Final Report to the Great Lakes Commission

FOR

An amendment to: A test of the variability and usefulness of SOLEC indicators in wetlands of Lakes Huron and Michigan*.

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*This document has been written to stand alone but makes substantial reference to the final report prepared for the Great Lakes Commission by Uzarski, Cooper, Burton, Albert and Rediske in 2003.

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. 2004 (also attached as Appendix B), 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 invertebrates. 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. 2004). 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. 2004). 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. 2004). We are also working to develop macroinvertebrate-based metrics for use in Lily and *Typha* vegetation zones. Progress on these efforts as well as analyses of coastal wetland macroinvertebrate communities (at the Great Lakes basin scale) is included in this report.

Another objective of this project was to develop a fish-based IBI for Great Lakes coastal wetlands. 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. (1994) 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. We have developed a preliminary fish-based IBI from data collected in 2002 (as part of the GLCWC pilot study). This IBI was included in a manuscript accepted to a special issue of the Journal of Great Lakes Research scheduled to be published in Spring of 2005. The manuscript is included in this report (Appendix A) and represents our current state of coastal wetland fish-based IBI development. Also included in the manuscript is a comprehensive analysis of coastal wetland fish communities at a Great Lakes basin scale.

The original RFP from the Great Lakes Commission, which initiated the GLCWC pilot studies, set forth seven criteria by which potential indicators were to be evaluated. These included: cost, measurability, basin-wide applicability, availability of complimentary data, indicator sensitivity, ability to set endpoints and statistical approach. We reported on each of these criteria in our initial report to the Great Lakes Commission. Our overall conclusions were that a monitoring program based on invertebrates, fish and plants could be conducted in a cost-effective manner by field staff with a moderate amount of training. The protocols for collecting biotic data did not have to be modified for use across the Great Lakes basin and only small adjustments to metric scoring schemes were anticipated from one lake/ecoregion to another. By the time the pilot studies were completed in 2003 we had developed and were testing an IBI based on macroinvertebrates for use in fringing wetlands of Lakes Huron and Michigan. Since that time we have developed and are testing an IBI based on fish (Uzarski et al. (in press)), as well as additional macroinvertebrate-based IBI metrics for use in *Typha* and lily vegetation zones.

Metrics for use in *Typha* vegetation zones have been identified as a need across the Great Lakes basin. The predominance of *Typha* stands in particularly impacted coastal wetlands further necessitates the development of indicators specifically designed for use in these areas. In the preliminary IBI of Burton et al. (1999), *Typha*-specific metrics were developed. However, Uzarski et al. (2004) found them unreliable and recommended that they be dropped from the IBI. Inherently high biological and chemical/physical variability in *Typha*-dominated wetlands has made metric development difficult. This report includes our current state of *Typha* metric development for both fish (Uzarski et al. 2005 in press) and invertebrates.

Objectives - Several indicators based on fish, macroinvertebrates, and plants were recommended for use in Great Lakes Coastal Wetlands at the 2000 SOLEC. Our objective has been to fully develop these and other indicators of ecosystem health for Great Lakes coastal wetlands. This project represents the continuation of a pilot study completed in 2003. **The objective of this portion of the project was to further develop and test 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. Specifically, we have developed and are testing metrics based on macroinvertebrates and fish for coastal wetlands of all five Great Lakes using data collected in 2002 as part of a pilot study funded by the Great Lakes Coastal Wetlands Consortium initiated by US EPA and facilitated by the Great Lakes Commission through.**

Methods

Sampling Sites

Twenty Lake Huron and Michigan wetlands were selected for study of flora and fauna indicators following procedures developed and/or agreed on by the Project Management Team of The Great Lakes Wetlands Consortium. Data from an additional 28 sites from Lakes Huron, Michigan, and Superior, sampled with identical protocols but funded elsewhere, were also used to develop and test indicators and are included in this report. Through collaboration with Joel Ingram of Environment Canada and Steve Timmermans of Bird Studies Canada, we acquired data from 25 additional sites on Lakes Erie and Ontario. With these additional data, we were able to apply a basin-scale approach to our faunal and chemical/physical analyses. All of these data were used in indicator development and testing and are included in this report. Site locations, sampling dates and vegetation zones for all sites are listed in Table 1.

We tested and developed indicators at open lacustrine and protected embayment wetlands selected from the U.S.A. shoreline of Lakes Huron and Superior and the northern shoreline of Lake Michigan. Drowned river mouth wetlands along the eastern shore of Lake Michigan were also sampled in an effort to include as many geomorphic wetland classes as possible. 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 June, July and August of 2002. 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. A map of general site locations is found in Figure 1 of Uzarski et al. (2005 in press) (included as Appendix A in this report).

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. To that end, we included 11 Lake Michigan and three Lake Superior drowned river mouth wetlands in our dataset.

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. 2004). 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. 2004). 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.

Since one of our goals in IBI development has been to keep the number of required protocols to a minimum, we developed and tested metrics at a number of different scales usually beginning with the broadest applicable scale (i.e., basin-wide) and reducing scale only when necessary (i.e., when ecoregional attributes overwhelm the community structure).

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. For a subset of samples chloride and sulfate were also measured. Quality assurance/quality control procedures followed protocols recommended by U.S. EPA and discussed in detail in the QAPP for the GLCWC pilot study.

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.

Since *a priori* determination of anthropogenic disturbance is critical to IBI metric development and is a particular challenge at the basin-scale, we placed increased emphasis on developing a method to accurately rank sites according to disturbance. In our preliminary invertebrate-based IBI for fringing wetlands of Lake Huron (Burton et al. 1999) and our subsequent validated IBI (Uzarski et al. 2004 and Appendix B), we assigned sites to one of three disturbance classes. This was done by direct interpretation of limnological and land use/cover data, by combining parameters with principal components analysis and by our own onsite account of impacts (e.g., boat traffic, dredging, etc.). This strategy for characterizing anthropogenic disturbance worked well for developing our initial IBIs. Additional information regarding the establishment of these disturbance classes and their use in IBI metric development can be found in Burton et al. (1999) and Uzarski et al. (2004) (Appendix B.)

With the expansion of our IBI development efforts to the basin-scale, we are faced with the task of accounting for many more classes of disturbance. Anthropogenic impacts affect wetlands at variable intensities and at varying spatial and temporal scales. Consequently, we worked to develop a new method for ranking sites that integrates a number of land use and water quality parameters simultaneously and accounts for the extremes in nutrient concentrations (both high and low) that we often observe at our most impacted sites. We feel that this new approach is the most accurate way of characterizing anthropogenic disturbance from the water quality and land use data collected as part of the 2002 project.

Disturbance gradients were established for each subset of sites (stratified by vegetation type and lake) using chemical/physical and land use and cover data. Stratification of the dataset by vegetation type for establishing disturbance gradients was necessary because we have found that vegetation type is the most important variable for structuring biotic communities and describing ambient chemical/physical condition (Uzarski et al. 2004, (in press), Burton et al. 1999). Further stratification by lake/ecoregion was done only when necessary (i.e., when ecoregional differences were overwhelming). Chemical/physical data were not available from a number of Lake Ontario and Erie sites. When data were absent, IBI scores were evaluated independently using surrounding land use data and onsite observations of disturbance.

Gradients were developed by transforming all available chemical/physical and land use data into ranks then combining these ranks to get a final grand rank. Turbidity, specific conductance, sulfate and chloride were ranked directly with the greater values indicating disturbance. Extreme values, either very high or very low, for pH, percent saturation of dissolved oxygen, nitrate-N, ammonium-N, and soluble reactive phosphorus concentrations were considered indicators of disturbance. Therefore, absolute values of the difference from the median concentration were used to establish a rank order for each of these parameters. We also combined all chemical/physical and land use and cover data using principal components analysis. When a principal component was found that could best be explained by anthropogenic disturbance, site scores for that component were also included in the overall disturbance ranking. Principal components analysis was conducted using SAS version 8.

Land use and cover data were analyzed at two scales for more than half of our sites and both were incorporated into final disturbance gradients when those data were available. The larger scale (20-km buffer) was chosen to represent the impacts to the nearshore area, or the water source of the wetland, and was double weighted in the final disturbance ranking. A finer scale (1-km buffer) was used to relate impacts much more locally and received a single weighting. Further explanation of our disturbance ranking practices can be found in Uzarski et al. (in press) (included as Appendix A).

Candidate IBI metrics were evaluated by testing for relationships with overall disturbance gradients, individual disturbance parameters or principal components of chemical/physical and land use and cover data. These three types of comparisons were used because we have found that certain attributes of biotic communities respond to only one type of disturbance while others respond to multiple disturbances (Uzarski et al. 2004). Our disturbance gradients are designed to account for many types of anthropogenic disturbance simultaneously.

Macroinvertebrate sampling (also see Uzarski et al. 2004 and Burton et al. 1999) - Macroinvertebrate samples were collected with standard 0.5 mm mesh, D-frame dip nets from late July through August for coastal fringing sites and mid June through July for drowned river mouth sites. In previous studies, we have demonstrated that samples taken from ice-out through mid-July in coastal fringing sites 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. In drowned river mouth wetlands, invertebrate and plant communities generally reach their peak earlier in the year because of warmer water temperatures in the rivers. 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 was included. In the field, samples were placed in white pans, and 50, 100, or 150 invertebrates were collected per replicate 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 a series of hoops forming a funnel to the trap end. These nets were constructed of 12.5 mm or smaller mesh (bar dimension). Smaller nets were set in water approximately 0.25 m to 0.50 m deep, the larger nets were set in water depths greater than 0.50 m. Nets were set adjacent to vegetation zones of interest with leads extending into the vegetation. Fish were identified and enumerated before being released. The occurrence of DELTs was noted.

Identifying metrics and developing IBIs (also see Uzarski et al. (2005 in press), Uzarski et al. (2004), and Burton et al. (1999) **-** Initially, correspondence analyses of invertebrate and fish community composition were used to determine if reference sites could be separated from impacted sites or if dimensions correlated with disturbance parameters or scores. When they did, 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 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 (Uzarski et al. 2004, Burton et al. 1999). Correspondence analysis was conducted with SAS version 8 and all other analyses were conducted with Systat verson 8.0.

We used medians in place of means for measuring assemblages of invertebrates in most cases. Occurrence, distribution and population size of invertebrates are highly variable in time and space. Highly variable data increase 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 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.

Testing existing IBI metrics for use across the Great Lakes basin- Since our invertebrate-based IBI for fringing coastal wetlands performed well in Lakes Huron and Michigan (Uzarski et al. 2004), we decided to test it on wetlands of other classes (drowned river mouths and barrier beach) from all five Great Lakes. Protocols used to collect data for part of the GLCWC pilot study of 2002 were compatible with our IBI protocol so scores were calculated based on Uzarski et al. (2004) and evaluated using our *a priori* disturbance gradients. IBI and Disturbance scores were converted to percent scores (percent of total possible for the IBI and percent of the highest score for disturbance scores) to allow site-to-site comparisons even when different combinations of vegetation zones were sampled. The relationship between IBI scores and disturbance gradients were evaluated using Pearson correlation of percent scores (Systat version 8.0).

