United States Environmental Protection Agency

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Washington, DC 20460

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METHODS FOR EVALUATING WETLAND CONDITION

#14 Wetland Biological Assessment Case Studies

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#14 Wetland Biological Assessment Case Studies

Edited By

Maine Department of Environmental Protection
Thomas J. Danielson

Connecticut Department of Environmental Protection
Douglas G. Hoskins

Prepared jointly by:

The U.S. Environmental Protection Agency
Health and Ecological Criteria Division (Office of Science and Technology)

and

Wetlands Division (Office of Wetlands, Oceans, and Watersheds)

Notice

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EPA acknowledges the contributions of the following people in the writing of this module: The members of BAWWG—without their hard work, this report would not have possible. Leigh Dunkelberger provided invaluable assistance during her internship with the U.S. EPA Wetlands Division.

This entire document can be downloaded from the following U.S. EPA websites:

http://www.epa.gov/ost/standards

http://www.epa.gov/owow/wetlands/bawwg

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Foreword

In 1999, the U.S. Environmental Protection Agency (EPA) began work on this series of reports entitled *Methods for Evaluating Wetland Condition*. The purpose of these reports is to help States and Tribes develop methods to evaluate (1) the overall ecological condition of wetlands using biological assessments and (2) nutrient enrichment of wetlands, which is one of the primary stressors damaging wetlands in many parts of the country. This information is intended to serve as a starting point for States and Tribes to eventually establish biological and nutrient water quality criteria specifically refined for wetland waterbodies.

This purpose was to be accomplished by providing a series of "state of the science" modules concerning wetland bioassessment as well as the nutrient enrichment of wetlands. The individual module format was used instead of one large publication to facilitate the addition of other reports as wetland science progresses and wetlands are further incorporated into water quality programs. Also, this modular approach allows EPA to revise reports without having to reprint them all. A list of the inaugural set of 20 modules can be found at the end of this section.

This series of reports is the product of a collaborative effort between EPA's Health and Ecological Criteria Division of the Office of Science and Technology (OST) and the Wetlands Division of the Office of Wetlands, Oceans and Watersheds (OWOW). The reports were initiated with the support and oversight of Thomas J. Danielson (OWOW), Amanda K. Parker and Susan K. Jackson (OST), and seen to completion by Douglas G. Hoskins (OWOW) and Ifeyinwa F. Davis (OST). EPA relied heavily on the input, recommendations, and energy of three panels of experts, which unfortunately have too many members to list individually:

- Biological Assessment of Wetlands Workgroup
- New England Biological Assessment of Wetlands Workgroup
- Wetlands Nutrient Criteria Workgroup

More information about biological and nutrient criteria is available at the following EPA website:

http://www.epa.gov/ost/standards

More information about wetland biological assessments is available at the following EPA website:

http://www.epa.gov/owow/wetlands/bawwg

List of "Methods for Evaluating Wetland Condition" Modules

Florida: Monitoring Across a Nutrient Gradient in the Everglades

Contact Russel Frydenborg

Organization Florida Department of Environmental Protection
2600 Blair Stone Road, MS 6511
Tallahassee, FL 32399-2400

Phone (850) 921-9821

E-mail russel.frydenborg@dep.state.fl.us

Purpose

This project was initiated to monitor biological assemblages across a nutrient gradient in the Florida Everglades in support of regulatory efforts to define a numeric water quality criterion for phosphorus. The goal is protection of natural populations of aquatic flora and fauna in the Everglades Protection Area.

Wetland Type

- Freshwater marshes

Assemblages

- Algae
- Macroinvertebrates
- Vascular plants

Status

- Revising sampling methods and analyzing data

Project Description

The historic Florida Everglades consisted of approximately 4 million acres of shallow sawgrass marsh, with wet prairies and aquatic sloughs interspersed with tree islands. Today, only 50% of the original Everglades ecosystem remains, primarily as a result of drainage and conversion of large portions of the northern and eastern Everglades to agricultural or urban land use. The remaining portions of the historic Everglades are located in the Water Conservation Areas (WCAs) and Everglades National Park (ENP) (see Figure 1).

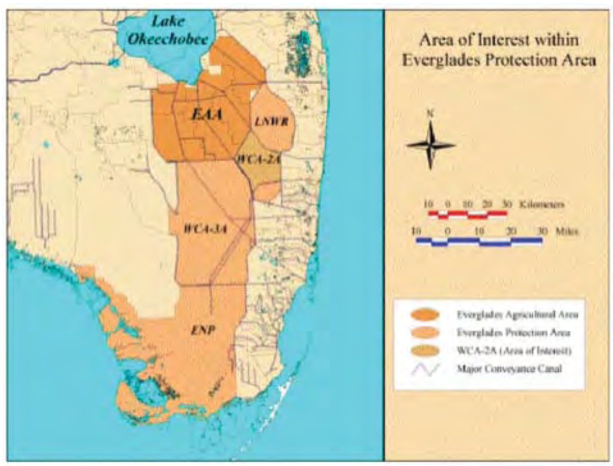


Figure 1: Area of interest within Everglades protection area.

The Everglades ecosystem evolved under extremely low phosphorus concentrations and is considered an oligotrophic ecosystem. A large body of evidence indicates that phosphorus is the primary limiting nutrient throughout the remaining Everglades. Introduction of excess phosphorus to the Everglades has resulted in ecological changes over large areas of the marsh. The Everglades Forever Act (EFA; Section 373.4592, Florida Statutes), passed by the Florida Legislature in 1994, stated that waters flowing into the part of the remnant Everglades known as the Everglades Protection Area (defined as Water Conservation Areas 1, 2A, 2B, 3A, 3B, and ENP) contain excessive levels of phosphorus and that a reduction in levels of phosphorus will benefit the ecology of the Everglades Protection Area. The EFA requires the Florida Department of Environmental Protection (FDEP) and the South Florida Water Management District (SFWMD) to complete research necessary to establish a numeric phosphorus criterion for the Everglades Protection Area.

The SFWMD Everglades System Research Division (ESRD) initiated a succession of studies, beginning in 1993 and continuing to the present, as part of the research and monitoring being conducted in the Everglades for phosphorus criterion development. Biological monitoring for the ESRD studies was initiated in early 1994 in WCA 2A. Data from this and other studies are being used by FDEP in the development of a numeric phosphorus criterion for the Everglades Protection Area.

Study Design

SFWMD ESRD initially selected 13 sites along 2 transects located downstream of canals discharging into WCA 2A and extending down the phosphorus gradient into the least affected areas of the marsh. Sampling sites ranged from the canal inflows (discharge structures on the northeastern margin of WCA 2A) to a site nearly 15 km downstream from the canal inflows. Three of the 13 main sites (sites U1-U3) were specifically chosen to represent the least affected area of WCA 2A with respect to anthropogenic disturbance. A series of 15 additional "intermediate" sites were added to the study later to obtain better spatial coverage of the lower portion of the transects. The sites have been monitored for water, sediment, and biological quality. Figure 2 shows the WCA 2A monitoring sites located along the phosphorus gradient.

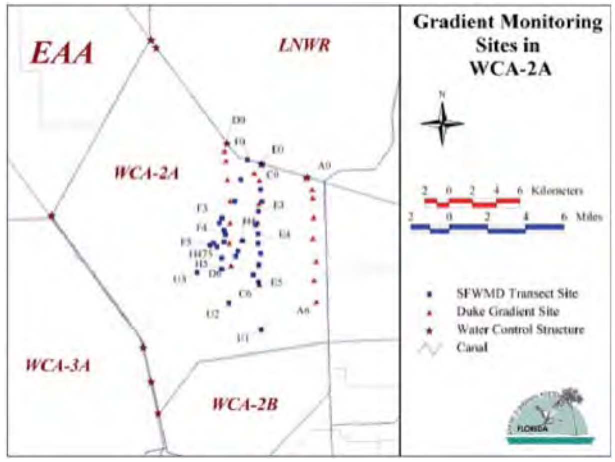


Figure 2: WCA 2A monitoring sites along the phosphorus gradient.

Sampling Methods: Algae

- *Water Bottles:* Phytoplankton samples initially were collected monthly and later collected quarterly using water bottles. Samples were preserved in the field and sent to the FDEP Central Biology Laboratory for taxonomic identification.
- *Diatometers:* Racks each containing six glass diatometer slides were deployed quarterly at each site. It was determined that an 8-week deployment was necessary for sufficient periphyton growth. Diatometers were collected and preserved and sent to the FDEP Central Biology Laboratory for processing and taxonomic identification.
- *Natural Substrate (benthic):* Samples of benthic periphyton were collected from surficial sediment cores at the main transect sites on several occasions. Samples were retained by SFWMD ESRD for processing and taxonomic identification.

Analytical Methods: Algae

- *Water Bottles:* Samples were processed and enumerated by FDEP Central Biology Laboratory staff according to FDEP standard operating procedures (SOPs) (e.g., AB-04 and AB-05; available at http://www8.myflorida.com/labs/sop/index.htm). Analysis from this study and other studies has indicated that Everglades phytoplankton are largely periphyton that has sloughed off into the water column. Thus, algal data analysis was focused on the periphyton data.

- *Diatometers:* Samples were processed and enumerated by FDEP Central Biology Laboratory staff according to FDEP SOPs (e.g., AB-02, AB-02.1, AB-02.2, and AB-03; available at http://www8.myflorida.com/labs/sop/index.htm).
- *Natural Substrate (benthic):* SFWMD processed and enumerated natural substrate samples.

Sampling Methods: Macroinvertebrates

- *Dipnet:* SFWMD staff conducted quarterly macroinvertebrate sampling using a standard D-frame dipnet with a 30-mesh bag from September 1994 through November 1995. The sampling method consisted of the collection of 20 0.5 m (in length) discrete dipnet sweeps from representative habitats in the area of each site on a given sampling date. The 20 dipnet sweeps for a given site were combined and sent to the FDEP Central Biology Laboratory for processing and taxonomic identification.
- *Quan Net:* Beginning in May 1996, SFWMD staff conducted quarterly macroinvertebrate sampling using the Quan Net method. The sampling method consisted of the deployment of a 1-m^2 frame at the site and the collection of net samples and all vegetation within the area of the frame. Frames were deployed in each of several representative habitats, where present, in the vicinity of each site. Samples from each site/habitat were kept separate. Representative habitats were labeled as cattail, sawgrass, or slough, depending on the predominant vegetation type. The collected material from each site/habitat was subsampled, preserved, and sent to the FDEP Central Biology Laboratory for processing and taxonomic identification.
- *Hester-Dendy:* SFWMD staff deployed Hester-Dendy artificial substrate samplers at each of the main transect sites on a quarterly basis. The samplers were deployed for a 1-month period, after which they were collected, preserved, and sent to the FDEP Central Biology Laboratory for processing and taxonomic identification.

Analytical Methods: Macroinvertebrates

- *Dipnet and Quan Net:* FDEP Central Biology Laboratory staff subsampled the dipnet and Quan Net samples from each site and analyzed them according to FDEP SOPs (e.g., IZ-02 and IZ-06; available at http://www8.myflorida.com/labs/sop/index.htm).
- *Hester Dendy:* FDEP Central Biology Laboratory staff processed and analyzed the Hester-Dendy samples from each site according to FDEP SOPs (e.g., IZ-03 and IZ-06; available at http://www8.myflorida.com/labs/sop/index.htm).

Sampling Methods: Macrophytes

- *Macrophyte Stem Density and Frequency:* In April 1997, SFWMD staff conducted a study of macrophytes at the WCA 2A transect sites. A 50-m tape was laid out at each transect site. A 1-m square frame was used every 2 m along the tape to delineate the sample area for calculation of macrophyte stem densities (stems/m^2) and frequencies (# plots where a species was found/total # of plots) by species.

- *Macrophyte Harvesting:* On the other side of the 50-m tape used for establishing stem densities and frequencies, SFWMD staff harvested macrophytes for biomass measurements, using the 1-m square frame at five predetermined locations to mark the sample area for harvesting.

Analytical Methods: Macrophytes

- *Macrophyte Stem Density and Frequency:* Stem densities (stems/m^2) and frequencies (# plots where a species was found/total # of plots) by species were counted at each site.
- *Macrophyte Harvesting:* SFWMD staff conducted biomass analysis of the harvested macrophytes for comparison of the relative biomass of several species present at each of the WCA 2A transect sites (e.g., Eleocharis, Nymphaea, Typha).

