance, pH, alkalinity, Redox potential, and land use/cover data in our analyses. We ran PCA using only the abiotic data to first determine if our sites ordinated on any of the levels of interest (Lake, ecoregion, wetland type, or vegetation zone). Results of these analyses (Figs. 7 and 8) showed that vegetation zone was the single most important factor ordinating the sites based on these chemical/physical and adjacent land use data. The sites grouped into three major categories: 1) bulrush sites with low respiration and relatively high proportions of adjacent forests; 2) high nutrient and high percentage of adjacent agriculture cattail sites, and finally, 3) cattail sites with relatively high urbanization and urban runoff such as chloride (Fig. 8).

We then performed correspondence analyses using the fish data to determine if those data alone grouped sites at any of our chosen levels (Lake, ecoregion, wetland type, or vegetation zone). Initially, rare taxa were removed from the data set leaving 42 species in the analyses. Bowfin and black bullhead overwhelmed the first and second dimensions of the analysis respectively (Fig. 9). These taxa tend to school and our nets happened to catch large schools at several sites. We observed large schools of these taxa at most of our sites, and therefore, could justify removing them from our subsequent analyses since we could attribute these large catches at a portion of our sites to happenstance alone. We continued this process, documenting the taxa removed and the justification for removal until 26 species remained (See Table 8 for fish taxa). Our goal was to use these iterations to reduce the number of taxa to a group that could represent a community typical of coastal wetlands of all five Great Lakes, and therefore, evenly distribute the sites in two-dimensional space. This even distribution of sites could then reveal the underlying factor(s) responsible for characterizing fish community composition in Great Lakes coastal wetlands, and in turn could be used to establish indicator taxa for these systems. The 26 species separated the sites based on vegetation zone similar to the PCA (Fig. 10). Pearson correlation was then used to relate CA dimensions, or fish community composition, to PCs, or chemical/ physical and land use/cover data. A significant correlation ($r=0.398$, $p < 0.001$) existed between $CA_1$ and $PC_1$ establishing a relationship between fish community composition and chemistry and land use. We then superimposed our four levels as a third dimension over this relationship to discover that our chemistry and land use data were most closely related to vegetation zone (Fig. 11).

In conclusion, plant community zonation was the most important variable associated with fish community composition, regardless of lake, ecoregion, or wetland type. Plant community zonation was most likely determined by hydrologic variables such as depth and duration of inundation over the growing season and across annual variations in lake levels. Within a particular hydrologic regime, nutrient concentrations and adjacent land use/cover as well as fetch and pelagic mixing probably were the driving variables associated with plant community dominance. Within specific vegetation zones, fish community composition seemed to respond to nutrient concentrations and/or fetch and pelagic mixing. However, this response could be correlative since fetch and pelagic mixing contribute to plant zonation and the dilution of nutrients and/or the amount of organic sediment accumulation. Changes in the invertebrate food base also occur in response to nutrients, fetch and pelagic mixing (Burton et al. 2002, Uzarski et al., submitted, Burton et al., submitted), and these changes may also contribute to the observed correlations between plant and fish community composition. In general, fish communities tended to move from a 'banded killifish, pugnose shiner, redear sunfish, smallmouth bass, whitemouth shiner, white sucker, and yellow perch community' to a 'brook silverside, brown bullhead, fathead minnow, golden shiner, green sunfish, and spotfin shiner community as nutrients and adjacent agriculture increased along an environmental gradient (Fig. 12).

*Aquatic Plants*

To date, analyses of the aquatic plants data at our IBI sampling sites have identified sensitive indicators, but these indicators are difficult to interpret over the entire study area. For example, the Saginaw Bay wetlands are distinctive floristically from those of northern Lakes Michigan and Huron, but they are also generally much more degraded. The combination of floristic differences and major land use differences may require that the sampling area be further stratified for meaningful patterns to be recognizable.

Bivariate analyses were conducted on Michigan data and Ontario data, attempting to identify strong relationships between the land use and flora at sites, as measured by Floristic Quality Indices, individual exotic plant species, groups of exotic and aggressive colonizing native species, and structural plant groups (primarily submergent species). Analyses were initially conducted on all sites (Michigan, Lake Ontario, and Long Point) simultaneously. Then regional analyses of Michigan data were conducted by separating the sites into Saginaw Bay and northern Lakes Michigan and Huron. Analyses were also conducted on individual geomorphic marsh types: open marsh (fringing wetlands), protected marsh, and barrier beach wetlands.

The R-Square values were extremely low when we compared all sites across Lake Ontario, Long Point (Lake Erie), and Lakes Michigan and Huron together by plant zone, regardless of which plant variables were included in the analyses. R-Square values were typically less than 0.02. Stratified comparisons of land use and vegetation relationships were not appreciably higher, when the strata were either regions (northern Lakes Huron and Michigan or Saginaw Bay) or regions and landform combinations (Appendix II, Figs. 2 and 3). This lack of strong land use - plant relationships may be partially a product of not identifying appropriate land use factors and/or conducting analyses at an inappropriate spatial scale.

Floristic diversity and Floristic Quality are regionally distinctive. The wet meadow zone at sites on Saginaw Bay have FQIs between 10.8 and 17.8, while those on northern Lakes Michigan and Huron ranged between 19.6 and 40.0, typically much higher than those on Saginaw Bay. The most degraded sites on Lake Michigan, Escanaba (Terrace Inn) and Ludington Beach, had the lowest FQI scores, but Search Bay and Nahma, both exposed sites, had FQIs nearly as low, even though neither showed evidence of being heavily degraded by human activities. Three other exposed sites, St. Ignace, Whitefish Bay (Alpena), and Squaw Bay (Alpena), have equally high FQIs as the highest quality protected embayment sites, Mackinac and Hessel Bays, even though St. Ignace and Whitefish Bay have roads and industrial management at their margins.

A combination of exotic plants (purple loosestrife and bull thistle), nutrient responding plants (cattail and tall reed), and aggressive native species (reed canary grass) characterize all or most Saginaw Bay sites. As individual species, these are less useful as indicators, but as a group they are effective. In northern Lakes Michigan and Huron, the most degraded sites had relatively low levels of these common exotic and aggressively colonizing species, but they supported the highest number of exotic species. The wet meadow zone typically supports the greatest diversity of exotic species, with reduced numbers of species and coverage values in the emergent zone.

Coverage values for submergent plants were not effective for identifying degradation. This may be partially a result of a nonlinear response of submergent species to one form of site degradation, nutrient enrichment. For northern sites, where nutrient levels are low, nutrient enrichment in the form of low levels of sewage effluent or golf course runoff may actually increase the coverage values of submergent species. This is best seen at Cedarville, at the mouth of a creek that carries sewage effluent. The increased levels of nutrients seem to have resulted in quantities of submergent plants much higher than in other local wetlands. Similarly, golf course fertilizer in Mackinac Bay (Hessel) may have resulted in increased plant cover in this bay as well. In contrast, highly degraded sites, with high levels of agricultural sediments and associated turbidity are almost devoid of submergent species. On Saginaw Bay, this turbidity was not encountered, partially because of low water conditions which left most of the marshes with only 20 to 30 cm of water.

*Contaminated Sediments*

Few sediment parameters measured proved valuable in partitioning sites along gradients of anthropogenic disturbance. Most potential sediment contaminants were in concentrations below detection limits at most of the 20 sites. Therefore, multivariate analyses could not be conducted. Pearson correlations were conducted to test for responses of biota to sediment contamination and to test for correlations between sediment contamination and water quality.

Percent solids ranged from 18.4% in the wet meadow zone of the Rapid River site to 84.0% in the wet meadow zone of the Big Fishdam site. A significant negative correlation ($p<0.05$, $r=-0.598$) was found between percent solids and family-level IBI scores. Percent solids also were correlated with PC 2 scores of the chemical/physical PCA ($p<0.05$, $r=0.598$). No general pattern in percent solids was detected between vegetation types within wetlands.

Percent Total Organic Carbon (%TOC) ranged from below detection limit of 0.05% at a number of sites to 7.66% in the wet meadow zone of the Rapid River site. The high %TOC at the Rapid River wet meadow zone was expected because sediment cores contained a layer of partially decayed wood, possibly sawdust from past lumbering operations. There was a significant negative correlation ($p<0.05$, $r=-0.493$) between %TOC and PC 2 scores of the chemical/physical PCA across all wetlands sampled. Percent TOC was also negatively correlated with %solids; this was expected since the inverse of %solids can be used as a surrogate measure of organic matter. Cadmium concentrations were significantly correlated with PC 2 scores of the chemical/physical PCA suggesting complimentary sources of cadmium and those parameters making up PC 2.