We also calculated IBI scores based on Uzarski et al (2004) for a subset of sites using data reduced to the family level. Significant taxonomic expertise is required to identify Great Lakes coastal wetland macroinvertebrates to the genus/species level. Therefore, greater applicability may be realized if the IBI can be applied to family level data.

Development of macroinvertebrate-based metrics for use in Typha zones- Macroinvertebrate-based IBI metrics for use in *Typha* zones were developed from data collected in 2002 as part of the GLCWC pilot study. Disturbance ranking was conducted *a priori* for all *Typha* sites at both the basin and lake scales according to the methodology of Uzarski et al. (in press)(Appendix A). These disturbance rankings along with individual disturbance parameters were then used to search for potential metrics.

Initially, detrended correspondence analysis (DCA) was used to search for gradients in *Typha* zone invertebrate data that could be explained by abiotic factors such as chemical/physical condition, land use, ecoregion, etc. These indirect gradient analyses served two purposes. First, they were used to determine the proper scale at which the communities should be analyzed. That is, if ecoregional variables overwhelmed the dataset at the basin scale, then the dataset should be stratified at a finer scale (e.g., lake). Second, if gradients could be identified that were best explained by anthropogenic disturbance, the ordinations would be useful in identifying attributes of the invertebrate communities (taxa or combinations of taxa) that were responding to anthropogenic disturbance. Specifically, if a dimension of the DCA was best explained by anthropogenic disturbance (water quality, land use, etc.), then the taxa contributing the most inertia to that dimension could indicate disturbance and subsequently be incorporated into IBI metrics (e.g. Uzarski et al. 2004, Uzarski et al. (in press) for more information on this technique). Data from the Lake Erie sites had to be excluded from ordination analyses because the sample totals from these sites were highly variable and did not coincide with the expected 50, 100 or 150 specimens (there were usually less than 25) that our protocol yields. This high variability in total abundance suggested that the sample protocol was not followed. DCA was conducted using Canoco for Windows.

Potentially useful attributes were also explored by testing 14 metrics from Burton et al. (1999) and Uzarski et al. (2004), 10 from Kashian and Burton (2000) and one from Wilcox et al. (2002). Few of the published metrics were developed specifically for *Typha* zones, but we tested them based on their potential as indicators of disturbance in Great Lakes coastal wetlands in general. Metrics were calculated for each individual replicate sample and then medians of these were tested for response to disturbance (Uzarski et al. 2004, Burton et al. 1999). Relationships between metrics and disturbance were evaluated with Spearman correlation and were deemed significant when $p<0.05$.

A third approach used to search for useful attributes of the *Typha* invertebrate communities included calculating correlation matrices between individual taxa abundances and disturbance parameters (land use and chemical/physical). Significant correlations were identified and explored for possible integration into IBI metrics. Spearman correlation was used because data did not approach a normal distribution. Correlations were conducted using Systat version 8.0.

All three approaches were conducted at multiple scales beginning at the basin-scale and down to lake- and ecoregion-scales when necessary to account for ecoregional effects on the communities. Community attributes that showed a response to anthropogenic disturbance were further evaluated for their potential use as metrics. Natural breaks in the data were identified and used as scoring thresholds for individual IBI metrics.

Great Lakes coastal wetland fish community analyses and fish-based IBI metrics- A preliminary fish-based IBI was developed using data collected in 2002. Metrics were developed for use in both *Scirpus* and *Typha* zones. We also attempted to develop metrics for lily (*Nuphar* and *Nymphaea*) zones. However, an insufficient number of these zones were sampled in 2002. This IBI along with global analyses of Great Lakes coastal wetland fish communities can be found in Uzarski et al. (in press) and Appendix A.

Great Lakes coastal wetland invertebrate analyses-Invertebrate data collected in 2002 from 62 coastal wetlands spanning all five Great Lakes were analyzed to determine the most important drivers of community composition. These data were collected as part of the GLCWC pilot study in addition to other studies using identical protocols but funded elsewhere. Detrended correspondence analysis (DCA) was used to search for gradients in the basin-wide invertebrate data that could be explained by environmental variables. The most important drivers of invertebrate community composition were then identified by laying a number of additional dimensions on biplots of the first two dimensions of the DCA. These additional dimensions represented the following 'scale' variables: lake, ecoregion, wetland type and vegetation zone. 'Scale'variables were evaluated for their ability to structure invertebrate communities. These were evaluated by color-coding sites in the biplot of the first two dimensions of the DCA according to lake, ecoregion, wetland type and vegetation zone. These color-coded plots were then visually inspected for structuring patterns.

The relative influence of lake, ecoregion, wetland type, and vegetation zone on invertebrate communities was further explored using Pearson correlation between DCA dimensions, principal components of chemical/physical data, and individual water quality parameters. These relationships were evaluated to determine if invertebrate community composition was related to chemical/physical and land use and cover data. Our third 'scale' variable was then superimposed on these relationships (only those relationships that were significant at $p<0.05$) to further explore the relationships between community composition and drivers at the basin scale.

A second round of DCA was conducted on a reduced dataset (taxa that were represented by less than 15 specimens in the dataset were lowered in taxonomic resolution). This was done to reduce the inertia of rare, or ecoregionally-distinct, taxa . Pearson correlation was again used to search for relationships between community data (DCA dimensions) and chemical/physical and land use data. The third “environmental” variables were again superimposed on the relationships that were significant in order to further explain the link between communities and drivers.

Since fish and invertebrates were both sampled at many of the 2002 sites, community comparisons were possible. Pearson correlations of fish and invertebrate DCA dimensions were used to link the two communities and biplots of fish and invertebrate dimensions were constructed to represent the relationships.

Results and Discussion (also see Uzarski et al. 2005 in press, Appendix A)

Chemical/physical and land use and cover parameters for all sites sampled in 2002
Chemical/physical and land use and cover parameters were measured every time biotic samples were taken. These data were used to characterize ambient conditions and relate biotic state with stressor. Table 2 lists all chemical/physical and land use and cover parameters for every vegetation zone at every site. Analysis of chemical/physical and land use and cover data is included for all fished sites in Uzarski et al. (in press) (Appendix A). Additional analyses of chemical/physical and land use and cover data are also included in the disturbance ranking for *Typha* sites and the ranking for *Scirpus* and wet meadow sites as well as the basin-wide invertebrate analyses.

In general, principal components analysis of chemical/physical and land use and cover data (at the basin and lake scales) indicated gradients that were best explained by vegetation zone (Uzarski et al. (in press), Appendix A). *Scirpus* zones were generally oriented on one end of the gradients and *Typha* zones on the other with the other zones placed in between. At the basin scale, the *Scirpus* end of the gradient was characterized as having higher dissolved oxygen and pH as well as having higher percent surrounding land use in forest. The other end of the gradient included sites with high nutrients and a high percentage of land use in agriculture or high run off and a high percentage of land use in urbanization. See Fig. 2 of Uzarski et al. (in press), Appendix A) for an example of this gradient for all sites fished in 2002).

Principal components analysis was also used to search for patterns in water quality and land use and cover that could be explained by lake, ecoregion or wetland type. This was done by laying a third dimension (lake, ecoregion and wetland type) on top of the first two axes produced by principal components analysis. No distinct gradients could be explained based on these variables suggesting that plant zone was the best environmental variable to explain ambient chemical/physical condition and land use and cover data at the basin scale (Uzarski et al. (in press), Appendix A).

Determination of Anthropogenic Disturbance and Disturbance Ranking (also see Uzarski et al. (in press), Appendix A).

Disturbance gradients were established for *Scirpus, Typha* and lily vegetation zones that were fished in 2002 and for Scirpus/wet meadow and *Typha* vegetation zones that were sampled for invertebrates in 2002. Disturbance gradients were developed to correspond with each type of biotic data (separate gradients for fished sites and invertebrate sites). This was necessary because our method for establishing disturbance gradients uses ranks to place sites relative to one another and many of the sites that were sampled for invertebrates in 2002 were not sampled for fish.

The number of variables used to establish disturbance gradients varied with plant zone. In general, all available parameters that could be attributed to anthropogenic disturbance were included in the gradients. However, nitrate and soluble reactive phosphorus had to be excluded from a number of the disturbance gradients when median concentrations of these nutrients were below detection limit (see Table 2 for raw chemical/physical and land use data that were used to determine disturbance gradients).

Inclusion of two land use and cover buffer sizes proved important in accounting for the differing impacts at these two spatial scales. The 20-km land use buffer showed, for example, that all Saginaw Bay sites tended to be more impacted than N. Lake Huron sites (large-scale differences in water quality), while the 1-km buffer was important in ordering sites within Saginaw Bay and the other regions.

By converting our nutrient concentrations to the absolute value of the difference from the median, we were able to emphasize the extremes in the respective datasets and incorporate these extreme values into the disturbance ranking. This method seemed to work well in ranking sites according to disturbance. For example, many sites with a high percentage of land use in agriculture actually had non-detectable dissolved nutrients in the water column. This may have been because these systems tend to have higher productivity and efficiently store excess nutrients in biomass. If sites with extremely high nutrients were removed from the analyses, results would have shown: 1.) that agricultural sites either had very high or very low nutrient concentrations as was found for invertebrate populations (Uzarski at al. 2004), and 2.) relatively pristine sites had moderate nutrient concentrations.

We included as many disturbance parameters as possible when developing gradients in order to account for many types of disturbances. Formulas used to calculate disturbance gradients for each plant zone can be found in Table 3 and additional discussion on the use and performance of our disturbance ranking methods can be found in Uzarski et al. (in press), Appendix A).

Disturbance gradients were developed for *Scirpus* zones that were fished in 2002 by averaging ranks for inner protected and outer wave-swept *Scirpus* to correspond with the combined fish data from these zones (Uzarski et al.(in press), Appendix A). Disturbance gradients were also developed for *Typha* and lily zones that were fished in 2002. Disturbance rankings and overall disturbance gradients for fished sites are found in Tables 4a-c and in Uzarski et al. (in press), Appendix A). Additional disturbance gradients were established using PCA for each plant zone. These were used to search for metrics that were not apparent from the primary gradients. Those variables that weighted the heaviest in PC1 of each analysis were identified and taken into consideration when searching for metrics. Those variables that weighted heaviest in PC1 for *Scirpus*, *Nuphar/Nymphaea*, and *Typha* were nitrate, chloride, and specific conductivity, respectively (see Uzarski et al. (in press) for additional information on PCA of physical/chemical and land use and cover variables for sites fished in 2002).

Two disturbance gradients were also established for sites where invertebrate data were collected. A gradient was established for sites where at least one *Scirpus* and/or wet meadow zone was sampled by calculating percent disturbance scores for each vegetation zone then averaging these scores per site. This gradient was used to evaluate the invertebrate-based IBI for coastal fringing wetlands developed previously (Uzarski et al. 2004, Burton et al. 1999). An additional disturbance gradient was also established for *Typha* zones where invertebrate data were collected. This gradient was used to develop and evaluate new invertebrate-based IBI metrics for *Typha* zones. Disturbance rankings and overall disturbance gradients for invertebrate sites are found in Tables 5a-b.