Lessons Learned

Periphyton, macroinvertebrate, and macrophyte communities in WCA 2A change substantially from reference conditions at approximately 7 to 8 km downstream of canal discharges into WCA 2A (see Figures 3–5). Data analysis has shown that biological populations at the two stations (E5 and F5) nearest to the three initial reference sites (U1-U3) are very similar in terms of biological community structure. This suggests that these areas, despite slight phosphorus enrichment, still support reference condition biota. The somewhat higher phosphorus regimes at the next stations (E4 and F4 and beyond) are associated with greater biological changes. Experimental field dosing studies (microcosms) have been conducted by SFWMD ESRD and show that the addition of phosphorus causes changes in periphyton assemblages consistent with those observed in the transect study.

The WCA 2A transect periphyton data for each site/date have been analyzed using the entire taxonomic assemblage encountered and using lists of pollution-sensitive and -tolerant species based on available literature and experimental phosphorus addition studies (the microcosms) in WCA 2A. Macroinvertebrate data have been analyzed using the Florida Index and the macroinvertebrate component of the Lake Condition Index (LCI), measures of the numbers of pollution-sensitive taxa in a sample that are routinely used by FDEP in bioassessments of streams and lakes. The use of these methods with the WCA 2A transect data has demonstrated a clear signal of biological disturbance along the nutrient gradient in WCA 2A. FDEP is using this information as well as information from other studies conducted in the Florida Everglades to develop a numeric phosphorus criterion for the Everglades Protection Area.

Additional Comments

The information provided here is based solely on the transect study by SFWMD ESRD in WCA 2A. Research and monitoring of Florida Everglades water, sediment and biological quality is being conducted by several research groups in WCA 2A, WCA 1 (Arthur R. Marshall Loxahatchee National Wildlife Refuge), Everglades National Park (ENP), and WCA 3A, including studies by SFWMD ESRD similar to the WCA 2A transect study.

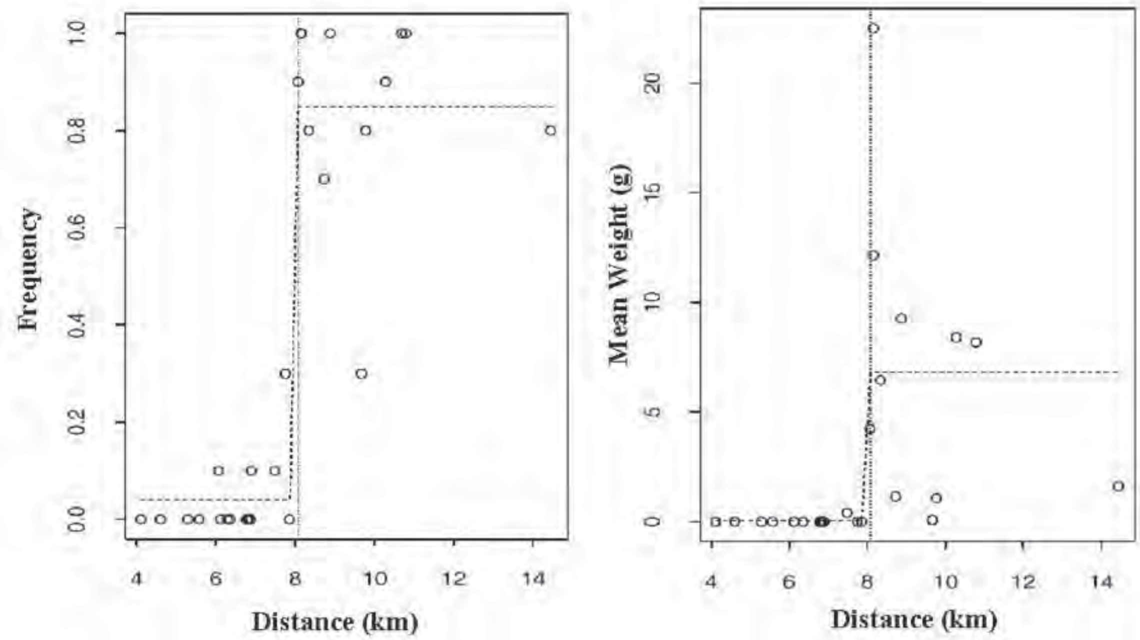


Figure 3: Change point analyses of *Eleocharis* frequency of occurrence and biomass data along the SFWMD transects. Collected April 1997.

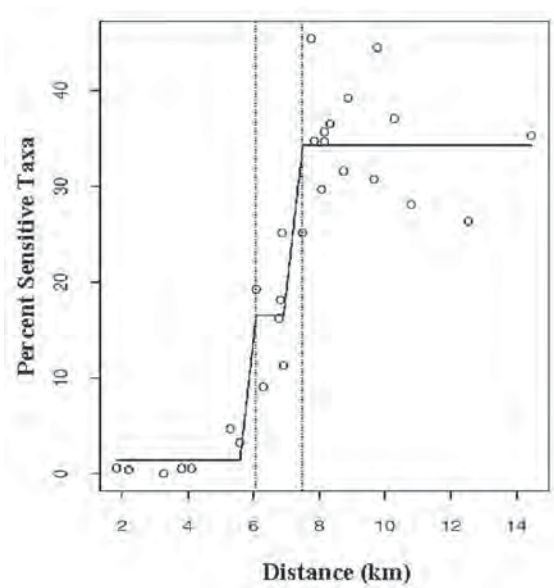


Figure 4: Results of change point analyses performed on median total percentage of pollution-sensitive (literature determined) periphyton taxa.

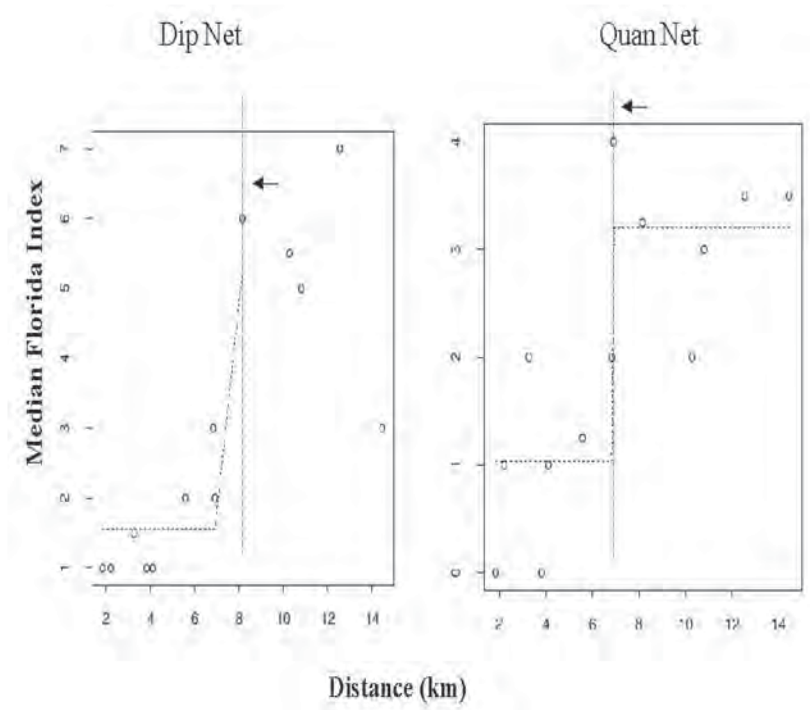


Figure 5: Results of change point analyses on median Florida index values.

Florida: Development of a Biological Approach for Assessing Florida Wetland Integrity

Contact Mark Brown

Organization University of Florida, Center for Wetlands
Phelps Lab – PO Box 116350
Gainesville, FL 32611-6350

Phone (352) 392-2309

E-mail mtb@ufl.edu

Website http://www.enveng.ufl.edu/homepp/brown/syseco

Purpose

To develop "bioindicators" of ecosystem health for wetlands in Florida. To achieve this goal, the project team has developed wetland ecoregions using GIS technology, a classification scheme for Florida wetlands, sampling protocols for herbaceous and forested wetlands, and a quantitative index of the human disturbance gradient.

Wetland Type

- Freshwater marshes
- Forested wetlands

Assemblages

- Algae
- Macroinvertebrates
- Vascular plants

Status

Developed field protocols, sampled more than 150 wetlands statewide, developed candidate plant, macroinvertebrate, and algae metrics for marshes. Currently sampling and developing metrics for forested wetlands.

Project Description

The University of Florida Center for Wetlands is involved in a multiyear wetland research project funded by the Florida Department of Environmental Protection (FDEP) to develop an integrated biological approach for evaluating Florida's wetlands. The project goal is to develop an assessment approach that recognizes the utility of both biological and functional assessments, and is rapid, reproducible, and meaningful.

The Center for Wetlands began development of the assessment approach in 1997. Now in its fifth year, the project, titled "Development of a Biological Approach for Assessing Wetland Function and Integrity," has four main tasks:

Task 1. Review and synthesize all current and relevant literature.

Task 2. Develop a wetland classification system for wetland types in the State of Florida.

Task 3. Develop a GIS-driven methodology for classifying bioregions within the State of Florida that identify climatic, geologic, and geophysical provinces that are sensitive to wetland classes.

Task 4. Develop a bioassessment methodology, biocriteria, and metrics for wetlands in the State of Florida.

To date, Tasks 1–3 have been completed, and work continues on Task 4. Nearly complete is a set of metrics for herbaceous depressional wetlands, and data are currently being analyzed to develop metrics for forested depressional wetlands.

The development of the approach has included a review of technical and scientific literature, a wetland classification and crosswalk, wetland regionalization of Florida, and two wetland biological surveys in summer 1998 and summer 1999. The biological surveys were designed to test the validity (and necessity) of the proposed wetland regions, to identify the appropriate biological indicator taxa, and to quantify the gradient of human disturbance.

Study Design

The approach has included a review of technical and scientific literature, development of a wetland classification system and crosswalk with other classifications, wetland regionalization of Florida, and 4 years of wetland biological surveys in the growing seasons of 1998, 1999, 2000, and 2001 (a fifth season of field surveys is under way for 2002). The biological surveys were designed to test the validity (and necessity) of the proposed wetland regions, to identify the appropriate biological indicator taxa, and to quantify the gradient of human disturbance.

The wetland classification scheme was organized with major classes defined from three variables: (1) dominant vegetation (forested, shrub, herbaceous); (2) geomorphic position (stream channel [floodplain], flat topography, sloped topography, lake fringe, depressional); and (3) primary water source (rainfall, surface water, ground water). Subclasses are discriminated by modifiers (hydroperiod and plant community associations). Eleven wetland classes were identified: forested (river swamp, slough/strand/seepage swamp, lake swamp, depression swamp, flatland swamp); shrub dominated (shrub-scrub swamp); and herbaceous (river marsh, wet prairie, lake marsh, depressional marsh, seepage marsh). An HTML electronic database has been completed that crosswalks existing wetland classifications to the new simplified classification scheme developed for the bioassessment project.

Regionalization of the State was necessary because there is significant variation in climatic and physical features of the Florida peninsula and it was believed that these regional differences would equate to variations in bioindicator "signals." Map coverages of physical and climatic variables of the Florida landscape were used to develop regions that had different characteristic driving energies and landscape structural characteristics.

The map coverages were combined with GIS map algebra to create a spatial hydrologic budget equation for the State. The equation modeled the movement of water on the landscape during the ecologically sensitive spring growing season. The output of the model provided a value for a Potential Soil Moisture Index (PSMI). The PSMI was separated into four regions based on both the critical depth of saturation and on a statistical clustering of the PSMI values (see Figure 6). The classified regions were tested for similarities and differences to determine if between-region variation in wetland type and environmental variables was greater than within-region variations.

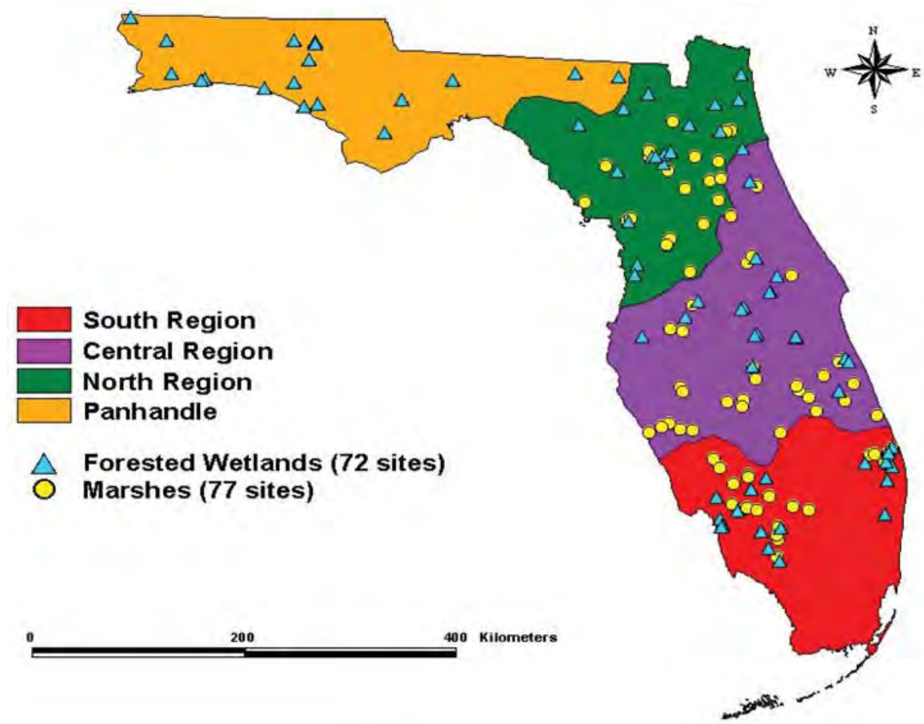


Figure 6: Florida's wetland regions and field site locations.