The correlation between sediment organic matter (measured by %TOC and %solids) and IBI scores may indicate a response of macroinvertebrate communities to high productivity relative to decomposition, but is most-likely spurious, resulting from the more degraded sites of Saginaw Bay having higher %solids and lower %TOC due to differences in wetland morphology as these sites generally receive more physical disturbance from wave action and ice scour than do northern sites. The correlation between %TOC and PC 2 could also be an effect of underlying differences in wetland morphology between sites of Saginaw Bay and those of northern Lakes Michigan and Huron.

Dibenzo(a,h)anthracene, DDD, DDT and total PCB's were not in concentrations above detection limits in any of the 20 sites. The highest concentrations of the remaining 17 organic contaminants occurred at the Rapid River and Ogontz Bay sites. No significant correlations occurred between sediment contamination and biotic integrity as measured by IBI scores. The inability of our IBI to predict sediment contamination may reflect the very low concentrations of most contaminants measured. Thus, analysis of sediment contamination does not appear to be of significant value as part of an ongoing monitoring program. However, baseline data on wetland sediment contamination may be useful in determining historical sources of wetland disturbance.

## 6. **Ability to Set Endpoint or Attainment Levels**

*Faunal analyses:*

Data from reference sites established and sampled in the past were used to establish endpoints or attainment levels for each indicator. We had already established attainment levels for several metrics and/or indicators for these systems during invertebrate IBI development for fringing wetlands (Burton et al. 1999; Uzarski et al., submitted). The fish data collected for this and other projects will be used to establish a fish based IBI with levels of attainment specified. We expect to complete a manuscript that will meet this objective some time in 2003. We will continue to work on development of IBIs for wetland types not included in the earlier papers and foresee no complications in doing so.

Consensus-based guidelines have been published for metals and organic chemicals in sediments in the Great Lakes (MacDonald et al. 2000). These guidelines contain Threshold Effect Concentrations (levels below which no environmental effects are expected) and Probable Effect Concentrations (levels above which adverse effects are possible). The presence of published sediment quality guidelines serve as attainment levels.

*Plant analyses:*

Meaningful end points have not yet been identified based on plants for metals and organic chemicals. Plants are generally recognized to require much higher concentrations of metals or organic chemicals than animals before an end point is identified. Plants are recognized to be sensitive to high levels of turbidity, reducing or eliminating photosynthesis, and also to sediment destabilization caused by feeding or mating carp.

Measurable end points for plants are most likely to be identified for nutrient enrichment. At least one site, Cedarville, provides an excellent opportunity to measure response of aquatic plants to increased nutrient loads. Sediments and plant tissue can be measured for nutrient enrichment at increased distances from the source of enrichment, evaluating both amount of enrichment and spatial rate of nutrient change. Additional sites with increased levels of enrichment are needed to identify thresholds that result in increased turbidity and major loss of species diversity.

## 7. Statistics

*Faunal analyses:*

Initially, correspondence analyses of invertebrate and fish community composition were used to determine if reference sites could be separated from impacted sites. When they could, individual taxa containing the most inertia responsible for separation were deemed potential metrics. Mann-Whitney U tests and Pearson correlations were used in conjunction with PCA to determine if densities of these taxa at reference sites were significantly different from densities at impacted sites.

Attributes that showed an empirical and predictable change across a gradient of human disturbance were chosen as metrics to be included in our multi-metric IBIs. Correlation analyses were then used to link state with stressor by relating potential metrics to specific parameters impacted by anthropogenic disturbance. Finally, stressor-land use relationships were explored to aid in management decisions. Graphic analyses were also used to detect differences among wetlands with respect to individual metrics and to set endpoints. The variance of each metric was used to predict the robustness and resolution that could be obtained using a given metric. Metrics that distinguished between two sites with relatively similar exposure to anthropogenic disturbance were said to have high resolution.

We used medians in place of means for measuring assemblages of invertebrates. Occurrence, distribution and population size of invertebrates are highly variable in time and space. Highly variable data increases the chance that an area sampled may be unusually depleted or concentrated in constituents of a metric. If this occurs, it may be that the area is: (1) more or less isolated from anthropogenic disturbance than is the rest of the wetland, (2) receiving more or less disturbance than is typical for the entire wetland or plant zone, or (3) characterized by some unique "natural" chemical/physical component of the environment not found in the rest of the wetland. Regardless of cause, data from such unique areas are outliers and not representative of the entire wetland. Using the median in place of mean as a measure of central tendency dampens the influence of these outliers.

*Plant analyses:*

Bivariate linear comparisons were utilized to evaluate plant response to differing levels of human land use. R-square values were consistently low, probably as a result of the complexity of wetland degradation by human activities. Two different scales of disturbance were observed in Michigan sites, complicating the interpretation of land use data. In Saginaw Bay, the scale of human modification of the landscape was so great, that no site was identified as lightly altered. Instead, all sites were highly degraded, with some being excessively so. Even though a site looked relatively undisturbed because of a large forest buffer, the overall level of degradation from the remainder of the Saginaw Basin, results in an exotic rich, depauperate flora. The exotics are spread along the entire shoreline of Saginaw Bay by littoral processes. Propagules and nutrient loading enter the bay via drainage ditches on half mile or mile intervals, creating a relatively uniform level of degradation in all wetlands. Recent studies of swamp forests demonstrated a similar basin-wide level of degradation.

In contrast, wetlands along northern Lakes Huron and Michigan are characterized by having intact buffers with high levels of forest and other natural vegetation. Photo interpretation indicates a highly intact landscape, although locally the number of houses can be high. During plant sampling on these sites, exotics were encountered in several wetlands, but the number and coverage of exotic species were typically low. A closer look demonstrated that exotic plants often had a local source, such as introduced soil at the end of a road or adjacent to a private boat ramp. Even though aerial photos showed a pristine site, very small disturbances had introduced exotic species; thus the land use analyses did not account for presence of exotic plants.

Field sampling demonstrated that many sites had low levels of exotic plant species, but that levels were too low to be detected during photo interpretation of plant zones. Thus, photo interpretation can not be relied upon to identify most populations of exotic plants. Even in areas where exotic plants were abundant, such as along Saginaw Bay, exotics were not necessarily abundant or dense enough to be consistently identified from aerial photography.

Problems associated with both photo interpretation and plant sampling jointly reduce the effectiveness of statistical evaluations.

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Appendix I

# VALIDATION AND PERFORMANCE OF AN INVERTEBRATE INDEX OF BIOTIC INTEGRITY FOR LAKES HURON AND MICHIGAN FRINGING WETLANDS DURING A PERIOD OF LAKE LEVEL DECLINE

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## Abstract

Development of indicators of "ecosystem health" for the Great Lakes was identified as a major need at the State-of-the-Lakes Ecosystem Conference in 1998, 2000, and 2002. Our goal was to develop an invertebrate-based index of biotic integrity (IBI) that was robust to water level fluctuations and applied to broad classes of lacustrine wetlands across wave-exposure gradients. Our objectives were to evaluate the performance and test the robustness of our preliminary IBI (e.g., Burton et al. 1999) at a range of water levels, eliminate any problems with the IBI, remove the preliminary status, test the IBI on similar wetlands of Lake Michigan, and establish stressor:ecological-response relationships. Twenty-two sites, both open- and protected-fringing lacustrine marshes of Lake Huron and Michigan were selected for study. Correspondence analysis and Mann-Whitney U tests were used to test the robustness of existing metrics and search for additional metrics. Wilcoxon Signed Rank tests were used to determine if metrics were responding to inter-annual water level fluctuation. Principal components analysis and Pearson correlations were used to establish stressor : ecological response relationships. Analyses confirmed the utility of most of the metrics suggested in our preliminary IBI, but we recommended several improvements. With improvements, the IBI was able to place all sites in comparable order that we placed them a priori based on adjacent landuse/landcover, limnological parameters and observed disturbances. The improved IBI worked very well from 1998 through 2001 despite the substantial decreases in lake level over this time-period. Analyses of 2001 data collected from similar fringing wetlands along the northern shore of Lake Michigan suggested that the IBI could also be used for fringing wetlands of northern Lake Michigan. We are confident that our IBI is ready for implementation as a tool for agencies to use in assessing wetland condition for Lakes Huron and Michigan fringing wetlands.