Great Lakes coastal wetland invertebrate analyses- Invertebrates were sampled from 62 coastal wetland sites spanning all five Great Lakes. These sites included five from Lake Superior, 19 from Lake Michigan, 23 from Lake Huron, 3 from Lake Erie, and 12 from Lake Ontario. Within these 62 sites we sampled 132 plant zones yielding nearly 56,000 invertebrates representing 237 taxa.

DCA was used to determine if sites would group according to Great Lake, ecoregion, wetland type or vegetation zone. All taxa were included in the first iteration of DCA. The first two dimensions of the DCA explained 10.7% of the variability in the dataset. The DCA revealed that Great Lake, ecoregion, wetland type and vegetation zone were all responsible for a portion of the variability in community composition across the basin. Figure 8 shows the first two dimensions of the DCA with sites coded according to Great Lake. In this case, the first dimension is driven by Lakes Huron and Ontario having low dimension 1 scores vs. Lake Michigan having higher dimension 1 scores. The second dimension is best described as Lake Huron vs. Lake Ontario. We then explored the relationship between community composition and ecoregion by identifying sites according to ecoregion. Figure 9 shows that dimension 1 is best explained by Eastern Lake Ontario and Thunder Bay vs. Northeast Lake Michigan. The second dimension is best explained by Eastern Lake Ontario vs. Thunder Bay.

To explore the relationship between community composition and wetland type, we identified sites in the DCA as either fringing, barrier beach or drowned river mouth wetlands. With sites identified according to wetland type, dimension 1 appears to represent a gradient from fringing and barrier beach sites with low dimension 1 scores to drowned river mouth sites with higher dimension 1 scores (Fig. 10). Dimension 2 can not be explained by wetland type. While wetland type appears to explain a great deal of the variability in the invertebrate dataset, it is unclear if this variable is structuring the communities or is simply correlated with ecoregion as most of the drowned river mouth wetlands were found on the eastern shore of Lake Michigan.

The role of vegetation type in structuring communities was also explored with the DCA. Sites were identified according to vegetation type (Fig. 11) and dimension 1 represented a gradient from *Juncus* and wet meadow zones to *Typha*, lily and *Sparganium* zones with the *Scirpus* zones falling in the middle. Dimension 2 appears to indicate a gradient of communities from *Juncus* to wet meadow zones with the others falling in between.

Great Lake, ecoregion, wetland type, and vegetation type all appeared to contribute to the structure of invertebrate communities in coastal wetlands across the Great Lakes basin. There was substantial variability in communities and none of the environmental variables explained an overwhelming amount of this variability. We therefore, examined the relationship between communities and ambient chemical/physical condition and adjacent land use and cover. Pearson correlation coefficients were calculated between DCA site scores (which represented invertebrate communities) and individual chemical/physical and land use and cover parameters as well as between DCA site scores and principal component scores (from a PCA of all chemical/physical and land use and cover parameters).

A significant correlation was found between dimension 1 scores and adjacent land cover as forest (r=0.260). Dimension 2 had the best relationship with adjacent land use as agriculture (r=-0.298). While these results seem to indicate adjacent land use is driving invertebrate community composition, the greatest differences in land use and cover were determined by ecoregion (i.e. all Saginaw Bay sites had much higher adjacent agriculture than northern Lakes

Huron and Michigan sites). Therefore, these analyses did not allow us to partition the variability in communities that was due to surrounding land use from that of ecoregion.

DCA revealed that, at high taxonomic resolution, some of the variability in invertebrate community composition could be explained by Lake, ecoregion, wetland type, vegetation type, and surrounding land use and cover. At this high taxonomic resolution, however, relatively rare taxa contributed the most inertia to the ordinations. Since the occurrence of rare taxa seemed to be dictated mostly by ecoregion, the relationships identified do not answer the question: functionally, what is shaping invertebrate community composition? Therefore, taxa represented by 15 organisms or less (0.03%) were lowered in taxonomic resolution, and the analyses were repeated.

After lowering taxonomic resolution, a DCA was conducted on the resulting 138 taxa. A Pearson correlation matrix was then calculated to identify relationships between community composition and chemical/physical and land use and cover parameters. Dimension 1 was still most related to surrounding land cover as forest ($r=0.301$), but a relationship with turbidity ($r=0.258$) also became apparent. Dimension 2 scores were found to correlate significantly with pH ($r=0.533$), chemical/physical PC 1 ($r=-0.424$) (this PC can be decomposed to decreasing pH and DO, and increasing SRP) and SRP alone ($r=0.260$).

The correlation between PC 1 and dimension 2 represents the best relationship between community composition and an environmental gradient. PC 1 is best described as increasing SRP and decreasing pH and DO, and this combination of parameters suggests that sites with high PC 1 scores are those where daytime productivity vs. respiration (p:r) is relatively low compared to sites with low PC 1 scores. Higher respiration at these sites is probably associated with organic sediment accumulation (personal observation) and a lower degree of fetch and pelagic mixing.

To further explore the relationship between community composition and ambient chemical/physical condition, we laid our third environmental dimensions (lake, ecoregion, wetland type and vegetation type) over the relationship. Vegetation type appeared to explain the relationship best with communities shifting from those found in *Scirpus* zones (with seemingly higher p:r, less organic sediment accumulation and increased fetch and pelagic mixing) to those found in lily to those found in *Typha* (with seemingly lower p:r, more organic sediment accumulation and decreased fetch and pelagic mixing) (Fig. 12). These relationships were corroborated, in part, by the proximity of similar vegetation types in the plot (Fig. 12). For example, *Juncus* is very similar to *Scirpus* in structure and is usually found in the same type of wetland habitat. *Juncus* sites were also plotted close to *Scirpus* sites in the relationship and had relatively low PC 1 and dimension 2 scores. On the other end of the gradient, *Sparganium* sites plotted very close to *Typha* sites, and these two vegetation types are also very similar to one another and are often found in similar wetland habitats.

The relationship between PC 1 and dimension 2 also revealed the varying responses of invertebrate communities of different vegetation zones to chemical/physical condition. In *Scirpus* zones, a small increase in PC 1 score (increasing respiration and SRP) was associated with a dramatic shift in community composition (increase in dimension 2 scores). In *Typha* zones, a relatively large gradient of ambient conditions was found (PC 1 scores). However, the respective shift in community composition over this chemical/physical gradient was relatively limited (Fig. 12). These varying responses suggest that invertebrate communities of less impacted, *Scirpus* dominated wetlands with little accumulated organic sediment may be more sensitive to new or increasing anthropogenic impacts than *Typha* dominated wetlands already subjected to relatively harsh anthropogenic impacts.

Since both invertebrates and fish were sampled at most of the sites, comparisons between the two communities could be made. Figure 13 shows the correlation between dimension 1 of the fish DCA and dimension 2 of the invertebrate DCA (r=0.289). While this correlation was significant, our data do not suggest that the fish and invertebrate communities were necessarily shaping each other but that both communities seem to be responding to plants and abiotic factors primarily and interacting with each other on a secondary basis.

Use of existing IBI metrics across the Great Lakes basin-

IBI scores from Uzarski et al. (2004) were calculated for 41 sites from across the Great Lakes basin. These included eight fringing wetlands from Saginaw Bay, two drowned river mouth wetlands from the eastern shore of Lake Michigan, 13 fringing wetlands from northern Lake Huron, three fringing wetlands from Lake Erie near Long Point, nine fringing wetlands from northern Lake Michigan, two fringing wetlands from Lake Ontario, two drowned river mouth and two barrier beach wetlands from southern Lake Superior. The IBI was applied to sites where at least one Inner or Outer *Scirpus* or a wet meadow zone was present.

A number of sites contained zones dominated by *Juncus* spp. and/or *Eleocharis* spp. IBI metrics were applied to zones not included in Uzarski et al. (2004) based on similarity in structure and sediment accumulation. For example, the *Juncus* zones at the Wigwam Bay and Big Fishdam sites contained shallow water and dense vegetation making them more similar to wet meadow zones, whereas the *Juncus* zone at the Epoufette Bay site was deeper, had slightly less dense vegetation and was best classified with Inner *Scirpus*. These determinations were made in the field whenever possible. Table 6 lists dominant vegetation type as well as vegetation zone classifications.

Table 6 lists IBI metric values and scores for the 41 wetland sites. Resultant IBI scores were similar to what we expected and categorized most sites according to the disturbance class determined *a priori.* Disturbance gradients were used to evaluate the IBI scores and a Pearson correlation coefficient of 0.674 was found between the overall disturbance gradient and IBI scores (Figure 2).

Both the IBI and the disturbance ranking seemed to account for the anthropogenic disturbance found at sites across the Great Lakes basin. A number of sites did, however, have IBI scores that were either higher or lower than expected from the disturbance ranking. For instance, the IBI seemed to underestimate the condition of the Big Fishdam and Epoufette Bay sites placing them in the "moderately impacted" category while the disturbance ranking placed them among the least impacted sites. It is unclear from the data collected in 2002 whether these discrepancies were a result of the disturbance ranking not accounting for certain disturbances that were detected by the IBI or whether the IBI was not sufficiently representing the "reference condition" of these sites.

The IBI was useful in measuring wetland condition in multiple wetland types. The IBI was applied to data from four drowned river mouth sites (Tahquamenon, Pentwater, Lincoln, and Portage). The Tahquamenon site was considered one of our least impacted drowned river mouth sites (95.1 disturbance % score) and received the highest IBI score of the four drowned river mouths. The Pentwater and Lincoln sites received moderate disturbance scores (60 and 61 disturbance % score, respectively), and the IBI placed these two sites in the "mildly impacted" category. Further testing of the IBI for use in drowned river mouths may reveal a need to adjust category scoring as these sites were expected to receive a "moderately impacted" score.

There was a relatively large discrepancy between IBI and disturbance scores at the Portage River site. The IBI placed this site in the "moderately degraded" category while our disturbance ranking placed this site in the top 10 most pristine sites. This discrepancy may be a result of either a relatively anomalous growth pattern of the vegetation at this site, (small, fairly isolated stands of *Juncus* sp.) or of the primary impact to the site being boat traffic (the site was adjacent to a busy navigation channel) which may not have been represented in our disturbance ranking.

The IBI was also applied to data from two barrier beach wetlands along the southern shore of Lake Superior (Lightfoot Bay and Ojibwa Bay). These sites were considered to be relatively pristine by our disturbance ranking and on-site observations. The IBI placed both of the sites among the least impacted sites sampled in 2002 (in the "mildly impacted" category). Metrics developed for use in Inner *Scirpus* zones were applied to these two barrier beach sites because the vegetation was similar in structure and density to typical Inner *Scirpus* zones. A wet meadow zone was also sampled at the Ojibwa Bay site and was scored accordingly. While the IBI placed these two sites where the disturbance ranking predicted, further testing may reveal a need to adjust category scoring for use in barrier beach wetlands.