The importance of hydrology in determining wetland type and location (the premise behind the PSMI) was then tested using a hierarchical classification technique (TWINSPAN) and ordination (DCA and CCA) with variables of seasonal and annual rainfall, seasonal and annual potential evapotranspiration, slope, geology, drainage class, and runoff. TWINSPAN, DCA, and CCA tested the relationships between wetland type and climatic and physiographic landscape characteristics. Based on the geostatistical output, hydrology is indeed a major determinant of wetland type and location, and supports the use of the spatial hydrologic budget equation in delineating wetland regions.

Four years of biological surveys of wetlands throughout the State were designed to test the regionalization techniques as well as the metric and bioindicator development. The 1998 pilot field research involved surveying 24 herbaceous and forested depressional wetlands in north and central Florida. Major taxonomic assemblages were characterized and ranked along a gradient of disturbances. Sites were located within multiple land uses such as parks, preserves, pastures, farm fields, well fields, silviculture plots, and urban areas. Impacts that were assessed included hydrological modifications, nutrient loading, and altered hydroperiod. The first year of sampling resulted in development of standardized sampling procedures, design and implementation of a statewide sampling program, and identification of community attributes and candidate metrics.

In the second and third field seasons, 77 herbaceous, depressional wetlands were surveyed in 3 regions (south, central, and north). Approximately half of the wetlands were impacted (agricultural setting) and half were reference locations. Many of the sites were paired sites (impacted and reference at close proximity).

In the fourth season, 72 forested, depressional wetlands were surveyed in four regions (18 sites in each of four regions: north, central, and southern peninsular as well as the panhandle; see Figure 6). Approximately ? of the wetlands were impacted (? in agricultural settings and ? in urban settings) and ? were reference locations. Many of the sites were paired sites (impacted and reference at close proximity).

Quantifying gradients of human disturbance

A Land Development Intensity (LDI) index is being used to quantify gradients of human disturbance for wetlands throughout the State. The LDI index is calculated using land use/land cover characteristics, from aerial photographs, of lands within a 100-m buffer surrounding the wetland. Land uses in the area surrounding a wetland are first characterized and then an intensity factor is assigned to each land use type. The LDI algorithm multiplies the percent area of each land use/land cover in the surrounding 100-m buffer by intensity coefficients. The intensity coefficients are scaled from 1 to 10 and represent intensity of environmental manipulation as measured by energy use per unit area per unit time. The LDI index is such that lower LDI values are indicative of a lower disturbance level.

Sampling Methods: Algae

Materials

- Three 100-mL collection jars
- 1-L collection jar
- Bottomless collection cup, knife, large pipette, bag, brush/scraper
- M3 preservative
- 1 L of deionized water (for dry sites)

Methods

1. Methods vary depending on whether there is water present at the site. We are experimentally collecting dry benthic algae samples when wetlands are dry. At this time, there is no information as to the usefulness of this sampling technique. Methods outlined below are for wet sites.
2. Samples are taken depending on substrate and separated as benthic, epiphyton, metaphyton, and phytoplankton.
3. For epiphyton, the 10 aliquots are divided equally among herbaceous and woody debris:
   a. For herbaceous debris, plant stems are cut from the soil to the water surface, and placed in a zip-lock bag with some wetland water. The plant matter is shaken and massaged thoroughly to dislodge the algae. Using a large pipette, 10 mL of sample are extracted into collection jar.
   b. For woody debris, a brush is used to dislodge algae. If the debris is small enough, it is placed in a bag, similar to the herbaceous methods above. If the debris is larger, a bottomless collection cup is used to confine the sample, and it is brushed while under water.
4. For benthic algae, a bottomless collection cup is used to isolate a spot on the sediment. Then a large pipette is used to gently stir the surface (top 1 cm) of the sediment, and extract a 10-mL aliquot that is placed in a collection jar. Sampling is repeated at different locations until a total of 100 mL is collected.
5. For metaphyton, a thumbnail size portion of the algal mat is collected from 10 different locations throughout the wetland.
6. For phytoplankton, a total 100 mL of surface water is collected at 10 locations throughout the wetland.
7. All samples are preserved with M3 using 5 mL per 100 mL of sample.
8. Samples are sent for later laboratory identification.

Sampling Methods: Macroinvertebrates

Materials

- Field Physical/Chemical Characterization Data Sheet
- Habitat Assessment Sheet
- D-Frame dipnet with no. 30 mesh
- 4-L sample jars
- Buffered formalin

Methods

1. The wetland is visually examined by walking throughout the wetland, paying close attention to its physical and habitat characteristics, and a Field Physical/Chemical Characterization Data Sheet and Habitat Assessment Sheet is completed. The percent coverage of substrate type and each habitat type is recorded.
2. A total of 20 sweeps with a D-frame dipnet are divided between the habitat types based on their percentage of total wetland area. Discrete 0.5-m sweeps are performed with the dipnet. The available substrates are sampled as determined by the above procedures in the following manner:
    a. For areas without flow, an area of substrate that is one dipnet width wide and approximately 0.5-m long is disturbed by sweeping the net over the area three times to ensure the capture of organisms.
    b. For heavily vegetated areas, the net is jabbed into the base of the vegetation, digging down to (but not into) the substrate, and dislodging organisms using a 0.5-m sweeping motion with the net.
    c. Sand, muck, mud, and silt (nonmajor habitats) are sampled by taking 0.5-m sweeps with the net while digging into the bottom approximately 1 cm.
3. The number of sweeps for each habitat is recorded on the Field Physical/Chemical Characterization Data Sheet.
4. The collected samples are reduced in volume after each discrete sample by dislodging organisms from larger debris (but retaining invertebrates in the net or sieve) and discarding the debris. The finer debris and organisms are saved in sample jars.
5. Samples are preserved with buffered formalin and shipped to the FDEP Laboratory for identification.

Sampling Methods: Plants

Materials

- Field data sheets
- Compass
- 100 m of tape
- FDEP's Florida Wetland Plant Identification Manual (Tobe et al. 1998)

Methods

1. Using a compass (or the GPS unit) four line-transects are located from four cardinal point directions (N, S, E, W) that run parallel to the slope of the wetland, beginning at the upland edge (0 m) and extending into the center of the wetland.
2. At the beginning of each transect, the approximate wetland/upland edge is located using a combination of wetland plants and hydric soils.
3. Preferably, all four transects are set at one time, with N, S and E, W transects meeting perpendicularly in the center, and dividing wetland into four equal sections. Each transect is started with the 0-m point at the wetland–upland edge and increasing in distance toward the wetland interior. A minimum of four data sheets is needed per site.

4. Two team members walk along each transect and record species presence of all plants within 0.5 m on either side of each 5-m interval (sampling area = 5 m^2).
5. Plant species names are recorded on the data sheets with full genus and species if known.
6. If unknown, the species is given a unique code identifying transect location and number of unknown encountered (N-1, N-2, N-3, E-1, E-2, etc.). Voucher specimens for all unknown species are collected, making sure to include plant inflorescence and roots. Each specimen is tagged with properly labeled masking tape, and put in collection bag then pressed for later laboratory identification.
7. Plant nomenclature follows FDEP's Florida Wetland Plant Identification Manual (Tobe et al. 1998).

Sampling Methods: Water Quality and Soils

Water quality samples are collected, preserved, and immediately sent to the chemistry laboratory for analysis. A composite soil sample is taken from each vegetation zone and later analyzed for nutrients, organic matter content, and physical properties.

Analytical Methods: Plants

Field data are entered into an MS Access database for analysis. After entry, each data sheet is checked by a second technician.

Plate 1: Cypress wetland. Photo: Elizabeth Spurrier

Plate 2: Macroinvertebrate sampling. Photo: Kelly Chinners Reiss

Maine: Developing a Statewide Biological Monitoring and Assessment Program for Freshwater Wetlands

Contact Jeanne DiFranco

Organization Maine Department of Environmental Protection
312 Canco Road
Portland, ME 04103

Phone (207) 822-6424

E-mail Jeanne.L.Difranco@state.me.us

Website http://www.state.me.us/dep/blwq/monitoring.htm#programs

Purpose

- Examine regional differences in wetland macroinvertebrate and algal assemblages
- Test and refine candidate biological metrics
- Diagnose stressors and identify risks to wetlands from human activities
- Develop impairment thresholds and biocriteria for use in State water quality standards

Wetland Type

- Freshwater marshes

Assemblages

- Macroinvertebrates
- Algae

Status

Expanding to Statewide program on a 5-year rotating basin schedule, testing and refining metrics, analyzing data, and developing impairment thresholds.

Project Description

In 1998, the Maine Department of Environmental Protection (DEP) began development of a biological monitoring and assessment program for freshwater wetlands. Between 1998 and 2000, DEP conducted a pilot study in the Casco Bay watershed to develop wetland biomonitoring protocols and identify candidate metrics related to wetland condition. During 2001 and 2002, DEP expanded monitoring to the Saco, Piscataqua, and Kennebec River watersheds using the methods developed in the pilot study. DEP uses aquatic macro-invertebrates as the primary taxonomic assemblage for this program. Algae and diatoms are also collected as part of a collaborative pilot project undertaken by Dr. R. Jan Stevenson of Michigan State University to develop algal indicators of wetland integrity. Based on the results of Dr. Stevenson's work, Maine DEP will evaluate the usefulness of algae to its wetland biomonitoring program. Assessment of algae has not been formally integrated into the Maine program at this time, however.

Study Design

The Maine wetland biomonitoring initiative has been incorporated into DEP's existing Biological Monitoring Program, and will be extended to assess additional major watersheds Statewide. Wetland monitoring is currently coordinated with the State's river and stream program using the following 5-year rotating basin schedule:

Kennebec, midcoast watersheds	2002
Androscoggin watershed	2003
St. John, Presumpscot watersheds	2004
Saco, southern coastal watersheds	2005
Penobscot, downeast watersheds	2006

Considerations for site selection include hydrologic regime, geographic distribution of sites, landscape position, human disturbance gradient, management significance, and accessibility. All wetlands sampled are semipermanently or permanently inundated, and range from minimally disturbed potential reference sites to poor-quality wetlands. As of 2002, DEP has conducted wetland biomonitoring at 88 different sites encompassing 115 sampling events. Some sites have been sampled repeatedly over multiple years.

Sampling Methods: Macroinvertebrates

Macroinvertebrates are currently sampled during June and early July. Three different approaches have been tested to develop both qualitative and quantitative methods. In addition, water samples are analyzed for a suite of physical and chemical parameters to help in wetland characterization, and to identify potential sources of human impact. Habitat information, dominant plant species, and a scoring of human disturbances are recorded in the field, along with measurements of water temperature, pH, dissolved oxygen, and conductivity.

Multihabitat sampling

A qualitative, multihabitat sampling approach was tested, with the goal of developing a screening level assessment tool. A standard D-frame net was used to sample all inundated microhabitats at each site, including emergent vegetation, aquatic macrophyte beds, pools, and channels. Samples were "picked" or sorted from detritus in the field. One to several organisms representing each different taxon found were placed into a vial of alcohol until no different taxa were observed.

Stovepipe sampler

Maine DEP designed its own stovepipe sampler for quantitative samples using a 5-gallon bucket with the bottom removed. In this method, the sampler was used to enclose three replicate plots to restrict the movement of organisms. The stovepipe sampler was pressed into the wetland substrate, and the contents of the sampler were then agitated. Vegetation and surface sediment were placed into a sieve bucket. The sampler was then swept 10 times with a small hand net. Large pieces of vegetation were washed and discarded; however, finer plant material and detritus were retained. Samples were preserved for later sorting and taxonomic analysis in the laboratory.

Dipnet measured sweep

A standard D-frame net is currently used to obtain a semiquantitative sample. A sample is collected by submersing the net and sweeping through the water column for a distance of 1 meter. The net is bumped against the bottom substrate three times (at the beginning, middle, and end of the sweep) to dislodge and collect organisms from the sediment. All material collected is placed in a sieve bucket. Large pieces of vegetation are washed and discarded; however, finer plant material and detritus are retained. Three replicate samples are collected in areas of emergent vegetation. Samples are preserved for later sorting and taxonomic analysis in the laboratory.