## Introduction

Wetlands of the Great Lakes are subject to multiple anthropogenic disturbances. These disturbances are superimposed on systems that experience a wide variety of natural stress resulting primarily from a highly variable hydrologic regime (Burton et al. 1999, 2002; Keough et al. 1999). These wetlands are classified into geomorphological classes, reflecting their location in the landscape and exposure to waves, storm surges and lake level changes (Albert and Minc 2001). Fringing wetlands form along bays and coves and leeward of islands or peninsulas. The location of the shoreline, with respect to long-shore current and wind fetch, determines the type of wetland found along the shoreline (Burton et al. 2002). The greater the effective fetch (e.g., Burton et al. In Press), the more the wetland is exposed to waves and storm surges until a threshold is reached where wetlands no longer persist. The separation of variation due to anthropogenic disturbance from variation due to natural stressors related to water level changes over long and short term periods is central to predicting community composition and in turn developing indices of biotic integrity (IBI) for these systems.

Development of indicators of "ecosystem health" for the Great Lakes was recognized as a major need at the State-of-the-Lakes Ecosystem Conference (SOLEC) in 1998 in Buffalo, New York and progress in developing indicators was the emphasis of the SOLEC Conference in 2000 in Hamilton, Ontario and again in 2002 in Cleveland, Ohio. Among the indicators listed by the task force at SOLEC 98 were indices of biotic integrity (IBIs) for coastal wetlands based on fish, plants and macroinvertebrates. These were also emphasized in the 2000 and 2002 conferences, but minimal progress in developing such indicators was reported at those conferences.

Wilcox et al. (2002) attempted to develop wetland IBIs for the upper Great Lakes using fish, macrophytes, and microinvertebrates. While they found attributes that showed promise, they concluded that natural water level changes were likely to alter communities and invalidate metrics. In an earlier paper, we developed a preliminary macroinvertebrate-based bioassessment procedure for coastal wetlands of Lake Huron (Burton et al. 1999). This system could be used across wide ranges of lake levels, since it included invertebrate metrics for up to four deep and shallow water plant zones with a scoring system based on the number of inundated zones present.

While Great-Lakes wide studies of aquatic macrophytes indicate that similar geomorphic wetland types support distinctively different plant assemblages in geographically distinct ecoregions (Minc 1997, Minc and Albert 1998, Chow-Fraser and Albert 1998, Albert and Minc 2001), several plant zones are common to many of these systems. In our preliminary invertebrate-based IBI, we (Burton et al. 1999) collected invertebrates from four plant zones characteristically inundated in fringing lacustrine wetlands of Lake Huron and northern Lake Michigan during high water years, and used invertebrate metrics from each of these zones in the IBI (Burton et al. 1999). By developing metrics for each wetland plant zone across a water level gradient from wet meadow to deep-water emergents, we assumed that we could compensate for absence of the higher elevation zones (e.g., wet meadow) during low lake level years by placing more emphasis on metrics from zones that remained inundated. As lake levels have fallen sharply since 1998, we have tested this assumption and report the results in this paper.

Our goal was to develop an IBI that is robust to water level fluctuations and applies to broad classes of lacustrine wetlands across natural wave exposure gradients. The broad class of wetlands we chose for the first stage of IBI development was fringing, lacustrine marshes (Burton et al. 1999). Fringing, lacustrine marshes are the most common type of wetlands of Lake Huron and the northern shore of Lake Michigan. They were included in three classes, Northern Great Lakes marshes, Northern rich fens, and Saginaw Bay lakeplain marshes, in the classification of Great Lakes wetlands by Albert and Minc (2001). All of the wetland types included in our broader definition of fringing, lacutrine marshes are characterized by having a species of Scirpus (e.g., S. acutus, S. pungens, or S. validus or combinations of two or more of these species) as the dominant plants in the two outer emergent zones (Burton et al. 1999) and by having wet meadow zones dominated by a combination of Carex spp. (C. stricta, C. lasiocarpa, and/or C. viridula) and Calamagrostis canadensis. We initiated IBI development for this broad wetland class in Lake Huron (Burton et al. 1999) and have begun test it in other lakes and wetland types. However, the data presented in this paper are only from open- and protected-embayment marshes (fringing) of Lake Huron, and northern Lake Michigan.

**The objectives of this study were to: 1.) evaluate the performance and test the robustness of our preliminary IBI (e.g., Burton et al. 1999) at reduced water levels when fewer plant zones per site were inundated; 2.) identify and eliminate any problems and make improvements to the IBI where necessary 3.) remove the preliminary status from the Burton et al. (1999) IBI; 4.) test the applicability of the IBI in similar wetlands of Lake Michigan, and 5). establish stressor : ecological-response relationships that could be used to manage high quality wetlands and restore degraded ones.**

## Methods and Materials

*Study Sites*

Both open- and protected-fringing lacustrine marshes of Lake Huron and Michigan were selected for study (Fig. 1a & 1b). Site selection was based primarily on site access, inundation status, and degree of human disturbance to the marshes. Depths rarely exceeded one meter and were as shallow as 10 cm. The plant communities at each site changed along a depth gradient from open water to shore and typically included an outer Scirpus zone in deep, wave swept areas of the marsh, an inner Scirpus zone in deep areas subject to less wave impact, a transitional zone that sometimes included Typha angustifolia as a dominant, and a wet meadow zone. The wet meadow zones extended to upland ecosystems directly or graded into shrub and forested wetlands depending on topography of the site. The disturbance status of each site is summarized in Tables 1 and 2; the general description of each site is listed below.

*Saginaw Bay Study Sites*

The Wildfowl Bay sites were located on the windward and leeward sides of Maisou and Middle Grounds Islands in Wildfowl Bay, a bay on the eastern shore of Saginaw Bay (Figure 1). The sites were located approximately 1.5 km northeast of the Sumac Island public access, Huron County (T16/17N R9E). While adjacent land use and the Saginaw River undoubtedly impacted all of Saginaw Bay, these impacts were likely diluted in Wildfowl Bay due to its proximity to the outer bay. The water quality of the outer bay is better than the inner bay because of dilution of the high agricultural, urban and industrial runoff into the inner bay with Lake Huron water in the outer bay. The islands are State wildlife management areas with little direct shoreline development, although they are impacted somewhat by development on the nearby mainland. Sparse Scirpus pungens Vahl zones (the outer Scirpus zone) dominated the outer wave exposed area at the southwest end of the island. Large and distinct Typha angustifolia L. complexes extended along a shallow bar south from the southwest end of Maisou Island, and one of these wave-swept stands was also sampled. In 1997, the protected interior of the island contained an extensive wet meadow dominated by Carex spp. and Calamagrostis canadensis, this zone was nearly devoid of water during 1998. Stands of Scirpus (including S. pungens, S. validus, and S. acutus), and and Typha (both T. angustifolia and T. latifolia L.) were also present on the lee side of the islands.

The Vanderbilt Park site (Figure 1) was located near the park approximately 2 km north of the Michigan Department of Natural Resources (MDNR) public access near Quanicassee Road, Tuscola County (T14N R6E) (Figure 1). The site contained large, dense stands of Scirpus pungens that were intermixed with several other species near shore. S. pungens was the primary species found in the outer marsh. The emergent zone extended about 500 m into Saginaw Bay from the sandy ridge that separated the fringing wetland from swale wetlands located between outer and inner sandy ridges. A large Typha angustifolia stand was located in the middle of the Scirpus complex just north of the sampled area. There was no wet meadow area at this site. Vanderbilt Park may be among the most impacted sites in Saginaw Bay due to its proximity to dwellings and inputs from the adjacent Quanicassee River and large drainage ditches draining intensely farmed fields of potatoes, beans, and sugar beets.

The Cotter Road site located on Saginaw Bay, Bay County, (T14N R6E) (Figure 1) closely resembled the Vanderbilt Park site with respect to number of dwellings/development and inputs from agricultural drains and the Saginaw River. A narrow wet meadow containing Carex stricta and Calamagrostis calamagrostis, monodominant and mixed stands of Scirpus pungens, Pontederia cordata, Phragmites australis (Cav.) Trin. ex Steud., and nearly monodominant stands of Typha angustifolia were present in this marsh complex. Extensive Typha complexes near the open water protected the Scirpus zone; thus, an outer Scirpus was too sparse to be included as a distinct zone.

Almeda Beach is located on the western shore of Saginaw Bay approximately 20 km north of Saginaw River (Figure 1). Samples were collected south of Almeda Beach at the end of Coggins Road. An agricultural drainage ditch emptied into the bay approximately 100 m from the sampled area. The site had a substantial Inner Scirpus zone with no distinct Outer Scirpus zone. The outer portion of the marsh was dominated by Typha angustifolia and Eleocharis spp. The area upland of the Inner Scirpus zone was a mixed vegetation zone containing Juncus spp., Scirpus spp., Salix spp., and Populus spp. seedlings. Only a few dwellings were located within 1 km of this site, but there were no other obvious, contiguous sources of disturbance.