The correspondence between calculated IBI scores and anthropogenic disturbance for drowned river mouth and barrier beach sites from Lakes Michigan and Superior shows that this set of metrics can potentially be applied to coastal wetlands across the Great Lakes basin in multiple wetland types as long as an inner and/or outer *Scirpus* or a wet meadow zone is present. Additional testing should continue throughout the Great Lakes basin and in all coastal wetland types before the IBI of Uzarski et al. (2004) is considered "validated" for use in these additional wetland types.

The IBI was also applied to data from four Lake Erie sites (Hahn, Lee Brown, Port Rowan and Booth's Harbor). These sites could not be included in our disturbance ranking because of insufficient chemical/physical data. However, based on surrounding land use and onsite observations, we assumed that these sites were receiving disturbances similar to our Lake Michigan drowned river mouth and Saginaw Bay fringing sites (i.e., relatively high percentage of surrounding agriculture in the 20 km buffer). The IBI placed these sites into the "moderately impacted" category, also similar to the Saginaw Bay sites. We suspected that the invertebrate sampling protocol was not followed as closely for Lake Erie sites as it was for sites of Lake Huron, Michigan and Superior (total sample abundances from Lake Erie sites varied significantly from the expected 50, 100 or 150 organisms that the protocol requires). However, IBI scores placed these sites where we expected, suggesting that the IBI is robust to a certain degree of protocol deviation (also see Uzarski et al. 2004 for more on deviations from protocol) and that the IBI could be useful in evaluating conditions in coastal wetlands of Lake Erie.

Data from one site on Lake Ontario (Robinson Cove) was subjected to the IBI of Uzarski et al. (2004). Again, this site was not included in our disturbance ranking for *Scirpus* and wet meadow sites but appeared to receive disturbances similar to our Lake Michigan drowned river mouth and Saginaw Bay fringing sites. The IBI placed Robinson Cove in the "moderately impacted" category, which corresponded to our expectation that this site was receiving impacts similar to our other sites in this category.

While the IBI of Uzarski et al. (2004) seemed to perform well in Lakes Erie and Ontario, testing should continue. Our application of the IBI to sites of these lakes was limited to the five sites containing *Scirpus* and/or wet meadow zones sampled in 2002. Furthermore, our quantification of disturbance was limited to our interpretation of a limited set of chemical/physical data for these sites. Testing of the IBI should continue in order to validate the IBI for these lakes and reveal any adjustments to the scoring scheme necessary to account for inherent differences in community composition between the upper and lower Great Lakes.

We also applied our modified IBI [modified from Uzarski et al. (2004), Appendix B to enable family-level macroinvertebrate identification] to data from 18 wetland sites that contained *Scirpus* and/or wet meadow zones. When modified IBI scores were calculated using family level data, sites separated along a 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 (Table 8). 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 8). Wigwam Bay was categorized as 'moderately impacted' and was placed among the northern Lake Huron sites. This was expected *a priori* because Wigwam Bay is located closest to open Lake Huron where anthropogenic disturbances would be diluted. This site had a largely forested watershed and was located farthest from the mouth of the Saginaw River, a known source of pollution for Saginaw Bay.

The modified IBI scores were 2% lower on average than the unmodified IBI scores for the 18 sites on which it was tested (scores were lower for 13 sites and higher for 5). This changed the disturbance category to the next lower category for 6 of the 18 sites (Table 7). The lower scores were the result of lower metric values for metrics measuring taxa richness and diversity (eg., *Odonata* richness, Shannon diversity, etc.). IBI scores could be adjusted to utilize family level data when higher resolution data are not available. For instance, by adding 2% to the family-level IBI scores, five of the six sites that had category shifts were shifted back to the categories assigned by the unmodified IBI. Individual metric scoring schemes could also be adjusted to account for the lower expected values for diversity and richness metrics when family-level data are used.

Development of macroinvertebrate-based metrics for use in Typha zones-

Macroinvertebrate data from twenty-eight sites (6 from Lake Huron, 10 from Lake Michigan, 11 from Lake Ontario and 1 from Lake Superior) yielded 12,153 organisms representing 168 taxa (genera and species). Our goal was to identify attributes of the *Typha* zone invertebrate communities that were responding to anthropogenic disturbance and incorporate these attributes into IBI metrics for use across the Great Lakes Basin. Data from the seven Lake Erie sites containing *Typha* zones were excluded from these analyses because of suspected deviations from sampling protocol.

Community composition and candidate IBI metrics were analyzed with chemical/physical and land use and cover parameters as well as with our *Typha* zone disturbance ranking (Table 5b). It is important to note that disturbance types and intensities in *Typha* zones were generally not unique to ecoregions or wetland types (Table 5b) though some correlation existed between ecoregion and land use parameters. Our disturbance ranking included as many parameters as were available from the 2002 data, and we feel it is our best possible estimate of the overall anthropogenic disturbance affecting these sites.

Initially, community composition among sites was explored using an indirect gradient analysis (DCA). As was the case for other plant zones, there were two primary objectives of using DCA. First, we wanted to determine whether community structure at the basin scale was best explained by lake/ecoregion, wetland type or anthropogenic disturbance. Second, if gradients corresponding to disturbance could be identified, this technique would reveal those taxa responding to the disturbance. These taxa could then be tested for their use in IBI metrics. To test whether dimensions could be explained by anthropogenic disturbance, we calculated Pearson correlation coefficients between DCA site scores for the first three dimensions of each analysis and disturbance scores (including our overall disturbance gradient).

The first DCA was conducted on means of all 168 taxa from the 28 sites. Dimension 1 of this DCA indicated a gradient from Saginaw Bay to Lake Ontario to Lake Michigan to our one Whitefish Bay site (Figure 4). This gradient indicated that at high taxonomic resolution (genus and species in most cases) invertebrate communities in *Typha* zones were relatively unique among Great Lake and/or wetland type (these were correlated in our dataset because all but one of the Lake Michigan sites were drowned river mouths while nearly all of the Saginaw Bay and Lake Ontario sites were fringing wetlands). Rare taxa were important in this ordination and the 6 taxa contributing the most inertia to dimension 1 made up less than 0.5% of the total invertebrate abundance. The second dimension in this DCA could not be explained by ecoregion or anthropogenic disturbance (Figure 4).

After establishing that at high taxonomic resolution, Great Lakes coastal wetland *Typha* zones were best described by lake/ecoregion and/or wetland type, additional DCAs were conducted to search for gradients that could be explained by other variables such as anthropogenic disturbance. We attempted this by excluding taxa that contributed significantly to the ecoregional gradient. Taxa were removed and DCAs were conducted in a stepwise fashion until only 30 taxa remained. These remaining taxa represented 72.4% of the original invertebrate dataset, and the resulting gradient was still best explained by lake/ecoregion and/or wetland type (Figure 5).

Additional DCAs were conducted using medians of the triplicate samples in place of means. Medians are more robust to outliers and have been used in our IBI metric development practices in the past. An advantage of using medians as a measure of central tendency in ordination analyses is their discounting of particularly rare taxa. In our dataset, many rare taxa were only found in one of the three replicate samples at a site. When using medians, a taxon had to be found in at least two of the three replicates at a site for it to be retained. When the original dataset was reduced to medians, 89 taxa were represented in the dataset from the 28 sites.

In a DCA of these 89 taxa, the first dimension was again best explained by ecoregion and consisted of a gradient from Whitefish Bay to Lake Michigan to Lake Ontario to Saginaw Bay sites. The five taxa with the lowest dimension 1 scores were found in overwhelmingly higher abundance at the Whitefish Bay site. The taxa with the highest dimension 1 scores were found in much higher numbers at the Saginaw Bay sites. These taxa were removed and the analysis was performed again with the remaining 73 taxa. A similar gradient was found in dimension 1 from Lake Michigan to Lake Ontario to Saginaw Bay sites. The one Whitefish Bay site had a dimension 1 score similar to the Lake Michigan sites.

The next approach was to lower the taxonomic resolution of our dataset. Many genera and species were unique to specific ecoregions while their respective families were cosmopolitan. Therefore, reducing the dataset to the family level could potentially uncover environmentally driven gradients in community composition irrespective of ecoregion. In addition, family-level taxonomy would be more reliable and require less labor by laboratory staff. Thus, lower resolution taxonomy may be more appropriate for incorporation into IBI metrics.

In a DCA of 69 taxa at the family level, dimension 1 was still best explained by ecoregion and formed a gradient from Saginaw Bay to Lake Ontario to Lake Michigan sites (Figure 6). Taxa contributing the most inertia to dimension 1 (ecoregion) were then removed and the analysis was repeated. DCAs were conducted in a stepwise fashion with 69, 58, 46, 35 and 27 taxa. An ecoregional gradient overwhelmed the first dimension in every analysis.

The overwhelming influence of ecoregion on Great Lakes coastal wetland *Typha* invertebrate communities was evident in this series of ordination analyses. When means were used and the dataset was reduced to 30 of the most abundant taxa, the communities were still relatively unique among ecoregions. When medians were used as a measure of central tendency, the results were similar. At the family level, when the 47 most ecoregionally-unique taxa were removed, community composition was still best explained by ecoregion. The apparent uniqueness of *Typha* invertebrate communities among lakes/ecoregions suggests that *Typha* zone IBI development may need to account for the inherent variability due to ecoregion. However, certain attributes of the *Typha* zone invertebrate communities may still be responding to anthropogenic disturbance predictably across the Great Lakes basin. While these attributes were not strong enough to be represented in the first dimension of the DCAs, they could be contained in other dimensions or found by testing candidate metrics from the literature.

Site scores from dimensions 1 through 3 of all DCAs were tested for correlation with disturbance parameters. Table 9a shows all significant correlation coefficients that were found and the disturbance class that the relationships suggest (either agriculture, urbanization or nutrients). Dimension 1 scores from six of the DCAs were significantly correlated with % Agriculture and % NonForest. While this result appears to suggest a community response caused by upland land use, it is difficult to partition the effects of ecoregion from that of land use. There is a significantly greater proportion of land in agriculture surrounding sites of Saginaw Bay (61.1±11.5%) than surrounding sites of Lakes Ontario (40.8±2.8%) and Michigan (25.3±4.7%). Similarly, there is a higher percentage of forest in the watersheds of Lake Michigan sites (47.8±5.1%) than Saginaw Bay sites (19.2±9.0) and Lake Ontario sites (24.03±3.3%). Taxa forming these gradients (first dimensions) were evaluated for possible use as indicators, but it was unclear whether or not they were responding to disturbance or ecoregional effects.