Plate 3: Maine's stovepipe sampler and sieve bucket. Photo: Maine DEP

Plate 4: Picking macroinvertebrates from multihabitat sample. Photo: Maine DEP

Analytical Methods: Macroinvertebrates

Analyses performed to date reveal significant relationships between a number of candidate invertebrate metrics and watershed development. Many invertebrate metrics tested also appear to respond to changes in water quality typically associated with urban nonpoint source pollution, including elevated conductivity and concentrations of anions, cations, and nutrients. As new data are collected, candidate metrics will be tested and refined, and regional differences and ecological linkages among wetlands and other waters will be examined. DEP is also developing impairment thresholds for wetlands. This is a necessary first step to enable the State to use biological monitoring data in wetland management decisions and development of wetland-specific water quality standards (designated uses and biological criteria), and to assess wetland condition and attainment status.

Sampling Methods: Algae

Quantitative and qualitative algae samples were gathered from the same wetland sites as used for macroinvertebrate sampling. Four algae sample types were tested to determine which produced the best indicators. Samples were collected from the water column, plant stems, and sediments. Samples from multiple sites within each wetland were composited into one sample from each habitat. In addition, a multihabitat sample was collected from each site. Samples were examined microscopically to determine species numbers and relative abundances of different species in samples. Chlorophyll a was quantified from a separate water column sample as an indicator of algal biomass.

For sampling, garden shears were used to clip plant stems below the water line. A turkey baster was used to collect qualitative sediment samples; however, this method was revised in 2002 to obtain a more quantitative sample. Sediment is currently collected over a known surface area using a petri dish pressed into the substrate and retrieved with a spatula. Three replicates are collected and composited into a single sediment sample. A long handled dipper is used to collect water samples. For the multihabitat sample, a dose from each single-habitat sample was combined into one container.

Analytical Methods: Algae

Dr. Jan Stevenson of Michigan State University is using three disturbance indicators: the land use indicator developed by Maine DEP, trophic status indicators (total nitrogen, total phosphorus, and chlorophyll a), and hydrologic and sewage chemicals (Ca, Na, Cl). Dr. Stevenson is comparing a suite of algal indicators to determine which types of indicators respond to the three disturbance indicators. The algae indicators include biological integrity measures such as genus-species richness, Shannon diversity, and number of taxa in genera. Dr. Stevenson is also using European algal autoecology information to determine environmental characteristics for the taxa. This information will give an autoecological index that shows a relationship to variables such as moisture, organic N, low oxygen, pH, salt, and nutrients. Although algae was collected from all sites sampled between 1998 and 2002, only samples from 1998 and 1999 have been processed to date because of funding limitations.

Lessons Learned

- Incorporating a wetland monitoring component into Maine's existing biomonitoring program has been an efficient way to pool limited resources and build on Maine's successful river and stream biomonitoring experience.
- To implement a comprehensive biomonitoring program for wetlands, Maine DEP needs to build the capacity to assess multiple biological assemblages, including algae and vascular plants. This capability will improve the State's ability to evaluate wetland impacts from stressors such as nutrient enrichment and hydrologic changes, and will allow for the assessment of less frequently inundated wetland types where aquatic invertebrates are not naturally abundant. Current funding and staff levels prohibit expansion of the program at this time.

Maryland: Developing an IBI Assessment for Restored Wetlands in the Mid-Atlantic States

Contact Peter Lowe

Organization USGS BRD
Patuxent Wildlife Research Center
11510 American Holly Drive
Laurel, MD 20708-4017

Phone (301) 497-5705

E-mail peter_lowe@usgs.gov

Website http://www.pwrc.usgs.gov/wli/

Purpose

- Develop sampling methods for different assemblages
- Develop a yardstick of biological metrics to assess the progress and condition of reconstructed wetlands in Maryland, Delaware, and Virginia
- Compare the suitability of different assemblages (plants, macroinvertebrates, amphibians) for assessing wetland condition
- Evaluate the sources and magnitude of variance in data collected for biological metrics
- Evaluate seasonal and annual biological fluctuations for the wetland sites

Wetland Type

- Delmarva Bays (depressional wetlands with emergent, scrub/shrub, or forested vegetation)

Assemblages

- Amphibians
- Macroinvertebrates
- Vascular plants

Project History

The project is a joint effort among the USGS, Patuxent Wildlife Research Center, the USDA Natural Resources Conservation Service Wetland Science Institute, and EPA. Project development started in 1995 in response to a lack of information about the success of wetland mitigation projects, especially those associated with wetland restoration on farmlands under set-aside programs. From 1996 to 1998, fieldwork focused on a set of restored and existing wetlands. In 1999, a second set of wetlands was studied to evaluate the robustness of the metrics developed from the first set. All fieldwork has now been completed and metric development is under way.

Study Design

Because one of the objectives was to assess the sources and magnitude of variance in the different measurements, we intentionally oversampled our sites compared with what would be done under typical regional or statewide assessments. For 3 years, work was done on a single set of 30 wetlands, including 22 restored and 8 natural wetlands or Delmarva bays. All wetlands in the database were depressional, semipermanent, or seasonal. Macroinvertebrates were sampled one to four times each year, depending on hydrology; macrophytes during spring and late summer; and amphibians almost continuously during the breeding season.

Sampling Methods and Analysis: Macrophytes

Data on plant species composition, abundance, and dominance were collected through the use of line transects. Pairs of 50 m transects were placed on opposite sides of the wetland at four different hydrological levels as determined at "full pool" levels. The hydrological levels were buffer, less than 25 cm, 25 to 45 cm, and greater than 45 cm. In wetlands larger than 5 acres, the number of transect pairs was doubled. Each transect was divided into five runs of 5 m each, alternating with 5 m of unsampled transect. Along each run, sampling occurred at specific points (as in point intercept methods) at 1-m intervals. Thus, each run of 5 m contained five sample points. Each transect consisted of five runs of five sampling points each. Each hydrological depth was sampled by 2 transects, bringing the total number of sample points per hydrological zone to 50. There were:

(4 hydrological zones) × (2 transects each zone) × (5 runs per transect) × (5 points per run) = (200 points per wetland)

This was done in spring and fall for each wetland. At each point, the species name and number of individuals were recorded.

In addition to point data, incidental species along the line were recorded to include the species not intercepted at any points along the line. These species were included in species richness and attributes derived from presence or absence data, but not in dominance calculations.

Sampling Methods and Analysis: Macroinvertebrates

Aquatic invertebrates were sampled at approximately 6-week intervals beginning in late May and continuing until October and were conducted in association with the sampling of water quality, aquatic plants, and hydrological and wetland dimensions. Invertebrate samples were collected along transects following compass coordinates originating from markers placed in the deepest part of every wetland before the sampling seasons began. Transect coordinates were randomly selected for each wetland and sampling time. The method of compass points was adapted to each wetland's morphology.

Samples were collected from three depth ranges along the transects to determine invertebrate relative abundance, diversity, and relative biomass in each wetland. As long as water depths were adequate, samples were collected from along the transects at the following water depths: less than 15.0 cm, 15.1 to 45.0 cm, and greater than 45.1 cm.

Samples were collected using a modified Gerking box sampler, which is a sheet aluminum box with a sliding screen door (1-mm mesh) at the bottom. The sampler has the advantage of allowing simultaneous collection of benthic, pelagic, neutonic, and plant-associated invertebrates. The sampler was lowered to the floor of the sampling area with the screen door open. The vegetation in the sampler was then cut at the mud-water interface and put into prelabeled plastic bags. Then the screen door was slowly closed as the sediments just in front of the advancing screen were stirred into the water column. After the screen was closed, soil materials were sieved through the screen by shaking the box. All invertebrates caught on the screen were then placed in prelabeled plastic bags. The bags of vegetation and invertebrates were placed on wet ice as soon as possible and stored in a refrigerator until the samples could be picked. Each individual was keyed to the lowest taxonomic level feasible, typically species or genus. Data on species composition and abundance were used to generate metrics in a manner similar to that for plants. An approved quality assurance/quality control process was followed, which included independent validation of 20% of our samples.

Sampling Methods and Analysis: Amphibians

Each site was sampled for amphibian larvae once every 4 weeks. Sampled areas consisted of the perimeter of the open-water portion of the wetland and lightly vegetated areas that allowed a seine to pass. We used a 6×8-m nylon mesh (1/16") seine to sample each wetland by wading out 3 to 5 m away from the shoreline and then moving in towards the shoreline in one continuous sweep. Sampling was time-constrained to 2 hours. If new species were caught during the last two sweeps, the sampling period was extended until no additional new species were found. Amphibian larvae were identified using published keys and by temporarily housing tadpoles until they metamorphosed and could be identified.

Drift fences were also used to supplement seining data and obtain information on adults and metamorphs. We initially considered surrounding each wetland with a drift fence but realized the impracticality of that idea. Therefore, each wetland was provided with 50-cm-tall and 15-m-long drift fences. If possible, the fences were placed along drains, travel corridors, and other likely points of amphibian use in order to maximize capture of individuals entering and exiting the wetlands. Five-gallon plastic buckets were buried in pairs at the ends and midpoint on the inside and outside of the fence to capture adults entering the ponds and juveniles leaving the ponds. Wet sponges, rocks, and vegetation were placed in the buckets to prevent desiccation and provide some cover and refugia to captured individuals. All amphibians were identified, sexed, and returned to the inside of the fence at the wetland from which they were captured. Juveniles and metamorphs departing the wetlands were identified, counted, examined for malformations, and released on the outside of the drift fence.

General Analytical Methods

A fundamental component of this study is to devise a gradient of physical factors (e.g., land use in drainage area, management techniques, landscape features, method of restoration) that affect wetland health. From there, data on the frequency of occurrence and relative abundance of species, guilds, or trophic classes will be used to develop attributes for an index of biological integrity (IBI) for each of the assemblages. An attribute will be considered a valid metric if it relates either positively or negatively to the physical gradient. We will then compare the IBIs developed and determine if they are consistent in ranking wetlands. Once acceptable IBIs have been developed on the initial wetland base, we will apply them to the second set of wetlands to validate the model. In addition, the variance within our sampling methodology will be assessed.

Lessons Learned

Overall

- Many of the wetlands in our bases were only a few years old when we started and may not have had sufficient time for ecological and anthropogenic factors to separate them along a physical gradient.

- As a result, development of a reliable and ecologically meaningful gradient has been one of the most difficult parts of this project.
- It would be very instructive to revisit these wetlands after 10 or 15 years and see how they have changed.

Macrophytes

- There can be considerable differences between mid- and late summer in the ability to easily record and identify plants. This is especially true for graminoids, which are primarily identified by fruiting body characteristics. In addition, many legumes, composites, and warm-season grasses are present in late summer but not apparent in spring.
- The inclusion of incidental species added appreciably to the number of species identified in a particular wetland. We are evaluating whether this inclusion has an effect on the resulting metrics.
- Deep-water areas (greater than 45 cm) have much lower species richness than shallower zones and do not need to be sampled at the same level of intensity at the same site.
- Permanent transects are preferred if data collection can continue over several years. This will allow for the annual and seasonal changes that occur over time through shifts in hydrology.

Macroinvertebrates

- Picking, sorting, and identifying aquatic insects was one of the most laborious aspects of the study. Investigators wishing to use invertebrates in their bioassessments should allocate sufficient resources to accomplish the task.
- Invertebrate species presence, and especially abundance, are seasonally quite variable. June to early July before the drying of mid- to late summer begins seems to be the best months for finding the greatest diversity and abundance of macroinvertebrates.

Amphibians

- The reduced species richness of amphibians, compared to macrophytes and macroinvertebrates, may limit the number and types of metrics that can be developed from this assemblage.
- Adequate sampling for amphibians requires more trips and techniques than other assemblages. This is due to their mobility, multiple life history strategies, and variable breeding periods among species. Sampling for one life stage only is probably not as effective as sampling for adults and tadpoles in determining amphibian usage of a wetland.