Wigwam Bay is located on the northwestern shore of Saginaw Bay approximately 40 km north of Saginaw River (Figure 1). Samples were collected from areas near the mouth of the Pine River. A very narrow band of Outer Scirpus was present, but the majority of the marsh consisted of Inner Scirpus. A transition zone from Scirpus spp. to Juncus spp. occurred very near shore. A few residences were located within 1 km of the site, but there were no other obvious, nearby sources of disturbance.

*Northern Lake Huron Sites*

The northern Lake Huron sites were located in the Les Cheneaux Island complex and along St. Martin's Bay, a large bay located west of the Les Cheneaux Islands at the eastern end of Michigan's upper peninsula (Figure 1). Typical plant zonation at these sites included wet meadow vegetation dominated by Carex stricta and/or C. lasiocarpa and separated from the deeper Scirpus-dominated emergent marsh by Typha angustifolia-dominated transitional communities. The inner Scirpus emergent zones were dominated by Scirpus acutus, Pontederia cordata, and Eleocharis spp., interspersed with floating-leaved plants such as Nuphar spp. and Potomageton spp. and patches of often-dense submersed plants. The outer, deeper, nearly monodominant regions of the emergent marshes were characterized by fewer stems of wave-swept Scirpus acutus and a few sparse patches of submersed vegetation with sandier bottoms compared to inner regions. Exceptions to this general pattern are noted below. The obvious impacts to each Lake Huron site are listed in Table 1a, while Table 1b lists the sites in order of anthropogenic disturbance.

The Mackinac Bay site (Figure 1) is an island-protected bay with a low-gradient stream running through it. A paved two-lane highway separates the upper end of the wet meadow from the rest of the marsh. Several residences with private docks and boathouses line the shore southeast of the site. Boat traffic in the marsh is limited, even though the main dredged channel through the Les Cheneaux Islands is located near the outer edge of the marsh.

The Golf Course site is located east of Mackinac Bay along the heavily used boat channel adjacent to a golf course (Figure 1). The site consists of a narrow band of Scirpus at the base of a fairly steep slope from the golf course.

The Mismer Bay marsh (Figure 1) is similar to the Mackinac Bay marsh in that small streams run through it. It is more wave-swept than Mackinac Bay, having only partial protection from open-lake waves resulting in a sandier bottom and no Typha zone. Although, the wet meadow is well established at this site. The sampled area is on the east side of the marsh east of the smaller of the two stream channels that traverse the marsh. Two residences and a dirt road are located along the eastern border of the marsh. Boat traffic is limited since the primary channel through the Les Cheneaux Islands is well away from the outer edge of the marsh.

Duck Bay is well protected on the lee side of Marquette Island, the largest of the Les Cheneaux Islands (Figure 1). The marsh area sampled supported a sparse submersed plant community interspersed with a low density, low diversity outer and inner Scirpus zones, a well-developed Typha zone, and a narrow wet meadow zone adjacent to upland forest. There were no residences and only one private dock on the shore. Boat traffic was low, since the main boat channel around the island does not enter the bay.

Two additional bays on Marquette Island were sampled. Peck Bay and its wetland are similar to the Duck Bay site but are located further south toward the open lake (Figure 1). Human impacts are low with only one residence located along the channel that leads into the wetland. Voight Bay differs in that it is on the south, windward side of the island with direct exposure to open-lake waves from Lake Huron (Figure 1). The sampled area is partially protected by low sandbars. The emergent marsh supports sparser emergent vegetation and has a sandier bottom than most sites. There are no human developments near the marsh. Boat traffic in both bays is limited, since neither are near main boat channels.

Cedarville Bay (Figure 1) is generally considered to be the most human-impacted area in the Les Cheneaux Islands (Kashian and Burton 2000). The middle of the bay is occupied by a very large island with large numbers of residences, summer homes and docks on it. The bay actually resembles a U-shaped channel, which receives very high boat traffic. The town of Cedarville, its marina, and public boat launch occupy the northwestern shore of the bay, and many private residences, businesses, and docks (private and commercial) line the mainland near the marsh. The deeper emergent marsh surrounds the stream mouth, public launch, and several docks, but is cut off from its historic wet meadow by a paved road and a lumber yard built on fill. The only remaining aquatic connection between the wet meadow and marsh is the stream, which runs through a culvert under the road and carries discharges from an upstream sewage treatment lagoon. The lagoon is usually discharged twice each year, but discharge can be more frequent in wet years. The emergent marsh in this area supports unusually dense growths of submersed plants and filamentous algae including several species such as Myriophyllum spicatum and Elodea canadensis characteristic of nutrient enriched conditions.

A paved highway separates Prentiss Bay marsh (Figure 1) from most of its wet meadow. A narrow Typha zone extends along the bay side of the highway. A single culvert connects the wet meadow to the deep-water zones. Anglers often put boats in near the culvert and fish at the edge of the deep marsh. The dense emergent zone is narrow, giving way to a deeper, sparse, patchy emergent zone fairly near the road. The bottom tends to have more clay than is characteristic of most marshes in this area.

The St. Martin's Bay (Figure 1) marsh was located between two parallel sandbars on an unprotected shoreline in this large bay. The inner sandbar supported upland vegetation along the top of the ridge. A wet meadow was located between the inner sandbar and the adjacent forest. A dense inner Scirpus zone was located between the inner and outer sand bars. The bottom of this interdunal swale was relatively sandy, and submersed plants were sparse because of exposure to waves via an opening through the outer sand bar. There was no direct wet meadow/emergent interface. A Typha zone occurred at the inner edge of the outer sandbar. The outer sandbar also supported a narrow upland zone at the top of the ridge. At the outer edge of the outer sandbar, a very narrow and sparse outer Scirpus acutus patch was present.

The Pine River site was located on the east side of St. Martins Bay (Figure 1). Only a narrow band of Scirpus approximately 100 m wide was present at this site. While a narrow wet meadow zone was located upland from the Scirpus zone, the wet meadow was never inundated during this study. The Pine River entered the bay approximately 1 km west of the site. The river drains an agricultural region with red clay soils and is always quite turbid from eroded clay particles. The turbidity plume is usually pushed by prevailing winds along the shore into and past the sampled marsh. High turbidity levels at the site reflect this.

The Port Dolomite site (Figure 1) is located on Bush Bay on the east side of the port facilities for a dolomite mining operation. McKay Bay is located on the west side of the point (Figure 1) where Port Dolomite is located. Both sites contain inner and outer Scirpus with little or no wet meadow zone. The Port Dolomite (Bush Bay) site has seeps that are likely from adjacent dolomite mining settling ponds. Cladophora can often be seen growing in or near the seeps. A small stream draining the settling ponds enters the wetland via a culvert under the road. The Mckay Bay site does not have the obvious seeps or streams entering the marsh. Several dwellings are adjacent to the marsh, and boat traffic is common.

*Northern Lake Michigan Sites*

Fringing wetlands similar to the ones sampled in Lake Huron are common along the northern shore of Lake Michigan. We sampled a subset of these sites in 2001 to test whether the Lake Huron IBI would work for these wetlands (Figure 1b). The disturbance status for these wetlands is summarized in Table 2a. They are listed a priori in order of anthropogenic disturbance in Table 2b. General descriptions are given below.

The Point St. Ignace (Mackinac Bridge) marsh was located immediately northwest of the Mackinac Bridge in Lake Michigan near the mouth of the Straits of Mackinac (Figure 1b). The bridge is heavily used by cars and trucks while the Straits experience a large volume of large freighter, commercial and recreational boat traffic. There was a rural road adjacent to the marsh with less than five dwellings located across the road from the marsh. A newly constructed building and tollbooths were located near the east side of the wetland. A narrow wet meadow zone bordered the road. A dense inner Scirpus zone extended approximately 200 m from shore and was bounded by a 50 m wide sparse outer Scirpus zone.

The Nahma and Ogontz marshes were located on Big Bay de Noc (Figure 1b). There were less than five dwellings adjacent to the Ogontz Bay marsh, and most of the adjacent riparian zone was forested. A golf course was near and several houses/summer cottages were adjacent to the Nahma marsh. The Ogontz site was adjacent to a public boat launch that could only be reached via several km of rural roads. Both sites contained relatively narrow (approximately 100 m) inner and outer Scirpus zones with almost no wet meadow zone.