The second and third DCA dimensions did not appear to be structured by ecoregion so significant correlations between their site scores and disturbance parameters indicated a potential for these dimensions to be indicative of disturbance. Table 9a lists all significant correlations and table 9b lists the five taxa contributing the most inertia to each end of each gradient (five highest and five lowest taxa in each dimension). While taxa at the genus and species level were identified as potential indicators of disturbance by a number of the DCA dimensions (Table 9b), we decided to focus on DCA dimensions where family-level data were used. Taxa at the family level would not only be easier to identify by laboratory staff, but are also probably less affected by ecoregional influences. Families that: 1) contributed significant inertia to the DCA dimensions that correlated with disturbance parameters; and 2) were found at five or more sites were considered candidates for use in IBI metrics. Since the DCA dimensions were correlated with specific disturbance parameters, the candidate indicator taxa were considered indicators of specific types of disturbance (either agriculture, urbanization, or nutrients). Taxa from both ends of gradients were identified so that some taxa would indicate reference conditions and some would indicate impacted conditions. Table 9b lists the taxa that were chosen to be tested for use as metrics.

We also tested IBI metrics found in the literature for their use in Great Lakes coastal wetland *Typha* zones. Initially, IBI scores were calculated for all *Typha* sites sampled in 2002 based on metrics developed by Burton et al. (1999). A weak but significant Pearson correlation ($r=0.41$) was found between the resulting IBI scores and our overall disturbance gradient (Fig. 3). This preliminary evaluation suggested that development of a basin-wide IBI for use in *Typha* zones was possible. The metrics developed by Burton et al. (1999) were then reevaluated along with eighteen additional metrics from the literature and two new metrics (Table 10 lists all metrics from the literature that were tested).

All 27 candidate metrics were calculated and tested independently for correlation with our overall disturbance gradient. Pearson correlation (significance was set at $p<0.05$) was used to test metrics (Table 10). Metric values were calculated for every sample and medians were used as a measure of central tendency. Significant correlations were found with Crustacea+Mollusca Richness, Total Genera Richness and Relative abundance of Sphaeriidae ($r=0.429$, 0.410 and 0.419, respectively). The next approach was to search for correlations between metrics and individual disturbance parameters. These relationships were explored in an effort to identify metrics that were responding to individual types of disturbance rather than the cumulative disturbance that our overall disturbance gradient represented. Again, using Pearson correlations, 10 metrics were found to respond to anthropogenic disturbance parameters (Table 10). Negative correlation coefficients indicated that metric values decreased with increasing disturbance. This was the case for all but % Odonates and % Agriculture, % Crustacea+Mollusca and nitrate-difference from median, %Amphipods and nitrate, and Diptera Richness and % Forest^{-1}. The % Crustacea+Mollusca and % Amphipod metrics were negatively correlated with other disturbance parameters, therefore, we retained their orientation as indicators of reference conditions. The % Odonata metric was not negatively correlated with other parameters, thus we considered it a potential indicator of impact in the *Typha* zone.

The third approach used to search for indicators of anthropogenic disturbance in *Typha* invertebrate communities was to calculate a correlation matrix between taxa abundances (means per site at the family-level) and disturbance parameters. Spearman correlation was used because invertebrate abundances did not approach a normal distribution. Taxa that were found at five or more sites, and were significantly correlated with disturbance parameters, were identified and evaluated for their potential use in IBI metrics. Table 11 lists these correlations and their Spearman correlation coefficients.

A preliminary IBI for Great Lakes coastal wetland *Typha* zones was created by combining a) the taxa that were identified as indicators of disturbance or reference conditions by either DCA or correlation with disturbance parameters; and with b) the metrics from the literature that correlated with disturbance parameters. These metrics and the type of disturbance that they seemed to indicate are found in Table 12. A number of candidate metrics were found to increase with one type of disturbance and decrease with another. This is likely a result of the bimodal nature of our coarse land use and cover parameters (eg., if sites were low in % agriculture, they were more likely to be high in % urbanization). Metrics that behaved in this way were not included in our preliminary *Typha* IBI since it was our goal to rank sites according to overall disturbance. Preliminary scoring ranges were determined using quartile values as cutoffs and assigning values of 1, 3, or 5 to these ranges. Metrics that correlated with our overall disturbance gradient were assigned scoring ranges for scores of 1, 3, 5 or 7 (scoring ranges are found in Table 13). We used quartile values to determine cutoffs because we feel that the sites sampled in 2002 were representative of the disturbance gradient that *Typha* sites fall into basin-wide. While there probably are some *Typha* sites that would fall outside of this gradient (either more or less disturbed than any of our sites), we feel that our 2002 sites likely cover most of the gradient.

Thirty preliminary metric scores from sites sampled in 2002 were calculated and are found in Table 13. These metric scores were summed and the resulting scores were significantly correlated with our overall disturbance gradient (Pearson correlation coefficient=0.400, p=0.035). Next, in an effort to cut the number of metrics needed for *Typha* zones, we calculated a Spearman correlation matrix between metric scores (1, 3, 5, or 7) at each site and the overall disturbance gradient. The nine metrics that were significantly correlated with the overall disturbance gradient were retained. Overall IBI scores based on these nine metrics were correlated with the overall disturbance gradient (r=0.538 p=0.003) (Figure 7). Table 14 is a preliminary IBI worksheet for *Typha* zones.

Conclusions

The IBI of Uzarski et al. (2004) was tested across the Great Lakes basin on all wetland sites containing at least one *Scirpus* or wet meadow vegetation zone. IBI scores for these sites correlated significantly with calculated disturbance scores. The IBI also placed most sites into the disturbance class that our *a priori* disturbance rank placed sites prior to community analyses. Therefore, we recommend that this IBI be used across the Great Lakes basin. However, variability in community composition due to ecoregional effects (especially across latitudes) and natural chemical/physical water quality differences will inevitably affect IBI scores to a certain degree. If variability due to these natural factors is substantial, the IBI may need adjustment in the future. In light of this possibility, we recommend that all community data be retained so that if IBI metrics or metric scoring schemes must be changed, the changes can be applied retroactively. This approach will allow management agencies to implement monitoring programs immediately while portions of the IBIs are concurrently tested and validated.

The IBI of Uzarski et al. (2004) require data to be at the lowest operational taxonomic unit (genus and species-level). Since the taxonomy at this resolution requires a fair amount of expertise, we tested the IBI with invertebrate data at the family level. Acquiring family-level data would undoubtedly require less effort by IBI practitioners resulting in lower monitoring costs. IBI scores calculated with family-level data placed most sites into the correct disturbance class. Adjustments to IBI category scoring could probably be made to reduce or eliminate the differences in results between the two taxonomic levels. However, since the IBI requires ongoing testing and validation, we recommend that data be acquired at the lowest operational taxonomic unit whenever possible. These data can then be used retroactively if metrics requiring high-resolution taxonomy are found.

The inherent variability in invertebrate community composition of *Typha* zones has made IBI metric development in this zone difficult. We feel that this is at least in part due to the relatively harsh environment of the *Typha* as well as the presence of *Typha* itself sometimes indicating disturbance. This zone often contains the fewest organisms as well as those adapted to extremely low DO and a large amount of organic sediments regardless of anthropogenic disturbance. The large dataset collected in 2002 allowed us to identify nine preliminary *Typha* metrics. These metrics were identified and calculated across the Great Lakes basin and in multiple wetland types. We are presenting these metrics as preliminary and suggest further testing and validation. As stated for the IBI of Uzarski et al. (2004), data collected immediately as part of a basin-wide monitoring program could be applied to a validated IBI retroactively.

The dataset collected in 2002 also allowed us to identify fish-based metrics for use in an IBI. Preliminary IBIs were developed for *Scirpus* and *Typha* zones. These IBIs were developed for use at the Great Lakes basin scale and now require testing and validation. As with the invertebrate-based IBIs, data collection for monitoring purposes could begin immediately with changes to the IBI applied to the dataset retroactively.

We now have a set of both invertebrate- and fish-based IBIs for use in monitoring the ecological integrity of Great Lakes coastal wetlands. By monitoring the biotic communities in coastal wetlands, many anthropogenic impacts that would go unnoticed by traditional chemical and physical measures can be tracked. Since different biotic communities will respond to different impacts, the power of IBIs to detect anthropogenic disturbance will inevitably increase when more and different communities are monitored (e.g. fish, invertebrates, birds, reptiles, and amphibians). We therefore recommend using as many biotic indices as practical when implementing a monitoring program. In addition, traditional chemical and physical measurements should not be overlooked when establishing a monitoring program, as these measures are still useful in identifying both short and long-term anthropogenic impacts.

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Appendix A

Fish Habitat Use Within and Across Wetland Classes in Coastal Wetlands of the Five Great Lakes: Development of a Fish-Based Index of Biotic Integrity

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Index Words
Coastal wetlands, fish, IBI, fish community composition, Great Lakes, bioassessment, land use effects.

Abstract

The relative importance of Great Lake, ecoregion, wetland type, and plant zonation in structuring fish community composition was determined for 61 Great Lakes coastal wetlands sampled in 2002. These wetlands, from all five Great Lakes, spanned nine ecoregions and four wetland types (open lacustrine, protected lacustrine, barrier-beach, and drowned river mouth). Fish were sampled with fyke nets, and physical and chemical parameters were determined for inundated plant zones in each wetland. Land use/cover was calculated for 1- and 20-km buffers from digitized imagery. Fish community composition within and among wetlands was compared using correspondence analyses, detrended correspondence analyses, and non-metric multidimensional scaling. Within-site plant zonation was the single most important variable structuring fish communities regardless of lake, ecoregion, or wetland type. Fish community composition correlated with chemical/physical and land use/cover variables. Fish community composition shifted with nutrients and adjacent agriculture within vegetation zone. Fish community composition was ordinated from Scirpus, Eleocharis, and Zizania, to Nuphar/Nymphaea, and Pontederia/Sagittaria/Peltandra to Spargainium to Typha. Once the underlying driver in fish community composition was determined to be plant zonation, data were stratified by vegetation type and an IBI was developed for coastal wetlands of the entire Great Lakes basin.

Introduction

Great Lakes coastal wetlands provide critical habitat for more than 80 species of fish (Jude and Pappas 1992). More than 50 of these species are dependent upon wetlands while another 30+ migrate into and out of them during different periods in their life history (Jude and Pappas 1992, Wilcox 1995). An additional 30+ species of fish may be occasional visitors to coastal wetlands based on occurrence in adjacent habitats (Jude and Pappas 1992). Coastal wetlands also provide habitat for 20+ species of mammals, large numbers of amphibians and reptiles (Wilcox 1995, Weeber and Vallianatos 2000) and 80-90 bird species including 28 species of waterfowl (Prince et al. 1992, Prince and Flegel 1995, Weeber and Vallianatos 2000). We have identified more than 250 taxa of invertebrates which utilize coastal wetlands (Burton et al. 1999, 2002, 2004; Cardinale et al. 1997, 1998; Gathman et al. 1999; Kashian and Burton 2000, and unpublished data). Similar numbers have been reported by others (e.g., see reviews by Krieger 1992 and Gathman et al. 1999). The actual number of species may be 3-4 times greater, given the difficulty in identification of larval invertebrates. Coastal wetlands are occupied by many rare plants with over 40 species listed for Lake Huron alone (Wilcox 1995). Despite their importance as habitats for so many organisms, knowledge about the biota of these wetlands is limited.