Massachusetts: Use of Multimetric Indices to Examine Ecological Integrity of Salt Marsh Wetlands in Cape Cod

Contact Bruce K. Carlisle

Organization Massachusetts Coastal Zone Management
251 Causeway Street
Boston, MA 02114

Phone (617) 626-1200

E-mail bruce.carlisle@state.ma.us

Website http://www.state.ma.us/czm/wastart.htm

Purpose

The primary focus of the Cape Cod Salt Marsh Assessment Project is to advance and improve the salt marsh assessment approach developed by the Massachusetts Coastal Zone Management (MA CZM) team through the application and review of the existing protocol in two different assessment investigations. The first investigation, conducted in the 1999 field season (May to October index period), examined salt marsh indicators from six sites on the Cape Cod Bay coast; these sites had varying types and intensities of human land use or disturbance. The second investigation is a long-term comparison of indicators from three pairs of salt marshes, each pair having a marsh area with restricted tidal hydrology and a corresponding area with normal tidal hydrology. The intent of this work is to document differences in indicators before and after tidal restoration actions. Through the implementation of these two investigations additional objectives will be realized. The collection and compilation of data on the condition of relatively undisturbed sites is of critical importance to the evaluation and determination of impaired sites. This project will serve to expand the salt marsh reference site database. Another important aspect of this project will be to further examine the suite of existing metrics and attributes used for biological comparison and to explore new metrics, based on the project data and literature/information base. The long-term tide restriction study will provide insight as to the utility of this assessment approach as a measure of determining salt marsh restoration progress and trajectory.

Wetland Type

- Salt marshes

Assemblages

- Birds
- Fish
- Macroinvertebrates
- Vascular Plants

Status

The Cape Cod Salt Marsh Assessment Project is approximately two-thirds complete, with the land-use investigation finished and the longer-term tide restriction study entering its third year (or index season). The report for the 1999 land-use investigation is being finalized and should be available in early fall 2002 from this site or MA CZM's site (http://www.state.ma.us/czm/wastart.htm). EPA grant funds will support two additional years of field data collection, with project completion anticipated in March 2004. Field investigations from 2000 through 2004 are focused on the assessment of the effects of tidal hydrology alterations and the restoration of normal hydrology on salt marsh ecological integrity. The study involved three salt marsh sites with tidal hydrological restrictions. Of the project sites, one (EOBP) received restoration action in spring 2002, one (EMC) is predicted for fall 2002, and one (MSLP) is still uncertain for restoration action, with unforeseen complexities. In addition, the project team expects to sample one additional reference site each year to expand the regional reference dataset.

Project Description

Massachusetts Coastal Zone Management launched its Wetland Assessment Program with its first effort in wetland bioassessment in 1996-1998 at the Waquoit Bay National Estuarine Research Reserve. A subsequent project was implemented on Massachusetts' North Shore in 1997-1999.

The EPA Region I sponsored pilot, Cape Cod Salt Marsh Assessment Project, began in 1999 and will continue through 2004. The investigative team for this project includes Bruce Carlisle, Tony Wilbur, Jan Peter Smith, and Jay Baker from MA Coastal Zone Management, and Anna Hicks, an independent consultant specializing in aquatic macroinvertebrates. Partners for this project include the MassBays Program, UMass Extension, the MA Department of Environmental Management (DEM) and its Area of Critical Environmental Concern Program, the Waquoit Bay National Estuarine Research Reserve, the Cape Cod Commission, and the towns of Barnstable, Eastham, Mashpee, Orleans, and Sandwich.

The Massachusetts CZM project team will apply standardized sampling and surveying protocol to salt marsh study sites to gather biological, chemical, and physical data for the two investigations referred to earlier.

The project team will analyze and express the biological data through a series of existing metrics or attributes, or develop new metrics or attributes as necessary, based on the project data and literature base. Chemical and physical data will be utilized as supporting information sources.

The team will make recommendations for revisions, additions, or deletions to its current wetlands assessment approach.

Study Design

In the 1999 field season, six salt marsh sites with varying types and degrees of intensity of surrounding land use were selected. Two sites with minimal human land use (conservation land and no tidal hydrological alteration) were chosen as reference sites, representing the best attainable conditions in the immediate region.

The four other salt marsh study sites have varied land uses including residential, commercial, and transportation. The impacts associated with these land uses include direct stormwater outfalls, large impervious areas, septic systems, lawn fertilizer/chemicals, pet waste, automobile emissions/byproducts, and direct habitat alterations.

For the 2000 field season, three salt marshes with tidal hydrological restrictions will be studied. Measurements will be made both at the salt marsh affected by the tidal restriction (the restricted study site) and at the salt marsh below the restrictive feature (the reference site). The reference sites or marshes below the tidal restriction receive normal tidal influence and inundation. In addition, the 2000 field season will also include two additional long-term salt marsh reference sites. The continued development of a robust reference site database is critical to the continuing evolution and application of wetland bioassessment.

Sampling Methods: Macroinvertebrates

At each site, a habitat characterization form will be completed that summarizes the ambient salt marsh habitat conditions at the study site. The information collected includes water quality parameters (temperature, pH, dissolved oxygen, salinity, and color) and habitat descriptors on hydrology, vegetation, substrate, available food sources for invertebrates, and degree of human impact.

The sampling protocol will utilize tree devices for application in different habitats:

Intertidal zone

Plot sampling using wooden frame (18"×18")

Subtidal (permanently flooded) zone

D-net, plot sampling, and auger

At each sampling site, three sampling stations will be selected over a defined linear distance of the creek channel, stations will be located at 1/3 intervals. At each of the three stations a representative composite sample of macroinvertebrates will be collected as follows:

Intertidal bank zone at low tide: 1 plot sample

Subtidal estuarine zone at low tide: 1 plot sample, 1 D-net sample, and 1 auger sample

Each sample will be placed in a zip lock bag labeled with site number, site name, date of sampling, sample number, sampling method, and name of sampler. The site field sheets also record the relevant sample numbers. Samples will be preserved in 70% isopropenol alcohol and placed in a cooler ready to be transported to the laboratory for sorting and identification.

Invertebrate samples are taken once in May and again in August.

Sampling Methods: Vegetation

At each site, the salt marsh wetland vegetation will be surveyed according to this protocol. Six transects will be established based on a stratified random sampling approach. A defined linear distance of the salt marsh creek channel is established.

The evaluation area will be segmented into three subunits along equal sections of the creek channel. The first third of this length is subunit #1, the second third is subunit #2, and the final third is subunit #3. In each of the subunits, two randomly selected transects will be laid. The transect locations will be determined by a computer random numbers algorithm.

The transects will run roughly perpendicular from the channel to the upland edge, and each transect will be laid according to a consistent compass bearing. Along each transect, 1-m^2 quadrats will be located every 60 feet, starting at the creek edge and progressing along the entire length of the transect until the upland edge. The last quadrat will be located in the salt marsh fringe community, well within the wetland and not on the upland.

Using a standard data sheet, in each quadrat along each transect, every plant occurring within that quadrat will be identified by genus and species. For each unique species within the quadrat, the abundance of that species will be determined using standardized coverage charts. Investigators will also define the community type that the quadrat falls in: low marsh, high marsh, or fringe. To be as accurate as possible, coverage estimates include duff, leaves, bare ground, and open water, collectively designated as "other." Coverage estimates will be adjusted during the analysis stage to account for the coverage of this "other" category.

Vegetation surveys will be conducted once at each site during the peak growing period from mid-July to mid-September.

Sampling Methods: Birds

Point counts will be utilized as the primary sampling method, using visual and auditory cues. At least two expert observers, including the principal investigator, will sit quietly from a vantage point where all of the evaluation area can be viewed. Using a standard data sheet, all species and individuals will be counted and recorded by the observers, as they are heard or seen demonstrating any activity in the evaluation area or in a 100-foot buffer area surrounding the evaluation area. Counts will be conducted for a period of 20 minutes, separated into four 5-minute sample intervals. All individuals will be counted, with a concerted effort not to duplicate individuals. An additional 10 minutes will be allotted to allow observers to walk slowly along the perimeter of the wetland in order to detect any species not tallied in the 20-minute count. Several sites may be visited on the same day, with census beginning at approximately 6 am and ceasing at approximately 8:30 am in order to capture peak activity. Sites will be sampled in late August to capture migrating shorebird usage, as saltmarsh habitats are known to have comparatively fewer breeding species.

Sampling Methods: Fish

The sampling strategy will be to capture the channel habitat (sub- and intertidal). As detailed above, the evaluation area will be divided into three subunits along a defined length of creek channel. Stations will be established for each habitat as follows. In the creek channel, fixed stations will be determined by a computer random numbers algorithm producing a random integer between 0 and 100. The random integer will be the distance of the starting point for the seine haul in feet from the start point (0’) of each subunit.

Seines will be utilized to sample the creek channel. At three stations, as defined above, the seine will be dragged through the water column along the creek bank and substrate for a length of 5 meters. Seines will be carefully withdrawn from the creek and the collected fish will be carefully extracted from the seine into a processing bucket. The fish will then be removed by dipnets and individuals will be identified and measured. Abundance and total biomass by species will be enumerated. All data are recorded on a standardized field sheet.

One sample run will be conducted each month from April to October.

Analytical Methods

Biological data collected at wetland study sites are compared to data collected at the wetland reference sites. Multimetric data analysis techniques are employed to examine attributes and variables of biological data, and these metrics are combined into a quantitative final index. A metric is a parameter or variable that represents some feature, status, or attribute of biological assemblage, chemical state, or physical condition. In a multimetric approach, several different metrics are chosen in order to effectively capture and integrate information from individual, population, guild, community, and ecosystem levels and processes. Metrics are selected on the basis of literature reviews, historical data, and professional knowledge. The quantitative output from each metric is then combined to produce an index. An index is the aggregate of weighted metric scores that serves to summarize the biological condition.

Lessons Learned

Through the three pilot projects, the MA CZM project team has been able to learn from each application and as a result has made several small, incremental revisions to many components of the protocols, including adjustments to sampling methods and shifts in the attributes and metrics examined. Each application has also generated results that indicate decreasing biological integrity with increasing land-use stressors.

Results from 6 years of wetland assessment experience have led the MA CZM team to conclude that this assessment approach has significant potential for a number of management applications, including:

- Inventory efforts such as identifying and protecting unique and important habitat
- Identification of degraded salt marsh sites and presence of invasive (nonnative) species in order to evaluate restoration potential and/or report on wetland status (i.e., 305b reports)
- Monitoring and evaluating the effectiveness of restoration actions
- Long-term tracking of salt marsh condition and documenting the effects of disturbances (i.e., stormwater pollution, eutrophication, hydromodification)

Many of these applications have appeal to watershed-based organizations and agencies as well as volunteer groups. The techniques of the MA CZM salt marsh assessment approach have been successfully taught through a comprehensive volunteer training program on Massachusetts' North Shore. At the time of writing, the Wetland Health Assessment Toolbox program is entering its fourth year, guiding and supporting volunteer groups as they monitor salt marsh restoration sites in their region (http://www.salemsound.org/wetlands.htm).

Results from this Cape Cod Salt Marsh Assessment Project are still being analyzed and written up, but some initial details are available: The 1999 land-use investigation has confirmed the trend seen in the previous two studies where increasing levels of land use around a given salt marsh site (wetland) correspond with decreased ecological condition, in this case indicated by the average of the Plant Community Index and the Invertebrate Community Index.

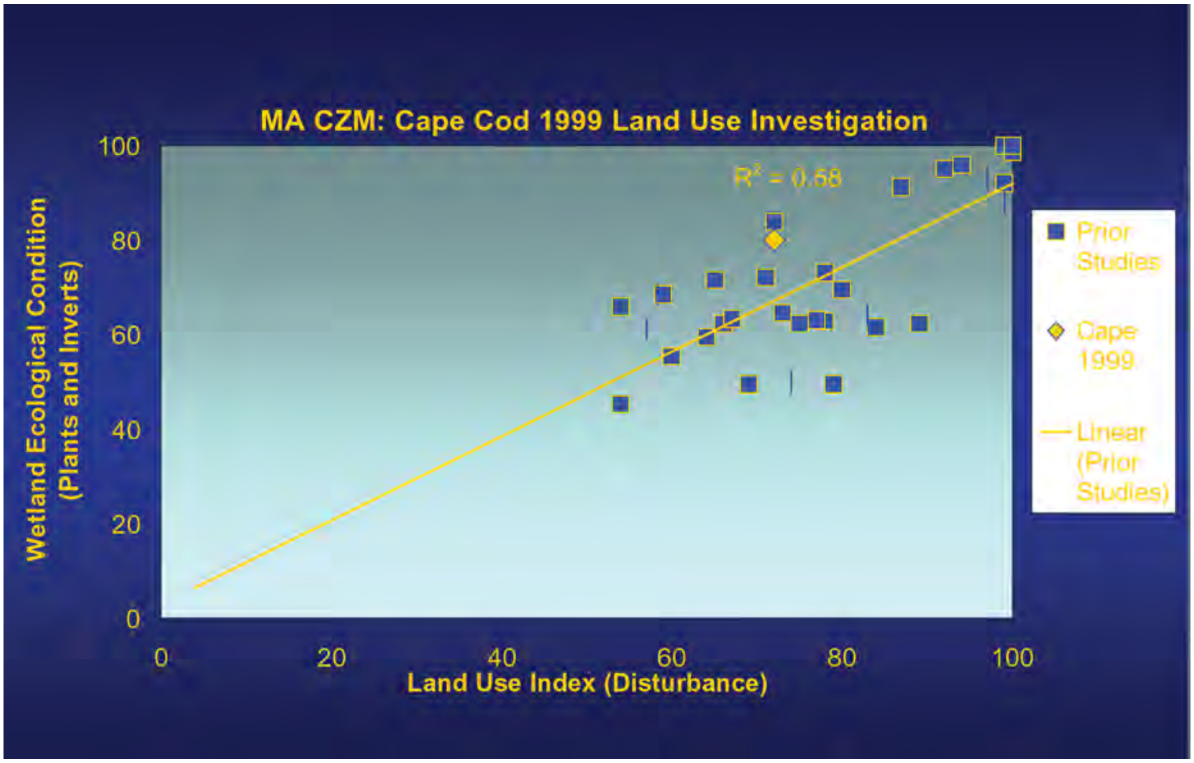


Figure 7: Relationships between wetland ecological condition and land use. The Land Use Index (LUI) score is a measure of human disturbance. With increasing land use types and intensities, the LUI score declines. Similarly, the Wetland Ecological Condition (WEC) is a measure of biological integrity. A lower WEC score indicates increasing biological impairment.