The Escanaba/Highway 2 site was located in Little Bay de Noc adjacent to an urban area along U.S. Highway 2 approximately 2 km north of the Escanaba River. A large paper mill located just upstream of the dam near the mouth of the Escanaba River may influence this site via the river plume along the shore. Inner and outer Scirpus zones were sampled. There was no wet meadow zone and only a few patches of Typha in this wetland.

The Ludington Park wetland was located approximately 10 km south of the Escanaba Highway 2 site in Ludington Park in downtown Escanaba. This park is located near the Escanaba waterfront in the midst of industrial, residential and commercial areas of the city. The park includes a large marina on an island. The sampled area consisted of patches of inner Scirpus located near a beach parking lot just west of the island near a channel that was connected to the marina via a culvert under a road. No other plant zones were inundated at this site.

*Chemical and Physical Measurements*

Basic chemical/physical parameters were sampled from each plant zone each time biological samples were taken. Analytical procedures followed procedures recommended in Standard Methods for the Examination of Water and Wastewater (APHA 1998). These measurements included soluble reactive phosphorus (SRP), nitrate-N, nitrite-N, ammonium-N, turbidity, alkalinity, temperature, DO, chlorophyll a, oxidation-reduction (redox) potential, and specific conductance. Quality assurance/quality control procedures followed protocols recommended by U.S. EPA.

*Determination of Anthropogenic Disturbance*

Wetlands that experienced a wide range of anthropogenic stressors were chosen for study. The extent of disturbance was determined using surrounding land use data in conjunction with limnological data and site-specific observations such as evidence of dredging, point-source pollution, and discharge into the wetland from drainage ditches or streams. If streams entered the wetland, land use from the stream catchment was considered when determining anthropogenic disturbance.

Land use data were obtained from existing digitized maps, topographic maps, and personal observations; the primary data source was the Michigan Resource Information System (MIRIS) Land Cover Maps based on 1978 aerial photography. These data included: percent urban and agricultural area, number of adjacent dwellings, percent impervious surface, total length of adjacent roads, and the number of connecting drainage ditches. The MIRIS data were the most recent data available to us. Visual observations of these data and current land use suggested that land use had not changed substantially for most of the wetlands included in our study.

*Macroinvertebrates sampling*

Macroinvertebrate samples were collected with standard 0.5 mm mesh, D-frame dip nets from late July through August. July-August is when emergent plant communities achieve maximum annual biomass and larger and easier to identify, late instars of most aquatic insects are present in the marsh.

Dip net sampling consisted of sweeps at the surface, mid depth and just above the sediments. Nets were emptied into white pans and 150 invertebrates were collected by picking all specimens from one area of the pan before moving on to the next area. Special efforts were made to ensure that representative numbers of smaller organisms were picked to minimize any bias towards picking larger, more mobile individuals. Invertebrates were picked from plant detritus for a few minutes after 150 specimens were collected to ensure that sessile species were included. Beginning in 1999, we modified this procedure to limit the amount of picking-time required at each site and to semi-quantify our samples. Individual replicates were picked for one-half-person-hour, organisms were tallied, and picking continued to the next multiple of 50. Therefore, each replicate sample contained either 50, 100, or 150 organisms. This procedure made it easier to compare samples on a catch per unit effort basis. Three replicate dip net samples were collected in each plant zone to obtain a measure of variance associated with sampling.

Specimens were sorted to lowest operational taxonomic unit, usually genus or species for most insects, crustaceans and gastropods. Difficult to identify insect taxa such as Chironomidae were identified to tribe or family, and some other invertebrate groups including Oligochaetes, Hirudinea, Turbellaria, Hydracarina, and Sphaeridae were identified to family level or, in a few cases, to order. Taxonomic keys such as Thorp and Covich (1991), Merritt and Cummins (1996), and mainstream literature were used for identification. Accuracy was confirmed by expert taxonomists whenever possible.

Identify and combine metrics into an IBI; an analysis to identify new metrics and confirm metrics identified previously.

Burton et al. (1999) developed metrics for their published IBI by initially analyzing data graphically by constructing box plots including the 10th, 25th, 50th, 75th, and 90th percentiles as recommended by Barbour et al. (1996). When attributes showed an empirical and predictable change across a gradient of human disturbances, Mann-Whitney U tests were performed to test for significant differences between impacted and reference sites.

In Burton et al. (1999), we used 1997 data to develop IBI metrics for Lake Huron wetlands. We tested these metrics using 1998 data. We expanded on these analyses in this paper using the 1998 data and newly collected data from 1999 through 2001 to test the performance of the IBI during this period of rapid decline in lake levels. Additional analyses were employed to search for any new metrics that might have been missed in the initial analyses. Instead of the graphical approach used previously, we used correspondence analyses (CA) (SAS version 8, SAS Institute Inc., Cary, NC, USA) of invertebrate community composition to determine if sites would ordinate according to predetermined gradients of anthropogenic disturbance. CAs were performed individually on Inner and Outer Scirpus zone data. Taxa represented by less than 20 total individuals (from all replicates from all sites combined) per zone in any one year were eliminated from the analysis. This resulted in approximately 40 taxa being used in each analysis. A separate CA was conducted for each plant zone for each year for 1998, 1999, and 2000. The 1999 data were most complete and were used to identify key taxa. These key taxa were then analyzed for each of the three years from 1998 through 2000.When reference sites separated from impacted site, groups of individual taxa containing the most inertia responsible for the separation were deemed potential metrics. Mann-Whitney U tests (SYSTAT version 5.0, Evanston, Illinois) were then used to determine if density of each of these taxa at reference sites were significantly different from its density at impacted sites. This allowed us to confirm the utility of our initial metrics and identify additional ones.

Like Burton et al. (1999), we used medians in place of means as measures of central tendency for measuring assemblages of invertebrates. Invertebrate parameters are highly variable, and medians are more resistant to effects of outliers. Therefore, we used medians to dampen the influence of outliers.

*Testing and Validation of IBI*

We continued to collect data from a subset of the original sites of Burton et al. (1999), providing us with our best indication of temporal variability. We calculated IBI scores by site (all plant zones present) as well as by individual plant zones (simulating a situation where only one plant zone had been inundated) and compared these scores within and among years. This exercise was used to determine which, if any, individual plant zones were most subject to inter-annual variability and to identify problematic plant zones that could give conflicting results if sampled alone.

*Testing Metrics Robustness from Inter-annual Variation*

We used Wilcoxon Signed Rank tests (SYSTAT version 5.0, Evanston, Illinois) on individual metrics through time to search for metrics that may have been responding to water level fluctuations. Significance was set at $p < 0.05$. Analyses were only done on two Inner Scirpus data sets, since these two data sets were the only ones available that were large and complete enough to permit this type of analysis. The analysis comparing 1998 to 1999 metrics included data from Duck, Mackinac, Prentiss, Mismer, St. Martin's, and Cedarville (n=6). The second analysis was done using data from 1997 through 2000, but only included Duck, Mackinac, and Mismer (n=3), since these were the only wetlands sampled every year over this four-year period.

*Test the applicability of the IBI in similar wetlands of Lake Michigan*

We sampled five similar fringing wetland sites in Lake Michigan (Figure 1b). We applied the IBI with improvements to those data to see if the IBI would place the Lake Michigan sites in the correct sequence along a disturbance gradient that had been identified a priori with land use data and other observation following the procedures detailed below. This was in attempt to provide evidence that the Lake Huron IBI could be extended to similar fringing wetlands in Lake Michigan. As a reference, we sampled many of our Lake Huron sites during this time-period as well.

*Establishing Stressor - Ecological Response Relationships*

Principal Components Analysis (PCA) using SAS version 8 (SAS Institute Inc., Cary, NC, USA) was used to establish PCs based on chemical/physical parameters as well as surrounding (1 km buffer) land use / cover data (MIRIS 1978). PCA was performed using SRP, $NH_4$, $NO_3$, $SO_4$, Cl, turbidity, chlorophyll a, alkalinity, dissolved oxygen, REDOX, and specific conductance while additional analyses were done using percent adjacent agriculture, urbanization, shrub-range land, swamps, and the total length of roads within a 1 km buffer. Pearson Correlations (SYSTAT version 5.0, Evanston, Illinois) between individual metrics and PCs were used to establish stressor-ecological response relationships. PCs were then decomposed to explore relative contributions of individual stressors. These analyses were performed on 1999 and 2001 Inner and Outer Scirpus data sets because they were the most complete.