As transitional systems between land and water, coastal wetlands are among the first habitats impacted by disturbances from adjacent uplands and/or pollutants from upstream. Activities and pollutants that degrade wetland habitat often also pose threats to other near shore and deep water habitats if allowed to continue unabated. Since many pollutants accumulate in them and adjacent changes in land use tend to impact them first, coastal wetlands can provide "early warning" of potential threats to the Great Lakes ecosystem. The governments of Canada and the U.S.A. recognized this potential and initiated a process to identify and/or develop indicators of “ecosystem health” for wetlands and other Great Lakes habitats at the State-of-the-Lakes Ecosystem Conference (SOLEC) held in Buffalo, New York in 1998. Progress was reviewed and potential indicators were identified by working group members at SOLEC 2000 in Hamilton, Ontario. Potential indicators listed by the wetlands indicators working group included indices of biotic integrity (IBIs) based on invertebrates, fish, and plants even though no broadly accepted protocol was available at the time for any of these biotic groups.

Recognition of the need for a biotic-based assessment system accelerated our on-going research on development of invertebrate-based IBIs for coastal wetlands and culminated in publication of an invertebrate-based IBI for coastal wetlands (Burton et al. 1999, Kashian and Burton 2000, Uzarski et al. 2004). We also expanded efforts to obtain data on fish populations in coastal wetlands, with the goal of developing fish-based IBIs for major classes of coastal wetlands described by Keough et al. (1999) and modified by Albert et al. (2003).

Great Lakes coastal wetlands occupy a relatively small percentage of the Great Lakes shoreline (e.g., about 11 % of the shoreline of the U.S. side of Lake Huron - Prince and Flegel 1995). Conversion of wetlands over the last 100 years has reduced the area of Great Lakes coastal wetlands by more than 50% with losses greater than 95% in some areas such as Western Lake Erie (Krieger et al. 1992). Sustainable management of the remaining wetlands and efforts to restore the large number of wetlands that have been converted to other land uses are critical to the long-term viability of the Great Lakes ecosystem. An important tool needed for management and restoration of coastal wetlands is a system of assessment which will allow managers to monitor the health of these and adjacent coastal systems on a routine basis so that trends in wetland condition can be established and used to identify threats to these ecosystems. Our overall goal was to develop a system of indicators of biotic integrity for coastal wetlands based on fish, invertebrates, and plants. Our goal in this paper is to document and provide details of a fish-based IBI for wetlands of the Great Lakes.

Minns et al. (1994) developed a fish-based IBI for marshes of Great Lakes' Areas of Concern which included metrics sensitive to impacts by exotic fishes, water quality changes, physical habitat alterations, and changes in piscivore abundance related to fishing pressure and stocking. This system has not been extended outside of the limited and often highly impacted Areas of Concern. The work of Brazner (1997), Brazner and Beals (1997), and Minns et al. (1994) demonstrated relationships between fish populations and wetland and/or nearshore habitats which suggest that development of a fish-based IBI for coastal wetlands is possible. Recently, Randall and Minns (2002) used an IBI to assess habitat productivity of near shore areas (including coastal wetlands) of Lakes Erie and Ontario and compared results to those obtained using their Habitat Productivity Index. Thoma (1999) developed a fish-based IBI for near shore waters of Lake Erie. Despite such promising results, Wilcox et al. (2002) concluded that development of wetland IBIs for the upper Great Lakes using macrophytes, fish, and microinvertebrates was impractical. Even though some of their metrics showed potential, they concluded that natural water level changes from those that existed during data collection were likely to alter communities enough to invalidate metrics in subsequent years. We overcame this problem for invertebrates in fringing coastal wetlands by developing a method based on sampling any or all of four plant zones depending on the number of zones inundated (Burton et al. 1999, Uzarski et al. 2004). The IBI scores for a particular year were calculated by summing scores from each of the zones that were inundated when sampling occurred. As water levels decreased and zones were no longer inundated, the IBI scores changed, but metrics for even a single inundated zone proved to be effective in establishing wetland condition for fringing wetlands of Lakes Huron and Michigan as water level decreased by more than 1-meter from 1997 through 2002 (Uzarski et al., 2004). Based on these results, we hypothesized that fish-based IBI metrics developed using samples from each inundated plant zone, rather than using composited samples to develop one set of metrics for the entire wetland, would provide the flexibility needed to make the IBI useful over a wide range of lake levels. This makes our approach different than efforts of others including sampling associated with the REMAP project of U.S. EPA where composite samples for the entire wetland are used.

Objectives

The primary objective of this study was to explore relationships of fish populations among Great Lakes, ecoregions, wetland types, and plant zones and relate these differences to water quality and adjacent land use/cover. Using what we learned from these analyses, our second goal was to develop a fish-based system of biotic indicators of wetland ecological health that could be employed in a monitoring program by federal, state, provincial, and local agencies to detect effects of anthropogenic disturbance on the biotic integrity of Great Lakes coastal wetlands.

Methods

Study Sites

Sixty-one sites spanning all five Great Lakes were selected for study. Five sites were located on Lake Superior, 18 on Lake Michigan, 13 on Lake Huron, 13 on Lake Erie, and 12 on Lake Ontario (Fig. 1). Each site was assigned designators based on lake (Superior, Michigan, Huron, Erie, or Ontario) ecoregion (E. Lake Superior, N. Lake Michigan, N.E. Lake Michigan, S.E. Lake Michigan, N. Lake Huron, Saginaw Bay Huron, Long Point Erie, N.W. Lake Ontario, and N.E. Lake Ontario), wetland type (open lacustrine, protected lacustrine, barrier-beach, and drowned river mouth), and vegetation type (*Sparganium* (bur-reed), *Scirpus* (bulrush) (inner and outer; e.g., Burton et al. 1999 and Uzarski et al. 2004), *Nuphar/Nymphaea* (lily), *Pontederia/Sagittaria/Peltandra* (pickerel weed/arrowhead/arrow arum), *Typha* (cattail), *Zizania* (wild rice), and *Eleocharis* (spike rush)) See Appendix A, available from the corresponding author's web site (www.gvsu.edu/wri/staff/duzarski.htm), for specific site locations and classifications. Site selection was based on access and inundation. We sampled every site that we encountered if we were granted access and the site was inundated with enough water to set nets (approximately 25 cm).

Wetland Classification

Wetlands of the Great Lakes were classified into geomorphological classes that reflect location in the landscape and exposure to waves, storm surges, and lake level changes. Classes followed categories described by Albert et al. (2003) on behalf of the Great Lakes Wetland Consortium. Wetlands were categorized as *lacustrine* (fringing), *riverine*, or *barrier protected*. All 61 sites sampled fit into *open lacustrine*, *protected lacustrine*, *barrier-beach*, or *drowned river mouth* subcategories (Appendix A).

Chemical/Physical and Land Use/Cover Measurements

Basic chemical/physical parameters were sampled within each vegetation zone fished. Analytical procedures followed those recommended in Standard Methods for the Examination of Water and Wastewater (APHA 1992). These measurements included soluble reactive phosphorus, ammonium-N, nitrite/nitrate-N, dissolved oxygen, temperature, turbidity, specific conductance, pH, and total alkalinity at all sites. Additional measurements of chlorophyll a, sulfate, chloride, and redox potential were made at approximately half of the sites. Quality assurance/quality control procedures followed protocols recommended by U.S. EPA.

Land use/cover data were obtained from existing digitized maps. When land use/cover data from more than one year were available, on-site observations were used to determine the most accurate map. For example, we found that maps digitized from aerial photographs taken in 1978 (available from the Michigan Center for Geographic Information) were more accurate at coarse resolution for many of the Michigan sites than newer available versions. Coarse categories, including agriculture, urbanization, roads, idle lands, wetlands and forests, were calculated for 1-km buffers around all sites except drowned river mouths. Land use/cover was calculated for the entire watershed at drowned river mouth sites. These data were verified with on-site observations where possible. Additional 20-km buffers were calculated around approximately half of the sites; we were unable to acquire these data for all sites.

Fish sampling

Fish sampling was conducted using a minimum of three replicate fyke nets with 12.5-mm mesh in each dominant vegetation zone for one net-night. Sampling was conducted during the summer of 2002 and corresponded to the maturity of the vegetation in each system. Only dominant plant zones that could be definitively assigned to a dominant (i.e. visually much more than 50% composition by one species) type (*Sparganium, Scirpus, Nuphar/Nymphaea, Pontederia/Sagittaria/Peltandra, Typha, Zizania,* or *Eleocharis*) were sampled to partition variation due to structure or habitat type. We rarely encountered vegetation zones without an obvious dominant. However, when we did, these were avoided. Two sizes of fyke nets were used, 0.5-m x 1-m openings and 1-m x 1-m openings. Smaller nets were set in water approximately 0.25 m deep to 0.50 m; larger nets were set in water depths greater than 0.50 m. Leads were 7.3 m in length and wings were 1.8 m. The depth of water in each plant zone dictated the net size used since the only difference between large and small nets was the height. Each net was randomly placed perpendicular to the vegetation zone of interest with leads extending into the vegetation itself. Therefore, fishes either occupying the vegetation or using the edge were likely to be caught. Wings were set at 45^{o} angles to the lead and connected to the outer opening on each side of the net. Fishes were identified to species and enumerated. Catches per net per night were recorded. Ten to 20 specimens of each species were chosen randomly for measurement, but these data were not included in this paper; please contact authors regarding these data if they can be of use.

Statistical analyses

Chemical/physical and land use/cover data were analyzed using principal components analysis (PCA). Percentages were transformed using an arcsine square root before inclusion in the PCA. All variables entered into the PCA represented a normal distribution. Correspondence analysis (CA), detrended correspondence analysis (DCA), and non-metric multidimensional scaling (NMDS) were used to analyze fish data. All three indirect gradient analyses were used because an 'arch effect' can sometimes confuse the interpretation of CA. DCA and NMDS were used to determine if the arch was present. Fish data were not transformed. When CA, DCA, and NMDS all showed similar results, only CA was used to describe relative fish communities.