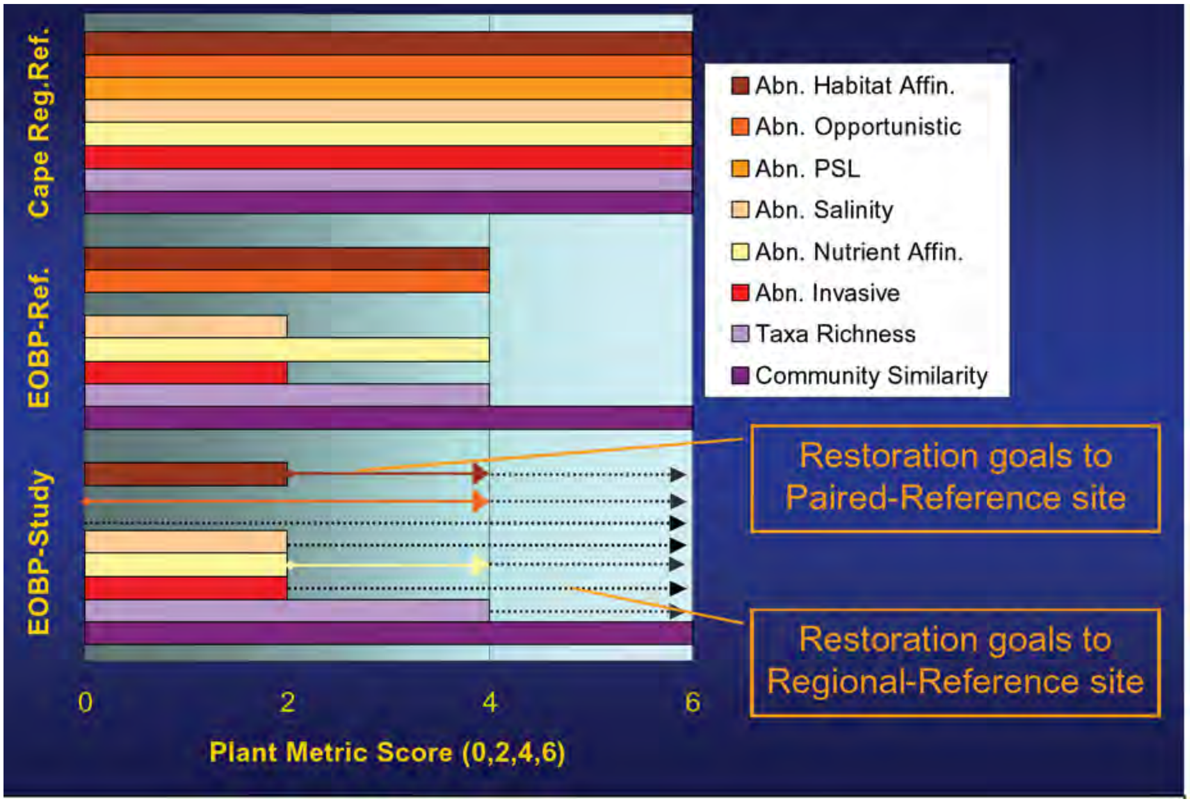


Figure 8: Two sets of multimetric goals for evaluating restoration. The restored site is on the bottom, its paired reference (directly below/seaward tide restriction feature) is the middle graph, and a regional reference marsh is displayed at top.

Plate 5: Cape Cod, MA, salt marsh plant survey:
1 m^2 along transect. Photo: B.K. Carlisle

Plate 6: Cape Cod, MA, salt marsh nekton: Watch your fingers!
(blue crab, *Callinectes sapidus*). Photo: B.K. Carlisle

Massachusetts: Involving Volunteers in Examining the Ecological Integrity of Coastal Wetlands in Cape Cod, Massachusetts

Contact	Britta Magnuson
Organization	Salem Sound 2000 201-203 Washington St., Suite 9 Salem, MA 01970
Phone	(508) 741-7900
E-mail	Brita.Magnuson@salemsound.org

Contact	Jan Smith
Organization	Mass Bays National Estuary Program 251 Causeway Street, Suite 900 Boston, MA 02114
Phone	(617) 626-1231
E-mail	jan.smith@state.ma.us
Website	http://www.state.ma.us/czm/wastart.htm

Purpose

- Training volunteers to do biological assessments
- Comparing results of volunteers to trained scientists

Wetland Type

- Salt marshes

Assemblages

- Birds
- Fish
- Macroinvertebrates
- Vascular plants

Status

Completed 3 years of sampling. Analyzing data.

Project Description

"Salem Sound 2000" and "8 Towns and the Bay," two regional subcommittees of the Massachusetts Bays National Estuary Program, participated in a salt marsh monitoring pilot project involving citizen volunteers in conjunction with UMass Cooperative Extension Service and Massachusetts Coastal Zone Management (MCZM).

During the summers of 1999 and 2000, more than 40 volunteers participated in training workshops and field data collection for a variety of parameters: water chemistry, land use index (a habitat assessment), aquatic macroinvertebrates, birds, tidal influence, and vegetation. During 1999, professional scientists did independent assessments, and the volunteers conducted assessments (using the same sampling protocols as the professionals) with the guidance of trained staff members. Data comparisons, as well as feedback from volunteer participants, were used to modify and improve training protocols for the 2000 field season. Field data were collected in 2000 at the same sites, which included salt marsh sites that were affected by tidal restrictions and sites impacted by stormwater discharges. The project is expected to be a model in other areas of New England. A volunteer training manual is currently being printed and should be available at the time of this printing. Funding from a private foundation has enabled the program to continue for two additional years, 2001 and 2002, and it is planned to add fish as an additional parameter for the volunteers to measure. The sampling protocols are summarized in the MCZM project summary.

Lessons Learned

Volunteers may need ongoing field instruction in order to ensure quality data collection. The initial comparison between data collected by volunteers and data collected by professionals indicated gaps, which improved field assistance was able to reduce. Also, asking the volunteers to review and comment on training workshops was helpful in improving program design. Although there is high turnover in volunteers from year to year, we are finding, after 4 years into the program, that some volunteers do come back after a year's break, and that the skills of the returnees definitely improve after the first year. Offering teacher training credits improves the interest of educators in participating in the program, and many get interesting ideas for future use in their classrooms.

Michigan: Bioassessment Procedures and Baseline Reference Data for Great Lakes Coastal Marshes and Inland Forested Wetlands in Michigan

Contact Thomas M. Burton

Organization Michigan State University
Department of Zoology
203 Natural Science
Last Lansing, MI 48824-1115

Phone (517) 353-4475

E-mail burtont@msu.edu

Contact Donald G. Uzarski

Organization Grand Valley State University
Annis Water Resources Institute
Lake Michigan Center
740 West Shoreline Dr.
Muskegon, MI 49441

Phone (616) 895-3989

E-mail uzarskid@gvsu.edu

Purpose

Subproject 1: Coastal wetlands

- Collect baseline data on water quality and adjacent land use, as well as plant, invertebrate, and vertebrate communities from Great Lakes coastal wetland sites experiencing a continuum of disturbance
- Continue development of invertebrate and fish-based indices of biological integrity (IBI's) by plant zone, for Great Lakes coastal wetlands
- Continue testing and validation of our IBIs

Subproject 2: Inland, forested depressional wetlands

- Collect baseline data on plant, invertebrate, and vertebrate communities with accompanying chemical/physical parameters from reference and impacted forested, depressional wetlands of southern Michigan
- Develop an IBI for forested depressional wetlands of southern Michigan based on invertebrates, plants, fish (if present), and birds

Wetland Type

- Great Lakes coastal
- Inland, forested depressional

Assemblages

- Birds
- Fish
- Macroinvertebrates
- Vascular plants

Status

Completed 3 years of sampling. Analyzing data.

Project Description

Subproject 1: Coastal wetlands

We developed a preliminary IBI for Lake Huron based on invertebrate data collected from coastal wetlands with funds provided by the Michigan Department of Environmental Quality, the Nature Conservancy, U.S. EPA, and the U.S. Geological Survey and published it in the journal *Wetlands* (Burton et al. 1999). Since publication, we have continued testing the IBI with data from additional Lakes Huron and Michigan fringing wetland sites. We also have continued monitoring a set of initial sites used in IBI development as lake levels declined in order to determine whether and how well the IBI works as lake levels decline substantially below levels that occurred during IBI development. We have collected extensive data on invertebrates, plant, fish, and bird communities on Lake Huron wetlands since the early 1990's. Much of our work has been in collaboration with the Great Lakes Science Laboratory (BRD-USGS), Michigan Natural Features Inventory (MNFI), and the Ohio Biological Survey (OBS).

Our preliminary IBI for Lake Huron fringing wetlands was developed using macroinvertebrate data collected in 1997 from six wetlands. It was tested using data collected from 11 Lake Huron wetlands (6 original and 5 additional) in 1998 at lake levels substantially lower than in 1997. We continued to test the IBI using data collected from 12 sites (7 original and 5 additional) at even lower lake levels in 1999 and from 5 additional sites at extremely low water levels in 2000. Even though some plant community zones used in IBI development were not flooded and could not be sampled in 1999 and 2000, the IBI functioned extremely well. In 2001, we sampled similar fringing wetlands of northern Lake Michigan along with northern Lake Huron sites. The IBI appears to work for fringing wetlands of northern Lake Michigan as well.

While testing and validating the Lake Huron IBI in 1999, 2000, and 2001, we developed modifications to simplify and improve it. A detailed explanation for the modifications was presented at "Wetlands 2000" in Quebec City in August 2000, and additional modifications to remove the "preliminary" status were presented in Lake Placid, New York, in June 2002. The modifications are as follows:

1. The Typha zone should be removed from the IBI.
2. The four diversity and richness metrics should be calculated by plant zone instead of combining data from all plant zones before calculations are made.
3. Two new metrics should be added to the Inner *Scirpus* Zone:
   - Relative abundance Isopoda (%)—Decreases with disturbance
   - Relative abundance Amphipoda (%)—Increases with disturbance
4. Use ½ person-hour count to determine number of individuals counted per replicate. Count either 50, 100, or 150.

A manuscript noting all of the above improvements is in preparation and will be submitted to the journal *Aquatic Ecosystem Health and Management* by September 2002 as part of a special issue on coastal wetlands.

We are optimistic that our Lake Huron IBI will work for northern Lake Michigan fringing wetland sites. These sites include most wetlands along the southern shore of the Upper Peninsula of Michigan from St. Ignace to the Wisconsin border. Most of these wetlands appear to be comparable in plant community composition and structure to the Lake Huron wetlands. Preliminary testing took place in 2001 and we expect to be able to recommend the IBI's use for these types of wetlands.

In 2000, we began development of new fish- and macroinvertebrate-based IBIs for the drowned river mouth wetlands of Lake Michigan. We collected data from eight sites representing a gradient of anthropogenic disturbance. Ten sites were sampled in 2001 and several new sites will be added in 2002. We expanded our work on drowned river mouth wetlands in 2001 to Lake Superior wetlands.

Subproject 2: Inland, forested depressional wetlands

We started our forested depressional wetland project in 1999. Invertebrates were collected three times per year from eight forested depressional wetlands during 1999 and 2000 using dipnets, activity traps, and black lights. Additional samples were collected from a subset of these and some additional wetlands in 2001. Up to 40 additional sites will be added in 2002, with emphasis on obtaining a greater array of impacted sites and extending the sites to isolated depressional wetlands in the coastal zone of the Great Lakes. Invertebrates are identified to the lowest taxonomic unit possible, and preliminary IBI development has begun. Accompanying chemical/physical samples taken from surface water and mini-piezometers have also been recorded and analyzed for potential metrics. Comparisons of the plant and invertebrate communities have been made using a subset of the sites.