## Results

*Testing and Validation of Preliminary IBI*

We calculated IBI scores using the preliminary IBI (Burton et al. 1999). The IBI ranked the majority of wetlands in order of anthropogenic disturbance, with only zero to four site placed out of order in any given year. We evaluated the metrics for each of the four plant zones individually to determine the efficacy of an IBI based on only a single zone. The inner and outer Scirpus and wet meadow zone metrics worked well when present. Metrics based on the inner Scirpus zone proved to be almost as effective as were metrics based on summing values from all inundated zones present, and would be the single zone to use if only one zone is to be sampled. Metrics based on the Typha zone did not work very well. The Typha zone was rarely sampled, due to lack of inundation or absence at a site, and IBI metrics for this zone did not consistently rank sites by degree of disturbance. In the preliminary IBI, we proposed four diversity and richness metrics based on combined data from all zones present. These combined zone metrics proved to be ineffective in ranking sites along a disturbance gradient. Based on these results, we recommend dropping the Typha zone metrics from the IBI and calculating the four diversity and richness metrics for each zone rather than calculating them using combined data for all zones.

*Correspondence Analyses*

Correspondence analyses were performed on data from the Inner Scirpus zone collected from 1998 through 2000 and for the Outer Scirpus zone from 1999 through 2000 (the 1998 outer Scirpus data were excluded because data were only collected from two sites ). We initially used 1999 data to identify taxa responsible for the most inertia in ordinations of the sites according to ecoregion. The 1999 data set was the most balanced with respect to number of sites sampled from each ecoregion (Saginaw Bay and northern Lake Huron sites are in two different ecoregions). Correspondence analyses ordinated 1999 Inner and Outer Scirpus zone site data by ecoregion (northern Lake Huron sites clustered separately from Saginaw Bay sites). We identified and removed taxa responsible for the most inertia separating the sites by ecoregion (Tables 3a and 3b) and ran the correspondence analysis again (Figure 2a). With taxa responsible for ecoregional differences removed (Table 3), the sites ordinated by disturbance (Figure 2b). The taxa showing ecoregional differences in 1999 were also removed from data from other years before running correspondence analyses, and sites for each year ordinated based on degree of disturbance after these taxa had been removed. In 2000, due to low water, we only obtained data from Northern Lake Huron. When the taxa identified as having ecoregional differences in 1999 (Table 3) were removed from the 2000 analysis, ordination based on anthropogenic disturbances was much improved even though no Saginaw Bay sites were included in the data set.

We used the CAs not only to search for additional metrics, but also to determine if any of our previous metrics may have included responses to ecoregion instead of disturbance. In the Inner Scirpus zone, few taxa removed due to ecoregional differences were major contributors to metrics. The caddis fly, Oecetis, was included in the Ephemeroptera plus Trichoptera taxa richness metric. Oecetis was more often found at Saginaw Bay, but was quite rare even in those sites decreasing its influence on the metric. Thus, its removal from the analyses did not have a significant effect on the metric. The Odonate, Enallagma, was generally common at all sites, but tended to be at higher densities in Saginaw Bay sites. Conversely, Libellula was more common in Northern Lake Huron than it was in Saginaw Bay marshes. Differences in these two taxa may have offset each other in the Odonata taxa richness metric and in Odonata relative abundance metric, since these metrics worked well with or without theses two genera included in the data set. The snail, Amnicola, tended to be more common in northern Lake Huron, and occurred in only one site in Saginaw Bay. Three other snails, Fossaria spp., Pseudosuccinea columella, and Physa gyrina were all more common in Northern Lake Huron than in Saginaw Bay, contributing to separation by ecoregion. However, these taxa also separated sites based on disturbance within each ecoregion. Even though we removed these taxa from the CA so that they would not pull ecoregions apart in the analysis, we still believe these taxa are likely to be valuable metrics for an IBI. Ecoregional differences in individual taxa did affect the Gastropoda or Crustacea plus Mollusca metrics enough to warrant removing either metric from the IBI. Dreissena was much more common in Saginaw Bay than in Northern Lake Huron and may have counter balanced differences in some gastropod taxa in the Crustacea plus Mollusca metrics. Decapods were rarely collected, but were more common in Northern Lake Huron than in Saginaw Bay. This may reflect differences in habitat between the two ecoregions rather than differences in anthropogenic disturbance. The Northern Lake Huron sites tended to have more cobble, pebble and boulder sized rocks and more submersed plants than did the Saginaw Bay sites. Decapods were relatively rare in samples from both ecoregions, and differences between the two regions did not affect the Crustacea plus Mollusca metric.

In most cases, a genus or species associated with one ecoregion was replaced by a closely related genus or species in the other, and therefore, had little effect on the diversity and richness metrics or metrics at coarser taxonomic resolution. Three insects taxa were removed from the CAs, the family, Ceratopogonidae, a ceratopogonid genus, Atrichopogon, and the genus Trichocorixa (Corixidae). Atrichopogon was collected only at Saginaw Bay. Trichocorixa was only found at two sites in Northern Lake Huron and not at all in Saginaw Bay.

In the Outer Scirpus zone, two amphipods, Crangonyx and Gammarus, were more common in Saginaw Bay than in northern Lake Huron sites. Neither was used in metrics other than richness and diversity in the Outer Scirpus. As was the case in the Inner Scirpus zone, in the Outer Scirpus zone, the gastropod, Fossaria, and the Hemipteran, Trichorixa, were much more common in Northern Lake Huron. They were not good indicators in either ecoregion. Tubificids were common at sites in both ecoregions, however two sites in Saginaw Bay had an over abundance of Tubificidae, one was a very impacted site, and the other was one of the least impacted in that ecoregion. Two Tricoptera were removed, Mystacides and Nectopsyche. Mystacides was more common in northern Lake Huron, while Nectopsyche was more common in Saginaw Bay. The Corixid, Sigara, was only found at one site in northern Lake Huron and was not found in Saginaw Bay in 1999.

Correspondence analyses of the data from 1999-2000 identified the same metrics that were proposed in the preliminary IBI based on 1997 and 1998 data (Burton et al. 1999), thus providing support for the importance of the preliminary metrics. Two new metrics for Inner Scirpus were suggested by the CA results: (1). relative abundance of Isopoda (%) which decreased with disturbance, and (2) relative abundance of Amphipoda (%) which increased with intermediate disturbance.

*Calculating IBI Scores with New Metrics and Category Score*

Using results from calculation of preliminary IBI scores and the CAs, we dropped Typha zone metrics from the IBI, calculated the four richness and diversity metrics by plant zone, and adopted two new metrics for the Inner Scirpus zone. When the IBI scores were calculated with these changes included, the IBI worked nearly perfectly from 1997 through 2001 (Table 4). Even without these changes, however, the preliminary IBI metrics suggested by Burton et al. (1999) performed reasonably well.

*Use of 1/2 person-hour count*

Most often, 150 organisms were collected. Occasionally 50 or 100 organisms were collected from the Outer Scirpus zone. While the timed count did not prove useful as a semi-quantitative metric, it did not negatively affect the IBI. We recommend its use, particularly for the Outer Scirpus zone where invertebrates are sparser than they are in the

Inner Scirpus or wet meadow zones making collection of 150 individuals too time consuming for wide spread use.

*IBI Response to Water Levels*

We used Wilcoxon Signed Rank tests on individual metrics. There were no significant differences at the $p < 0.05$ level in Metrics over time with changing water levels for either the 1998 vs. 1999 ($n = 6$) or 1997 through 2000 ($n = 3$) analyses (Table 5). However, with more power of detection, Odonata genera richness ($p = 0.08$) may have decreased with water level decline between 1998 and 1999.

*Relating Stressor to Ecological Response*

We used Pearson correlation matrices to search for relationships between chemical/physical and land-use/land-cover PCs and our metrics. We ran 302 total correlations and identified 53 significant ones (15 significant correlations would be predicted by chance alone at $p = 0.05$). We did not use a Bonferroni correction because n was low, ranging from 7 to 12. Therefore, these results should be viewed as suggesting hypotheses rather than being conclusive. These analyses suggest several possible relationships (Figure 3). Several examples of suggested relationships are also presented in Figures (4a, 4b, and 4c). Wetlands with high percentages of adjacent land use in agriculture tended to have relatively higher pH, temperature, turbidity, alkalinity, $DO_{(daytime)}$, redox $potential_{(daytime)}$ and sulfate compared to wetlands with high percentages of land use in forests. If urbanization and roads were adjacent, the wetland tended to have higher chloride, nitrate, and ammonium concentrations and higher specific conductance values. If the adjacent land cover was predominantly swamps, alkalinity and specific conductance tended to be higher while $DO_{(daytime)}$, sulfate, redox $potential_{(daytime)}$, turbidity and soluble reactive phosphorous tended to be lower in the wetland. Adjacent shrub land correlated with low turbidity in the wetland. Adjacent agricultural land use and/or urbanization and roads or wetland chemical conditions that correlated with these adjacent land use/land cover parameters correlated with reduced % Sphaeriidae, % Crustacea + Mollusca, % Gastropoda, Shannon Diversity, Evenness, and % Odonata and increased Simpson Diversity. Adjacent shrub lands or decreased turbidity was also associated with lower % Sphaeriidae, % Crustacea + Mollusca, and % Gastropoda. Adjacent swamps, or the correlated chemical/physical conditions, tended to be correlated with increased Shannon Diversity, Evenness, and % Odonata. Adjacent agricultural land use or wetland chemical conditions that correlated with agriculture reduced Crustacea + Mollusca richness, Odonata richness, and total genera richness. Adjacent swamps or the related chemical/physical parameters correlated with increased Ephemeroptera + Trichoptera richness, decreased total genera richness, and decreased Simpson Diversity. Adjacent agriculture correlated with decreased % Isopoda while adjacent swamps correlated with increased % Isopoda. Finally, as urbanization and roads increased adjacent to wetlands % Amphipoda in the wetland tended to decrease.