Indirect gradient analyses (CA, DCA, and NMDS) were used to determine if fish composition was mainly structured by Great Lake, ecoregion, wetland type, or plant zonation. This was determined by overlaying these variables as a third dimension onto the first two dimensions of the CA. If fish community composition was structured by one of these variables, fish community composition would in turn group the sites by either lake, ecoregion, wetland type, or plant zonation. Therefore, the first run of the CA contained all taxa represented by more than three individuals in the total dataset (15,000 + fish in total). Following each run of the analyses, sites were coded using Great Lake, ecoregion, wetland type, and plant zonation. Biplots were then visually inspected for groupings. Chemical/physical and land use/cover data were combined using PCA. Eigenvalues were then correlated with factor loadings from CA in an attempt to associate fish community structure with abiotic factors. If no groupings were observed and factor loadings did not correlate with eigenvalues, then taxa responsible for the most inertia in each dimension were identified. If these taxa were either very rare or had the tendency to school, they were likely to overwhelm the analysis and therefore were removed before the next iteration was performed. This process continued until a gradient (either Great Lake, ecoregion, wetland type, or plant zonation) could be identified. Once a gradient was identified, direct gradient analysis (canonical correspondence analysis) was performed to determine accordance between the two approaches (direct and indirect gradient analysis). The above statistical analyses were solely performed to determine the underlying forcing factors in establishing fish community composition in Great Lakes coastal wetlands. In turn, results of these analyses were used to determine proper stratification (either by Great Lake, ecoregion, wetland type, or plant zonation) for a fish-based IBI. Once the forcing factor was determined, the entire dataset, including those fishes removed from the CA, was stratified and analyzed under the confines of this stratification using Spearman or Pearson correlation to search for metrics. These data were correlated with disturbance gradients established *a priori* using land use and chemical/physical data. Statistical analyses were performed using Systat 8.0, SAS V8, and Canoco for Windows.

Establishing Disturbance Gradients

Disturbance gradients were established using land use/cover and chemical/physical data. They were established using both principal components (PCs) and calculating rank sums using all chemical/physical and land use/cover data (1-km and 20-km buffers). Turbidity, specific conductance, and chloride were ranked directly with the greater values indicating disturbance. Extreme values, either very high or very low, for nitrate-N, ammonium-N, and soluble reactive phosphorus concentrations, as well as percent saturation of dissolved oxygen, and pH were considered indicators of disturbance (reasoning for this assumption is explained in the discussion section). Therefore, absolute values of the difference from the median concentration were used to establish a rank order for each of these parameters. These data, as well as land use/cover data, were used to establish ranks. Ranks were then combined into a grand rank producing the final disturbance gradient.

Land use/cover data were analyzed at two scales for more than half of our sites and both were incorporated into the final disturbance gradient for this subset of sites. The larger scale (20-km buffers) was used to represent the impacts to the nearshore region or the water source of the wetland and was double weighted. These data were not available for all sites. A finer scale (1-km buffer) was used to relate impacts much more locally and received a single weighting. Metrics were correlated with this disturbance gradient as well as with PC1 of the chemical/physical, land use/cover PCA.

IBI Development

Community attributes were correlated with PCs and the rank-sum disturbance gradients using Pearson and Spearman correlations, respectively. When community attributes or specific taxa correlated with established disturbance gradients, they were deemed metrics. When attributes did not significantly correlate with the disturbance gradients but did show a dichotomy between pristine and impacted sites, Mann-Whitney U tests were performed and these too were maintained. Those attributes, including many from the literature that showed an empirical response to disturbance using one of the above methods, were deemed metrics. Natural breaks in metric values were then used as cut-offs for score categories.

Results

Chemical/Physical and Land Use/Cover Measurements

Our chemical/physical and land use/cover data suggested a wide range of ambient conditions among our sites. However, we were not able to obtain a complete matrix for all of our parameters; some sites had missing values, especially the 20-km buffer. The 20-km buffer could only be calculated for 33 sites, and therefore, ranks and disturbance gradients could only be calculated with this variable for a subset of our sites. Chloride data were also only available from a subset of sites (Table 1a-c)).

Principal Components Analysis

Principal components analysis, including all of the chemical/physical and land use/cover data, produced results very similar to the last iteration of the CA. That is, the PCA was structured by vegetation zone especially in PC1. *Scirpus* sites were given low PC1 scores and *Typha* sites scored high with the remaining vegetation zones ordinated somewhere in between (Fig. 2). In combination, the two dimensions accounted for 37% of the variation in the dataset. In general, the PCA can be viewed as having three groupings, those that scored low in both PCs and those that scored high in PC1 and either low or high in PC2. Those sites that scored low in both PCs tended to have higher dissolved oxygen and pH as well as a high percentage of forest in the 1-km buffer surrounding the site. This grouping included nearly all *Scirpus* sites. The second grouping of the PCA included those sites with high PC1 scores and low PC2 scores. These sites tended to be composed of *Typha* and generally had high nutrients and a high percentage of adjacent land use in agriculture. Finally, the third grouping was also composed of mostly *Typha* sites scoring high in both PCs and was indicative of high run-off and percent urbanization. Nearly all remaining vegetation zones were placed between the first and second groupings discussed (Fig. 2). Consistent with biotic data in the CA, no patterns were visually detected when sites were coded by lake, ecoregion, or wetland type.

Correspondence analysis

Rare or schooling taxa increased the variability in the data set and were often captured by chance alone. For example, schools of *Ameiurus melas* (black bullhead) and *Amia calva* (bowfin) were observed at nearly every site sampled, yet schools of these taxa were only caught at a few sites. When these taxa overwhelmed the first iteration of analyses, they had to be removed (see Table 2) and the entire process was repeated. Appendix B (available from the corresponding author's web site: www.gvsu.edu/wri/staff/duzarski.htm) includes the mean catch per net for all species. After several iterations, the first pattern appeared (Fig. 3). Just as in the case of the PCA of the abiotic data, plant zone was the major driving factor of community composition, more so than even lake, ecoregion, or wetland type. Fish community composition was ordinated from *Scirpus, Eleocharis*, and *Zizania* to *Nuphar/Nymphaea,* and *Pontederia/Sagittaria/Peltandra* to *Sparganium* to *Typha.*

Correlation Between Abiotic and Biotic Data

Once the pattern appeared in the CA, a significant ($p = 0.001$) Pearson correlation was found between the first dimensions of the CA and the first PC (Fig. 4). The third dimensions (Great Lake, ecoregion, wetland type, and plant zone) were then applied to the correlation analysis just as they were in each PCA and CA. Once again, plant zone showed the only apparent relationship. The order of the plant zones in the correlation was identical to the CA and the PCA as well. The relationship seems to be better represented by a quadratic function, suggesting that a threshold in fish community composition is reached, but a linear correlation was applied and was significant. Direct gradient analysis (CCA) supported these results suggesting that the underlying gradient in the fish community data was established by the plant and/or abiotic data. This relationship suggested that not only were plants, fish communities, and the associated abiotic factors related, but also that they were somewhat predictable. Therefore, a fish-based IBI for the entire Great Lakes basin appeared to be feasible. From this point on, additional data analyses were performed after stratifying by plant zone.

Disturbance Gradient

The number of variables used to establish primary gradients varied with plant zone. For example, *Scirpus* was stratified into an outer wave-swept area and an inner protected area (e.g., Burton et al. 1999; Uzarski et al. 2004; Burton et al. 2004) and ranks of these data were averaged for the overall *Scirpus*-zone gradient. In the inner *Scirpus* zone, the median nitrate concentration was below our detection limit (0.01 mg l^{-1}) so nitrate could not be used in the gradient. Formulae used to calculate disturbance gradients for each plant zone can be found in Table 3 and the overall rank order of the sites can be found in Table 1a-c. The 20-km buffer proved important in showing that, for example, all Saginaw Bay sites tended to be more impacted than N. Lake Huron sites (large-scale differences in water quality), and the 1-km buffer was important in ordering sites within Saginaw Bay and the other regions.

Additional disturbance gradients were established using PCA for each plant zone. These were used to search for metrics that were not apparent from the primary gradients. Those variables that weighted the heaviest in PC1 of each analysis were identified and taken into consideration when searching for metrics. Those variables that weighted heaviest in PC1 for *Scirpus*, *Nuphar/Nymphaea*, and *Typha* were nitrate, chloride, and specific conductivity respectively.

IBI Development

Once it was revealed that plant zone was the major driving factor in establishing fish community composition, and the above analyses suggested that an IBI could be developed for all five Great Lakes, we stratified the entire dataset (including those taxa removed from iterations) and began to search for metrics. We were not assuming that the taxa that were eliminated in these iterations were not responding to vegetation, nutrients, and/or agriculture as the remaining taxa did. The taxa removed were simply masking gradients and community structure because they tended to school or were uncommon. Our sampling effort was not great enough to determine how schooling or rare taxa contribute to overall community composition because the tendency is to catch either large schools or rare taxa more by chance than by their true abundances with only three nets per plant zone.

Fish of 38, 39, and 30 taxa were identified in the *Scirpus*, *Typha* and *Nuphar/Nymphaea -Pontederia/Sagittaria/Peltandra* zones, respectively. The *Nuphar/Nymphaea* and *Pontederia/Sagittaria/Peltandra* had to be combined post-hoc because sample size was simply too low for these communities and the CA showed very similar fish communities in these two vegetation zones. Community attributes and indicator species were evaluated based on their ability to order sites according to anthropogenic disturbance. Additionally, 41 published metrics were also evaluated (Wilcox et al. 2002, Minns et al. 1994, and Simon 1998). Correlation and graphical interpretation yielded 14, 11, and 2 metrics for *Scirpus,Typha* and *Nuphar/Nymphaea-Pontederia/Sagittaria/Peltandra,* respectively. Metric scores were established by searching for natural breaks in the metric values.

Fish data are inherently variable. In an attempt to remove some of this variability from the IBI, we eliminated data for plant zones when fewer fishes than a mean of at least 10 per net per plant zone had been caught before applying the IBI. Of the 22 *Scirpus*, 29 *Typha*, and 12 *Nuphar/Nymphaea- Pontederia/Sagittaria/Peltandra* sites fished, 5, 11 and 8, respectively, were excluded because of insufficient catches. We did not feel that these catches accurately reflected a 'typical catch' for these sites (we recommend that if the user feels that he/she collected an atypical sample for a given site, the site is fished for an additional night). After removing sites with insufficient data, metric scores correlated with disturbance rankings at r=0.891 for *Scirpus* and r=0.824 for *Typha* (Fig. 5). Table 4 contains the final set of IBI metrics for *Scirpus* and *Typha* zones. No significant correlation was found between the disturbance ranking for the *Nuphar/Nymphaea or-Pontederia/Sagittaria/Peltandra* sites and their respective candidate IBI metric scores. Therefore, no metrics could be developed for *Nuphar/Nymphaea* or *Pontederia/Sagittaria/Peltandra* either separately or together.