A list of potential metrics, a summary of our very preliminary analyses, and conclusions that we drew from the 2000 data set are as follows:

Significantly higher at reference sites (Mann-Whitney tests):

- Isopoda — $p < 0.001$
- Amphipoda — $p = 0.004$
- Diptera
- Culicidae — $p = 0.004$
- Ephemeroptera — $p = 0.015$
- Trichoptera — $p = 0.078$

Significantly higher at impacted sites (Mann-Whitney tests):

- Gastropoda
  - Lymnaeidae — $p = 0.004$
  - Planorbidae — $p < 0.001$
- Diptera
  - Chironomidae — $p = 0.074$
  - Chaoboridae — Collected from only one site
- Odonata
  - Libellulidae — $p = 0.039$
- Hemiptera
  - Lleididae — $p < 0.001$
- Coleoptera
  - Halipidae — $p < 0.001$
- Annelida
  - Hirudinea — Collected from only one site

Summary

Of the measured chemical/physical variables, only depth separated impacted and reference sites. Canopy cover was not measured in 1999 and 2000 but may have also separated impacted and reference sites. Estimates of canopy cover are currently being obtained for all sites. Eleven (+2 ?) taxa showed potential as metrics, but not during early inundation (April). During full inundation (May), the correspondence analysis grouped the wetlands using invertebrate community composition into distinct categories.

- Surface runoff-influenced impacted sites
- Groundwater-influenced reference sites
- Precipitation-influenced reference sites

Conclusions

- Increased runoff may increase water depth and subsequently kill trees, opening the canopy.
  - Some taxa such as *Libellulidae* may be responding to these changes in ambient conditions.

- Most traditional chemical/physical variables will not be useful as indicators of anthropogenic disturbance in these wetlands. These variables include turbidity, DO, pH, SRP, NH4, conductivity, alkalinity, temperature.
- An invertebrate-based IBI for (hardwood) forested wetlands of Southern Michigan appears to be feasible.

Study Design

Subprojects 1 and 2

Sites that experience a wide range of anthropogenic disturbance, or stressors, are chosen from each hydrogeomorphic class or subclass of wetland. The extent of disturbance is determined using surrounding land use/land cover and limnological data. Initially, correspondence analyses of invertebrate community composition were used to determine if reference sites separated from impacted sites. When they did, individual taxa containing the most inertia responsible for the separation were deemed potential metrics. Mann-Whitney U tests were then used to determine if densities of these taxa at reference sites were significantly different from densities at impacted sites.

We use medians in place of means in the IBI because medians are more resistant to the overwhelming effects of outliers. Our goal is to typify the wetland. If an area is sampled that is depleted or concentrated in the constituents of a metric, the area may be isolated from anthropogenic disturbance, receiving a dose of disturbance not typical of the entire wetland or vegetation zone, or may contain some "natural" chemical/physical component that is unique. Regardless of the cause, the area is not representative of the entire wetland. The influence of these outliers can be dampened by using the median in place of mean as a measure of central tendency.

After potential metrics were developed, principal components analysis (PCA) was used to establish principal components (PCs) based on chemical/physical parameters as well as surrounding (1-km buffer) land use/cover data. Pearson correlations were done between individual metrics and PCs to establish stressor-ecological response relationships. PCs were then decomposed to explore relative contributions of individual stressors.

Sampling Methods

Subproject 1: Coastal Wetlands

Macroinvertebrate samples. Macroinvertebrate samples were collected with standard D-frame, 0.5-mm mesh dipnets. All major plant community zones were sampled at each site, including a deep emergent and a shallow, wet meadow zone. If certain depths contained more than one dominant plant community along the shoreline, each plant community type was sampled.

Dipnet sampling entailed sweeps through the surface and middle of the water column and above the sediment surface to ensure that an array of microhabitats were included in each sample. Dipnets were emptied into white enamel pans, and 150 invertebrates were collected by removing all specimens from small areas of the pan. Special consideration was made to ensure that smaller organisms were not missed, as there is a bias toward larger, more mobile individuals with this technique. Plant detritus was left in the pan and sorted through for a few additional minutes to ensure that sessile species were included in the sample. If 150 individuals were not obtained after ½ person-hour of field picking, we collected to the next multiple of 50. The timed count was used to semiquantify sampling effort so that it could be used as a metric. Three replicate samples were collected in each plant zone to obtain a measure of variance.

Dipnet samples were collected from late July through August. Samples taken from ice-out through mid-July generally contained less diversity and a greater proportion of early instars of aquatic insects, making identification more difficult. The July–August time period also corresponded to the time when plant communities, characteristic of these wetland systems, achieved maximum annual biomass.

Fish sampling. Six fyke nets (0.5-in mesh) were set in each wetland for 1 net-night. Nets were set adjacent to specific plant zones with the leads bisecting the sampling area. Minnow traps were used as a secondary method.

Subproject 2: Inland, forested depressional wetlands

Invertebrate sampling. Macroinvertebrate samples were collected with standard D-frame dipnets containing a 0.5-mm mesh. Three replicate samples were collected from three locations in each wetland: (1) the deepest portion (usually the center), (2) near the upland, and (3) between these two areas. We attempted to incorporate habitat heterogeneity by sampling as many plant zones as possible at each location.

Dipnet sampling entailed sweeps through the water column at the surface, middle of the water column, and above the sediment surface. Dipnets were emptied into white enamel pans and 150 invertebrates were collected by focusing on small areas of the pan and removing all specimens. If 150 individuals were not obtained after ½ person-hour of field picking, we collected to the next multiple of 50. The timed count was used to semiquantify sampling effort so that it could potentially be used as a metric. Invertebrate sampling was conducted during early (April), full (May), and late inundation (June) at each site.

Fish sampling. The temporary pools that dominate most depressional forested wetlands are unlikely to contain fish. Thus, fish sampling was only conducted for permanent pools in depressional wetlands when they occurred. Small fish traps were placed in each of the permanent pools.

Birds surveys. Bird communities were surveyed using 10-minute, 50-m radius point counts (Ralph et al. 1995) by dual observers to sample the bird communities at each count location. The 10-minute counting period began when the observers reached the perimeter of the 50-m radius so that any birds flushed or silenced by the observer's approach were detected (Riffell et al. 1996). The bird communities of 30 forested wetlands were sampled in 2000 using a total of 6 count visits. A variety of habitat measures from each site were obtained as detailed under plant sampling below, and a manuscript on results is currently being prepared. No new sampling has occurred since 2000.

Plant sampling. The plant community and other habitat variables were described for each 50-m radius bird count area along four habitat sampling radii radiating from the center of each point count station following procedures detailed in Riffell et al. (1996). The understory habitat was described using a Wien's pole (Rotenberry and Wiens 1985) following procedures of Riffell et al. (2001), modified as appropriate to adapt them to forested habitat.

More traditional plant sampling, of a subset of forested wetlands, was done by Mike Kost and Dennis Albert of the Michigan Natural Features Inventory during 1999 and 2000 using quadrat sampling along transects running through each wetland. They have submitted a report on their results to the Michigan Department of Environmental Quality including recommendations on potential metrics for these wetlands.

Minnesota: Developing Wetland Biocriteria

Contact Mark Gernes

Organization Minnesota Pollution Control Agency (MPCA)
Environmental Outcomes Division
520 Lafayette Road
St. Paul, MN 55155

Phone (651) 297-3363

E-mail mark.gernes@pca.state.mn.us

Website http://www.pca.stat.mn.us

Contact Judy Helgen

Organization Minnesota Pollution Control Agency (MPCA)
Environmental Outcomes Division
520 Lafayette Road
St. Paul, MN 55155

Phone (651) 296-7240

E-mail judy.helgen@pca.state.mn.us

Website http://www.pca.state.mn.us

Purpose

- Develop the tools to assess wetland condition by studying vegetation and invertebrates in wetlands across a range of human disturbance
- Develop two separate Indexes of Biological Integrity (IBI) for Minnesota depressional wetlands

Wetland Type

- Depressional wetlands, large and small; riparian wetlands

Assemblages

- Macroinvertebrates
- Vascular plants

Status

Established indexes of biological integrity (IBIs) for plants and macroinvertebrates. Report completed on IBIs for 44 large depressional wetlands in May 2002. In 2002, evaluating data on statistical analysis of sampling methods in 9 (invertebrates) and 12 (vegetation) large depressions. Beginning June 2002, testing IBIs in new ecoregion, comparing IBI assessments with rapid assessment method and citizen monitoring data. Training of many citizen community teams in biological assessment of local wetlands continued in 2002; a pictorial guide will be produced in 2002.

Project Descriptions

Minnesota has been developing its wetland biological assessment program since 1992.

1992 – Small depressional reference wetland study

The first project was funded by the State legislature to develop biological reference conditions for small depressional wetlands in central Minnesota. Subsequent funding is primarily from EPA. The initial research studied the quantity and quality of macroinvertebrates primarily in highest quality, least disturbed reference sites and relatively few disturbed depressional wetlands.

1995 – Expanded small depressional wetland study

A second project funded by EPA was undertaken to develop multimetric IBIs for depressional wetlands in central Minnesota. During the 1995 sampling season, MPCA collected data on macroinvertebrates and vegetation for a larger set of depressional wetlands than the 1992 study, representing a wide range of impairment. MPCA's report on the 1995 IBI development was completed in 1999 and will be available on MPCA's web site (www.pca.state.mn.us).

1999 – Large depressional wetland study

MPCA sampled large depressional wetlands, including duplicate sampling at six of the sites, to validate metrics developed with smaller depressional wetlands in 1995. MPCA also developed a scoring system for human disturbance (HDS) that incorporates estimates of disturbances in the buffer area and in the near-wetland landscape, plus ranges of chemical pollution and alterations within the wetland. The HDS scores were used as the x-axis to calibrate the metrics and the IBI scores. Both the vegetation and the invertebrate IBI scores show significant relations to the human disturbance scores and to various water and sediment chemical factors. The invertebrate IBI was significantly related to HDS, turbidity, phosphorus, chloride, and other factors. The vegetation IBI was significantly related to HDS, phosphorus, and chloride in water; and to copper; zinc; and nickel in sediments. Minor changes were made in metrics compared with those used for the smaller depressions. The final report for this project, Indexes of Biological Integrity (IBI) for Large Depressional Wetlands in Minnesota, by M.C. Gernes and J.C. Helgen, was completed in May 2002. In 1999, 27 wetlands in riparian areas of small and medium-sized streams in the St. Croix River basin were sampled for the vegetation IBI. There data are currently under analysis.

2001 – Statistical assessment of wetland monitoring methods

MPCA sampled nine wetlands in three locations to test the methods for the invertebrate and vegetation IBIs with statistical procedures. These data are currently under analysis.

2002 – Citizen training

In 2002, MPCA expanded efforts for training citizens in biological assessment of wetlands, training 90 citizens in the invertebrate and vegetation methods (see Minnesota case study entitled "Dakota County Wetlands Health Evaluation Project"). A final guide for biological monitoring of wetlands by citizens will be produced by MPCA in 2002. This guide includes pictorial keys to wetlands invertebrates and plants. MPCA also trained school teachers in the assessment of ephemeral wetlands in the spring of 2001 and 2002.

In the summer of 2002, MPCA is validating the IBIs for depressional wetlands in 40 wetlands in southern Minnesota to test regional applicability of the methods. In addition, about 10 wetlands monitored by citizens will be assessed by MPCA using the technical IBIs. The data from the assessments by citizens using the modified IBIs and MPCA's technical IBIs will be compared. The IBI results will be compared with results from a Minnesota rapid assessment method carried out by consultants on the same 10 wetlands.

Study Design

In the 1992 project on reference wetlands, 32 least disturbed and 3 known disturbed wetlands were sampled for macroinvertebrates. In the second research phase in 1995, 27 wetlands were sampled for macroinvertebrates and vegetation. The 27 wetlands represented the full range of human disturbance typical of wetlands in this part of Minnesota, and were located in the North Central Hardwood Forest ecoregion (Figure 9). The sites included 6 least impaired reference sites and 21 sites highly impacted from human disturbances such as stormwater (12 sites) and agricultural influences (9 sites).

In the 1999 large depressional wetlands project, 44 sites were sampled, 6 of which were sampled twice on the same date. Included were 14 high-quality reference sites, 14 agriculture-influenced sites, and 16 urban wetlands (Figure 10). These wetlands were selected to represent the widest range of disturbance.