**Discussion**

*Performance of the IBI with New Metrics and Category Scores*

Calculating the preliminary IBI (Burton et al. 1999) using data collected from 22 sites during 1997 through 2001 and using correspondence analyses to search for disturbance related metrics confirmed the utility of most of the metrics suggested previously (Burton et al. 1999). Several improvements suggested by these calculations include: 1.) adding two new metrics to the Inner Scirpus zone, 2.) removing the Typha zone from the IBI, and 3.) calculating the four diversity metrics for each individual plant zone. With these improvements, the IBI was able to place all 22 sites in the same order that we placed them in based on adjacent land use / cover, limnological parameters and other observed disturbances. The improved IBI worked very well from 1998 through 2001 despite the rather substantial decreases in lake level over this time period. Analyses of 2001 data collected from similar fringing wetlands along the northern shore of Lake Michigan suggested that the Lake Huron IBI could also be used for fringing wetlands of northern Lake Michigan (Table 6).

One of the two new metrics suggested for use in the Inner Scirpus zone (relative abundance (%) of Amphipoda) does not increase or decrease with disturbance the way most of the metrics do. Instead, highest values for this metric occur at intermediate levels of disturbance. Conversely, the other metric, relative abundance Isopoda (%), decreased with disturbance. One possible explanation is that Isopoda and Amphipoda compete for resources when disturbance is low with isopods being the superior competitor. As isopod abundance decreases with increases in disturbance, amphipods, which appear to be less sensitive to disturbance, are subject to less competition and increase in abundance at intermediate levels of disturbance. As levels of disturbance continue to increase, the threshold for impacting amphipods is exceeded and amphipod relative abundance also decreases. Specifically, the relative abundance of isopods tended to decrease with increasing adjacent Agriculture and/or where wetland water chemistry included relatively higher pH, temperature, turbidity, alkalinity, $DO_{(daytime)}$, redox $potential_{(daytime)}$ and sulfate. Amphipods tended to decrease with increasing adjacent urbanization and roads and/or as chloride, nitrate, ammonium, and specific conductance values increased. Amphipods were much more common than isopods where sites experienced an intermediate amount of disturbance regardless of type of disturbance or ecoregion.

Due to low water, Typha zones were often not inundated during the period of rapid decline in lake levels from 1998 through 2001. Samples were collected from only two sites in 1998 and 1999, so our ability to test metrics for this zone was limited by sample size. Even so, the Typha zone metrics never ordinated the sites according to disturbance, and we recommend dropping the zone from the IBI. A possible reason for the failure of the Typha zone metrics to separate sites is that the Typha zone tends to occur in very different areas of the wetlands in the two ecoregions included in this study. Typha zones in the more pristine northern Lake Huron sites were located in a transitional zone between wet meadow and Inner Scirpus. This was not the case for the more impacted Saginaw Bay sites. Monodominant stands of Typha were found in areas exposed to direct wave action in Saginaw Bay as well as in protected wetlands behind islands or in the middle of Scirpus pungens stands. Exposure to waves can play a large role in determining invertebrate community composition regardless of the extent of anthropogenic disturbance (Burton et al. 2002). We did not have enough data from the

Typha zone to separate variance due to anthropogenic disturbance from that of wave exposure**.** It may be that Typha zone metrics would prove useful if location of the zone in relation to wave action were taken into account as it was in metrics for the two Scirpus zones.

We recommend calculating the four richness and diversity metrics by plant zone instead of combining all of the plant zones present (e.g., Burton et al. 1999) to calculate these metrics. Since the number of plant zones inundated varies by wetland and year, a combined calculation means that diversity is being calculated from a variable number of habitats for any given wetland or year. Since wetlands with the most structural diversity would be a function of the number of plant zones included in the calculation, and, since habitat diversity would likely be related to invertebrate diversity, the combined calculation should be dropped. By incorporating the metrics into each individual plant zone and adjusting category scores appropriately, we remove variation due to inequitable number of vegetation zones sampled.

With improvements incorporated (Table 7), we recommend dropping the 'preliminary' status from the initial IBI (e.g., Burton et al. 1999). Our data proved that this system could work well even during periods of rapid lake level decline as long as any of the three plant zones used in the improved IBI was present. The improvements and increased resolution also allowed us to introduce some new status categories. With these changes in place, we are confident that our IBI is ready for implementation as a tool for management and conservation agencies to use in assessing wetland condition for Lake Huron and Lake Michigan fringing, coastal wetlands.

*Deviation from Protocol*

Our protocol was developed for sampling macroinvertebrates, and field crews were told to only pick macroinvertebrates. However, it was common to have microinvertebrates such as Copepoda and Cladocera in samples. These microinvertebrates were identified and included in the IBI database. Inclusion of such animals by our sampling crews suggests that this might commonly occur when others use the IBI. To ensure that the IBI was robust to this common error, we used those data in calculations of metrics such as percent Crustacea plus Mollusca and the total richness and diversity metrics. Inclusion of the microinveretbrates had little effect on the IBI.

*Use of 1/2 person-hour count*

Use of the timed count did not improve the IBI, but it did not have any negative impact on it either. The timed count reduced time in the field. Without it, two or three individuals could spend up to four hours collecting three replicate samples from the Outer Scirpus zone alone.

*IBI Response to Water Levels*

Others have suggested that the IBI approach would not work for coastal wetlands because natural water level fluctuations of the Great Lakes would likely alter communities and invalidate metrics (Wilcox et al. 2002). By sampling only defined and inundated vegetation zones, we removed enough variation associated with water level fluctuation to maintain metric consistency from year to year even though annual average lake levels increased to above average and then fell 1.08 m to near historic lows over the several year period included in our sampling effort. Except for Odonata genera richness, there were no significant differences in metric scores among years even though water levels declined. With more power of detection, Odonata genera richness ($p = 0.08$) may have decreased with water level decline. The odonate metric played a crucial role in detecting anthropogenic disturbance within years, and the IBI was robust enough to accommodate among-year variation. Thus, we included this metric in the final IBI.

*Relating Stressor to Ecological Response*

It is important not only to detect anthropogenic disturbance, but also to identify which disturbance or suite of disturbances is likely to be causing most of the observed changes in IBI metrics. Once specific disturbances are identified, managers can use this information to decide on best management options. Biota usually respond to a suite of correlated ambient conditions. Multivariate analyses were used to combine parameters for more power of detection. Once relationships were established, we decomposed combined parameters to the original parameters. Such relationships are strictly correlative, cannot be used to infer causation, and must be used with caution. It is difficult to determine the impact of adjacent land use or land cover on a given fringing wetland. For example, figure 3 seems to suggest that urban areas contribute more $NO_3$ and $NH_4$ to wetlands than do Agricultural areas, since water in wetlands with adjacent urban land use contains more $NO_3$ and $NH_4$ than does water in wetlands with adjacent agricultural land use. An alternative explanation would be that increased inorganic N in the urban wetlands might not be processed as efficiently as it is in agricultural wetlands, so no conclusion about quantity of input from the adjacent area is warranted. We simply tended to find relatively higher $NO_3$ and $NH_4$ concentrations near urban areas where there was high run-off and lower productivity in the wetland. The conceptual drawing (figure 3) shows the relationships between the metrics and the appropriate land use and/or the chemical/physical parameters that correlate with that land use. It does not necessarily suggest that a given land use/land cover taken alone will create the associated chemical/physical conditions in the wetland. It does, however, provide some insight into what potentially might be causing the degradation. Confirmation of the causative agent would then need to be established using a more experimental approach.