Discussion

Principal Components Analysis

Uzarski et al. (2004) used a similar approach to examine invertebrate responses to human influences. They also used multivariate analyses to document relationships between chemical/physical and land use/cover variables and related these to invertebrate attributes. Both King and Brazner (1999) and Uzarski et. al. (2004) stressed that relationships between adjacent land use/cover and the chemical/physical conditions within the wetland are strictly correlative and cannot be used to infer causation. For example, Uzarski et al. (2004) data seemed to suggest that urban areas contribute more nitrate-N and ammonium-N to wetlands than do agricultural areas, since water in wetlands with adjacent urban land use tended to contain more nitrate-N and ammonium-N than did water in wetlands with adjacent agricultural land use. They explained that increased inorganic nitrogen in the urban wetlands might not be processed as efficiently as it is in agricultural wetlands. Therefore, no conclusion about quantity of input from the adjacent area was warranted (Jude et al. in press). They simply tended to find relatively higher nitrate-N and ammonium-N concentrations in wetlands near urban areas where there was relatively higher run-off from the upland and lower productivity in the wetland itself. It does not necessarily suggest that a given land use/cover taken alone would create the associated chemical/physical conditions in the wetland. Our PCA suggested that agriculture was associated with higher nutrients in wetlands. However, this relationship was driven by seven of the 61 sites having extremely high nutrient concentrations as well as adjacent agriculture. Many sites with a high percentage of land use in agriculture actually had non-detectable dissolved nutrients in the water column. This may have been because these systems tend to have higher productivity and efficiently store excess nutrients in biomass. If sites with extremely high nutrients were removed from the analyses, results would have shown: 1.) that agricultural sites either had very high or very low nutrient concentrations as was found for invertebrate populations (Uzarski at al. 2004), and 2.) that relatively pristine sites had moderate nutrient concentrations. Uzarski et al, (2004) sampled many of the same sites included in these analyses. They found an association between nutrients and urbanization because their (and our) most pristine sites had a relatively high concentration of cabins adjacent to the systems producing a relatively high 'urbanization' component to the 1-km buffers around the sites. However, by using an additional buffer of 20-km it became apparent that most of the watershed was intact forest. These sites rarely had either non-detectable or very high nutrient concentrations (unpublished data from 1996 through 2003). This is likely the reason for a long struggle with using chemical/physical and land use/cover variables to detect moderate disturbance in biotic populations in wetlands. We believe that an approach similar to our method of establishing disturbance gradients may be a valuable tool for detection.

Correspondence analysis

Rare or schooling fish taxa increased the variability in the data set and were often captured by chance alone. For example, schools of black bullhead and bowfin were observed at nearly every site sampled yet schools of these taxa were only caught at a few sites. When these taxa overwhelmed the first iteration of analyses, they had to be removed and the entire process was repeated. These data were not discarded from the study, but only from the exploratory analysis. There has been much debate in the literature regarding how to handle rare taxa. Studies involving fishes meet similar challenges when dealing with taxa that tend to school. The tendency is to occasionally capture rare taxa and to either catch many or no schooling taxa. When determining the importance in community composition of such taxa, studies will have to involve an enormous amount of sampling effort and this will likely be at the expense of the number of sites that can be visited. Our analysis suggests that, at least for the cosmopolitan taxa of the Great Lakes, plant zone or habitat structure was the major driving factor in shaping the community and we have no reason to believe that the same is not true for those rare or schooling taxa. However, our sampling effort was not great enough to establish such a relationship.

Correlation Between Abiotic and Biotic Data

Plant zones were ordered consistently in PC1 and CA1 suggesting that nutrients and the percent adjacent land use in agriculture were important in determining the plant zone found in the wetland. Fish community composition shifted with, and even within, plant zone with increasing nutrients and agriculture. However, it is also important to note that both the plant and abiotic data may also be correlated with parameters that we did not measure. These parameters include, but are not limited to, fetch and/or pelagic mixing and the accumulation of organic sediment. The order of the vegetation seems to represent an organic sediment gradient from *Scirpus* with the least amount to *Typha* with the most. Numerous studies have shown that macroinvertebrate communities also differ among plant zones (Burton et al. 1999; Burton et al. 2002; Burton et al. 2004; Uzarski et al. 2004). Fish community composition may be following a similar pattern based on food availability.

Disturbance Gradient

Turbidity, specific conductance, and chloride were ranked directly, with greater values indicating disturbance. Extreme values, either very high or very low, for nitrate-N, ammonium-N, and soluble reactive phosphorus concentrations, as well as percent saturation of dissolved oxygen and pH were considered indicators of disturbance. With respect to inorganic dissolved nutrients, we tended to find moderate concentrations at relatively pristine sites. Impacted sites often have either non-detectable values, because these systems are very productive and the nutrients are tied-up in organic matter and sediments, or nutrient concentrations that are so high that the communities do not assimilate them as quickly as they enter the system. Also, in a system experiencing cultural eutrophication, dissolved oxygen may be as high as 180% saturated during the day when we sample. In this case, percent saturation likely plummets at night when only respiration is taking place in the absence of photosynthesis. Likewise, a system with organic pollutants may have very low percent saturation (e.g., 50%) of dissolved oxygen due to decomposition of excess organic matter in the absence of photosynthesis. This can be caused by siltation, cloud cover, coverage of duckweed (*Lemna* or *Spirodela* spp.), and/or turbidity. Our pH measurements follow this relationship to some degree; a very high daytime pH may be indicative of extreme productivity, while very low daytime pH may be indicative of organic pollution.

Land use/cover data were analyzed at two scales and both were incorporated into the final disturbance gradient for a subset of our sites. The larger scale (20-km buffers) was used to represent the impacts to the nearshore region or the water source of the wetland and was double weighted. A finer scale (1-km buffer) was used to relate impacts much more locally and received a single weighting. The need for two scales was realized because the Saginaw Bay region represents the majority of agriculture in Michigan yet many areas around the bay have a relatively large forested area adjacent to the wetlands. This forested area undoubtedly intercepts excess nutrients that would have entered the wetland from the agricultural areas directly. However, impacts to the systems are coming through drainage ditches acting as conduits of pollution into the bay. These ditches, as well as the Saginaw River, often have extremely high nutrient loads, sometimes in excess of 40 mg L^{-1} nitrate-N (personal observation). When we determined land use for a 1-km buffer, land use for several of these sites was ~ 80% forested, yet when we determined land use for 20-km buffers, the same sites were ~ 80% agriculture. We felt it was appropriate to double weight the 20-km land-use buffer in the disturbance gradient because it better reflected the overall impacts of the adjacent landscape on the general water quality of the nearshore area . The nearshore water in turn inundated fringing wetlands.

IBI Development

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, Burton et al. 1999 and Uzarski et al. (2004) were able to remove 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 those studies. Since our analyses were stratified by plant zone, it seems unlikely that changes in water levels will invalidate the IBI. As plant communities shift in location or change all together, fish communities associated with specific zones should seek preferred structure. In some years, a few wetlands may dry out completely. During these times, a fish-based IBI could only be applied to wetlands with at least one inundated plant zone present but could still be used to assess overall water quality changes in a given Great Lake or region of one of the Great Lakes using data from such wetlands.

Unfortunately, we were only able to develop metrics for two plant zones. While at least one of these zones can likely be found in most Great Lakes coastal wetlands, some will certainly lack both. In that case, this IBI will not apply. However, by maximizing the number of available protocols, we are increasing the likelihood that one will be applicable. Furthermore, using several IBIs utilizing different organisms at a given site should prove most robust and we recommend doing so whenever possible.

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Table Titles

Table 1a-c. Anthropogenic disturbance gradients of Inner and Outer *Scirpus* (1a), *Typha* (1b) and Lily (1c) zones in Great Lakes coastal wetlands using land use and water quality parameters sampled during the summer of 2002. Data represent ranks for each parameter; sum of the ranks was used to determine the disturbance gradient. Land use parameters included percent developed land (%Dev), percent agricultural land (%Ag), percent forested land^{-1} (%For^{-1}) and percent wetland/meadow^{-1} (%Wet/Mead^{-1}) for both 1-km and 20-km buffers. Ranks for 20-km land use parameters were double weighted. Nitrate-N, pH and percent dissolved oxygen ranks were based on the absolute value of the measured value at a site minus the median of all sites. Principal components (PC) analysis was used to combine 13 chemical, physical and land use variables for *Typha* sites and the resulting PC1 scores represent increasing urbanization and agriculture.

Table 2. Fish species collected with fyke nets in coastal wetlands of the five Great Lakes in 2002. Fish species included in each iteration of the ordination analyses (42, 40, 34, 28 and 26-species analysis respectively) are indicated with ‘x’. Functional feeding groups include: insectivore (INS), molluscivore (MOL), omnivore (OMN), piscivore (PISC), zoobenthivore (ZOB).

Table 3. Parameters used to establish anthropogenic disturbance gradients for four vegetation zones in coastal wetlands of the five Great Lakes using land use/cover and water quality data collected in 2002. Disturbance gradients were determined using the sum of the ranks of the parameters identified with ‘x’ for each vegetation type. Principal component 1 represents increasing urbanization and agriculture from a principal components analysis combining 13 chemical/physical and land use/cover variables for *Typha* sites.

Table 4. Preliminary fish-based index of biotic integrity metrics for Great Lakes coastal wetlands derived from data collected in 2002. Scoring is to be conducted from mean values per net-night in *Scirpus* and *Typha* zones when a mean of at least 10 fish are captured per net per vegetation zone. If less than 10 are captured or a sample is suspected to be atypical, an additional net-night is recommended.

Figure Titles

Figure 1. Map of Great Lakes basin showing the locations of 61 coastal wetlands sampled during the summer of 2002.

Figure 2. Principal components analysis of 13 chemical/physical and land use (1-km buffer) parameters including specific conductance (SpC), ammonium-N (NH_4), turbidity (Tur), nitrate-N (NO_3), soluble reactive phosphorus (SRP), percent dissolved oxygen (%DO), pH, temperature (T), percent developed land (%Dev), percent agriculture (%Ag), percent idle lands and wetlands (%Id/Wet), percent forest (%For) and road density (RD) for 104 plant zones spanning all five Great Lakes sampled in 2002. Labels refer to vegetation type including *Typha* (Typha), *Scirpus* (Scirp), *Nuphar* and *Nymphaea* (Lily), Zizania (Ziz), *Sparganium* (Spar), *Pontederia/Sagittaria/Peltandra* (PSP) and *Eleocharis* (Eleo) with numbers referring to site location codes (available from the corresponding author as an appendix).

Figure 3. Correspondence analysis of 26 fish species in 104 plant zones in coastal wetlands of the five Great Lakes sampled in 2002. Site labels refer to vegetation type including: *Typha* (Typha), *Scirpus* (Scirp), *Nuphar* and *Nymphaea* (Lily), Zizania (Ziz), *Sparganium* (Spar), *Pontederia/Sagittaria/Peltandra* (PSP) and *Eleocharis* (Eleo) with numbers referring to site location codes (available from the corresponding author as an appendix). Fish codes are defined in Table 2.

Figure 4. Correlation between abiotic factors (combined in principle components analysis), and fish communities (represented by correspondence analysis), for 104 vegetation zones sampled during the summer of 2002. Labels refer to vegetation type including: *Typha* (Typha), *Scirpus* (Scirp), *Nuphar* and *Nymphaea* (Lily), Zizania (Ziz), *Sparganium* (Spar), *Pontederia/Sagittaria/Peltandra* (PSP) and *Eleocharis* (Eleo) with numbers referring to site location codes (available from the corresponding author as an appendix).

Figure 5. Sum of IBI metric scores for *Scirpus* and *Typha* sites based on fish collected with fyke nets in 61 Great Lakes coastal wetlands in 2002. Sites are ordered by increasing disturbance. See Table 4 for IBI metrics.

Appendix B

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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