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**Table titles**

Table 1a. Northern Lake Huron and Saginaw Bay sites listed with obvious impacts.

Table 1b. Northern Lake Huron and Saginaw Bay ranked in order of disturbance.

Table 2a. Northern Lake Michigan sites listed with obvious impacts.

Table 2b. Northern Lake Michigan sites ranked in order of disturbance.

Table 3. Taxa from the Inner and Outer Scirpus zone that contributed to the most inertia responsible for ordinating the sites based on ecoregion in correspondence analyses.

Table 4. IBI placement of Lake Huron sites from 1997 through 2000. Each year includes IBI ranking from least impacted to most impacted with an ‘X’ placed indicating which plant zones were sampled (WM = Wet Meadow; OS = Outer Scirpus; IS = Inner Scirpus) and which overall category each site was placed into.

Table 5. A summary of p values for each metric in Wilcoxon Signed Ranks Tests using Inner Scirpus metrics from 1998 and 1999 (n=6) Corresponding to a 46 cm decrease in water levels over this period. Nearly identical results were obtained using data from 1997 through 2000 (n=3).

Table 6. IBI placement of Lake Huron and Michigan sites from 2001. Each year includes IBI ranking from least impacted to most impacted with an ‘X’ placed indicating which plant zones were sampled (WM = Wet Meadow;

OS = Outer Scirpus; IS = Inner Scirpus) and which overall category each site was placed into.

Table 7. An index of biotic integrity (IBI) for Lakes Huron and Michigan fringing coastal wetlands.

**Figure Titles**

Figure 1a. Map of Michigan, USA including study sites located in Lake Huron.

Figure 1b. Map of Michigan, USA including study sites located in Northern Lake Michigan.

Figure 2a. Correspondence analysis including 1999 taxa collected from the Inner Scirpus zone of Lake Huron Sites. The solid line represents an ecoregion gradient with Saginaw Bay sites toward the left side of the gradient and Northern Lake Huron sites on the right. The dashed line represents the best disturbance gradient with the most disturbed sites towards the top and the least disturbed sites near the bottom. Circles are drawn around those taxa responsible for the most inertia separating the data based on ecoregion.

Figure 2b. Second run of a correspondence analysis including 1999 taxa collected from the Inner Scirpus zone of Lake Huron sites. Circled taxa from Figure 2a were removed from this analysis. The dashed line represents a disturbance gradient. The ecoregion gradient no longer exists. Circles are drawn around sites with different levels of disturbance.

Figure 3. Conceptual drawing established using chemical/physical principal components, land use principal components, and biotic metrics in a Pearson correlation matrix.

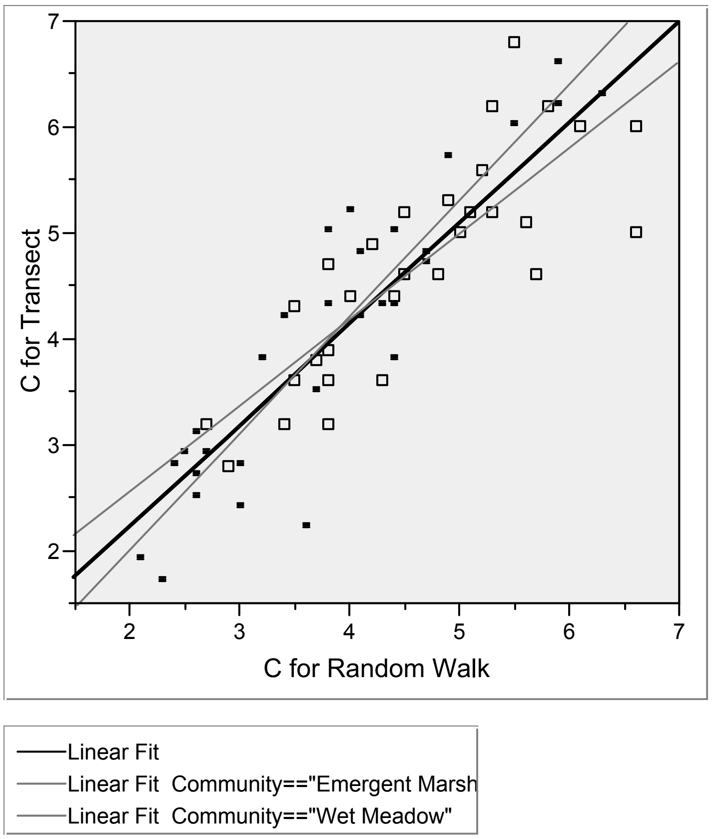
Figure 4a Principal components analysis using 1999 Inner Scirpus chemical/physical variables. Circles are drawn around sites with different levels of disturbance.

Figure 4b Principal components analysis of 1999 Inner Scirpus sites using land use / land cover variables. Circles are drawn around sites with different levels of disturbance.

Figure 4c Pearson correlation between the relative abundance of isopods and chemical/physical principal component two.

## APPENDIX II.1

**Figure 1. Total Coefficient of Conservatism (C) collected along transects compared to Coefficient of Conservatism collected during a fifteen minute random walk.**




### Linear Fit

Transect Total C = 0.3189925 + 0.9544252 Random Total C

### Summary of Fit

| | |
|---|---|
| R-square | 0.778299 |

### Linear Fit Community = "Emergent Marsh"

Transect Total C = 0.9364766 + 0.810598 Random Total C

### Summary of Fit

| | |
|---|---|
| Rsquare | 0.670227 |

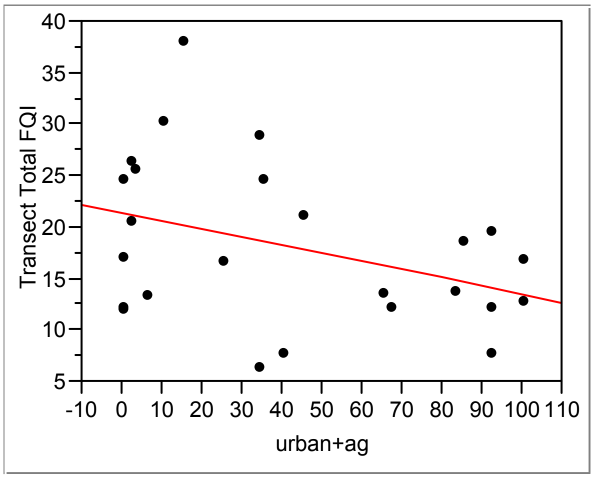
### Linear Fit Community = "Wet Meadow"

Transect Total C = -0.185108 + 1.0979393 Random Total C

### Summary of Fit

| | |
|---|---|
| R-square | 0.840279 |

**Figure 2. FQI along a gradient of Disturbance (% of urban+agricultural land use) within a kilometer of the sampling site. FQI computed for the Wet Meadow Zone of all Lake Michigan And Lake Huron sites.**



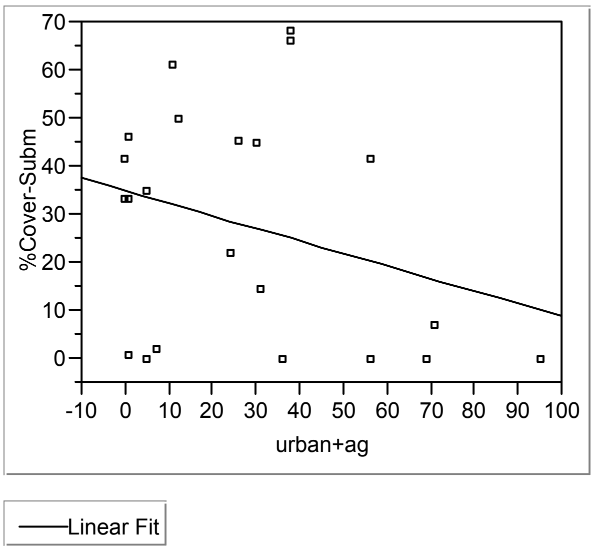

## Linear Fit

Transect Total FQI = 21.479172 - 0.0794346 urban+ag

## Summary of Fit

| | |
|---|---|
| R-square | 0.14545 |

**Figure 3. Percent cover of Submergent plants along a gradient of Disturbance (% of urban+agricultural land use) within a kilometer of the sampling site. Analyses for the Submergents within the Emergent Marsh Zone of all Lake Michigan And Lake Huron Protected Marsh sites.**




## Linear Fit

%Cover-Subm = 35.080225 - 0.2614436 urban+ag

## Summary of Fit

| | |
|---|---|
| RSquare | 0.089218 |