Figure 9: Locations of smaller depressional wetlands sampled in 1995.

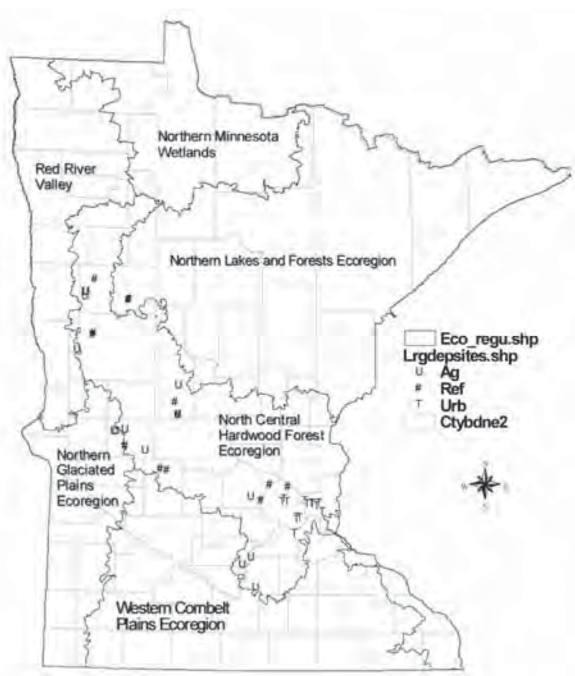


Figure 10: Locations of the 44 large depressional wetlands sampled in 1999.

In the 2001 statistical assessment of wetlands methods project, three sectors of nine wetlands were sampled for invertebrates and vegetation.

In 2002, approximately 50 large depressional wetlands representing a gradient of human influence are being assessed. Approximately 30 wetlands in the Western Cornbelt Plains and the Northern Glaciated Plains ecoregions are being analyzed to determine if the IBI developed in the North Central Hardwood Forest (NCHF) ecoregion can be used in other regions of Minnesota. Five of these sites will have duplicated sampling. Ten wetlands from previous projects in the NCHF ecoregion will be analyzed. Another 10 wetlands in the NCHF ecoregion are being sampled by citizens using the IBIs and by consultants using a rapid assessment method.

In the projects in 1995, 1999, 2001, and 2002, both invertebrate and vegetation sampling were done. Water chemistry samples were taken in June, and sediments were cored for metals and other factors in later summer.

In addition to invertebrate and vegetation sampling, wetlands were sampled for water pH, conductance, turbidity, dissolved oxygen, temperature, calcium (hardness), chloride, total suspended solids, total phosphorus, and total nitrogen. Sediments were analyzed for 15 heavy metals using ICP methods, as well as for total organic content, textural classes, carbonates, chloride, total phosphorus, and total nitrogen. Low-altitude aerial photographs were taken to support the scoring for the human disturbance gradient.

Sampling Methods: General Considerations

Stratification of the habitat for each study site was done so as to minimize the biological variability among the different strata of the wetland. For the depressional wetland projects, the following habitats were identified: nearshore emergent zone, open water submergent zone, and floating plant zone.

Sampling Methods: Macroinvertebrates

Sampling was done in the nearshore emergent zone during the seasonal index period of June to early July. This time frame ensured that optimal species maturity and richness were present. In previous fieldwork, MPCA had determined that sampling in May was too early because some invertebrates are too immature for identification. Once collected, invertebrate samples were preserved and analyzed in the laboratory. Macroinvertebrates were sampled using both the dipnet and the activity trap method. Dipnetting captured the greatest richness of invertebrates and the activity trap captured the active swimmers and night-active predators.

Dipnet

The D-frame aquatic dipnet with 600-micron mesh net was used. Two dipnet samples were taken within the emergent vegetation zones. A ½″ wire screen fixed to a wooden frame was used to keep the vegetation from the sample. After sweeping the net strongly through the water column four to five times and downwards to near the bottom, the contents of the dipnet are emptied onto the framed wire screen. The frame is set over a pan containing sieved water to catch invertebrates as they drop down from the vegetation. For approximately 10 minutes, the vegetation is gently spread and invertebrates are encouraged to drop or crawl down to the water in the pan. After separation from the vegetation, the water is then poured through a 200-micron sieve to concentrate the sample before preservation. Preservation of the samples is done using 80% ethanol, final concentration. Using a squirt bottle with the alcohol solution, the sample is back-flushed into the sample jar and labeled for later picking and identification.

Activity trap

The bottle trap works as a passive funnel trap that collects organisms as they swim into the funnel and pass through the neck into the bottle. Made from a clear 2-L round-bottomed plastic beverage bottle, the traps are nearly invisible underwater. The traps are supported on a 4.5′ wooden dowel with a flexible half section thin wall PVC pipe that allows raising and lowering the bottle trap on the dowel.

Ten bottle traps were placed in the emergent vegetation zone and left overnight for two consecutive nights. Placement of the bottle traps was from the nearest shallow shore edge to the inner side of the deeper emergent vegetation zone in water no greater than 1 meter. In the shallowest water, the traps were placed on the bottom just under the surface of the water. Traps were placed horizontally about 15-20 cm under the surface. The bottle traps were back-filled with water leaving no air bubbles inside to reduce the amount of predation within the trap.

Analytical Methods: Macroinvertebrates

After the invertebrates were sorted in the lab, the entire sample was identified and counted. The data were entered into an ACCESS database and scored for 10 metrics, which represent different measures of the invertebrate community. The metrics used for small depressions are in the 1999 report of the 1995 project. The metrics used for large depressions are in the 2002 report on the 1999 project (Table 1). For the 1995 data, the metrics were validated by plotting them against a ranking of the site disturbance based on professional judgment and against selected chemical variables. For 1999, the metric data were plotted against the human disturbance gradient scores (HDS) and some chemical factors to evaluate metric responses. In addition, linear regressions were done on metric data and IBIs against the measures of human disturbance.

Chemical (e.g., phosphorus, nitrogen, chloride, and heavy metals) and biological data were analyzed for statistically significant relationships to the metrics and IBIs. Of the 10 invertebrate metrics, intolerant taxa, chironomid taxa, and total taxa showed the strongest responses to the estimated disturbance gradient and water chemistry factors, followed by the Odonata and ETSD metrics. HDS scores, turbidity, and phosphorus and chloride in water were most significantly related to the invertebrate IBI; copper in sediments was significant.

Sampling Methods: Vegetation

Vegetation sampling techniques vary greatly for different wetland habitats and study designs. MPCA used a releve method for sampling vegetation. The releve method was chosen for several reasons. The primary reason is that it is easily adapted to widely varying habitats and vegetation community structure, which is typical for depressional wetlands. This adaptability in sampling methodology is needed for wetlands that receive significant quantities of water during storm events. A second reason that MPCA selected releve sampling over line transect or quadrat sampling was that the Minnesota Department of Natural Resources' Natural Heritage Program and County Biological Survey use releves, and Minnesota's academic researchers also use a similar releve method for collecting vegetation community data.

All vegetation sampling was done in July, which represents the typical period of maturity and the best time in Minnesota for determining community structure. In each wetland, a 100-m2 plot was established in representative sampling locations within the emergent and open water submergent zones. After establishing the releve plot, the vegetation cover classes were determined for each plant species occurring in the plot. Voucher specimens were collected at least once during the project for each species or taxon encountered. All taxa that couldn't be identified reliably to the species level in the field were also collected.

Analytical Methods: Vegetation

Ten vegetation metrics were developed and validated, using methods similar to those for the invertebrate IBI. Each promising attribute of the plant community was plotted against a disturbance gradient. In the 1995 small depressional wetlands project, the disturbance gradient index was developed from professional judgment ratings of several disturbance factors including stormwater input, agricultural practices, quality of adjacent buffers, hydrologic alterations, and historical disturbances. For the 1999 large depressional wetlands project, the HDS scores and chemical factors were used. Metric scoring criteria were then developed for the strongest responding metrics (Table 2). Metrics were also plotted against chemical variables to demonstrate their response to traditional water chemistry concerns.

Table 1: Scoring Criteria for 10 Invertebrate Metrics for IBI for Large Depressional Wetlands

Metric Description	Range	Score
Number of intolerant taxa: Leucorrhinia, Libellula, Tanytarsus, Procladius, Triaenodes, Oecetis	5-7	5
	3-4	3
	0-2	1
ETSD metric: # genera mayflies, caddisflies; presence of fingernail clams (Sphaeriidae) and dragonfly larvae	7-10	5
	4-6	3
	0-3	1
Tolerant taxa proportion of count of individuals to total sample count: Trichochorixa, Enallagma, Erpobdella, Physa, Cricotopus, Dicrotendipes, Endochironomus, Glypotendipes, and Paratanytarsus	<42%	5
	>42-69%	3
	>69%	1
Dominant three taxa: proportion of count of individuals in the dominant three taxa to the total sample count	<54%	5
	>54-74%	3
	>74 %	1
Corixidae proportion of beetles and bugs in activity trap samples: count of individual Corixidae to total beetle and bug count	<33%	5
	33-67%	3
	>67%	1
Chironomid genera	14 ->21	5
	7-13	3
	0-6	1
Total number of dragonfly and damselfly genera (Odonata metric)	6-10	5
	4-5	3
	0-3	1
Leech taxa	5-9	5
	3-4	3
	0-2	1
Snail taxa	7-9	5
	4-6	3
	0-3	1
Total number of invertebrate taxa: larval chironomids, caddisflies, mayflies, dragonflies, damselflies, beetles, bugs, leeches, snails, macrocrustaceans, diptera and fingernail clams	>52-77	5
	37-52	3
	<21-36	1
Total possible scoring range for invertebrate IBI		10-50
Range for excellent condition		35-50
Range for moderate condition		23-34
Range for poor condition		10-22

The 10 vegetation metrics showed significant responses to water (chloride and phosphorus) and sediment chemistry (zinc, copper, and nickel). Sensitive species were found to be the strongest and most reliable vegetation metrics.

Sampling Methods: Water and Sediment

Sampling water and sediment chemistry was conducted by MPCA staff. Water analysis was done by the Minnesota Department of Health, and sediment analysis was done under contract with the University of Minnesota Soils Analytical Laboratory. See April 1999 report for sampling methods for sediment and water chemistry.

Lessons Learned

- Vegetation IBIs show great promise for future applications in wetlands biological assessment. The wetland plant community is biologically rich and sensitive to a variety of human disturbances. Data are acquired in a short time.

Table 2: Scoring Criteria for 10 Vegetation Metrics for Large Depressional Wetlands

Metric	Metric Description	Range	Score
Vas genera	Number of vascular genera in the sample	>14 8-14 <8	5 3 1
Nonvas taxa	Number of nonvascular taxa	>1 1 0	5 3 1
Carex cover	Sum of all Carex species combined cover	>2 0.5-2 <0.5	5 3 1
Sensitive species	Taxa whose presence decreased with disturbance	>4 2-4 <2	5 3 1
Tolerant taxa	Proportion of tolerant taxa to total taxa	<25.98 25.98-44.8 >44.8	5 3 1
Grass-like taxa	Number of grass, rush, and sedge species	>6 3-6 <3	5 3 1
Perennials	Count of all perennial species in the sample	>17 6-17 <6	5 3 1
Aquatic guild	Number of aquatic guild species	>8 5-8 <5	5 3 1
Proportion of dominant 3 taxa cover class	Equitability of perennial cover within the sample	<0.35 0.35-0.68 >0.68	5 3 1
Persistent litter	Relative cover class (sum of individual cover class/total sample cover class) taxa with persistent litter	<17.3 % 17.3-34.6% >34.6%	5 3 1

- The invertebrate community is sensitive to many disturbances in wetlands. Responses differ from stream invertebrates because wetlands invertebrates are adapted to daily cycling of dissolved oxygen in wetlands. What may be a "pollution tolerant" invertebrate in streams may be a specialist in the wetland habitat.
- Biological metrics respond to many stressors and to other disturbance factors in the landscape and in physical aspects of the wetlands that can be readily measured.
- After applying appropriate stratifications, we find that wetlands are not chaotic or highly variable. They show clear patterns and predictable responses to human disturbances.

Minnesota: Dakota County Wetland Health Evaluation Project

Contact Daniel Huff
Environmental Education Coordinator

Organization Dakota County Environmental Education Program
4100 220th St. West, Suite 101
Farmington, MN 55024-9539

Phone (651) 480-7734

E-mail daniel.huff@co.dakota.mn.us

Website http://www.extension.umn.edu/county/dakota/Environment wetlands/wetld.html

Purpose

- Evaluate wetland health using biological data gathered by citizen volunteers using approved techniques developed by the Minnesota Pollution Control Agency
- Increase biodiversity in wetlands in urban areas by installing Best Management Practices (BMPs)
- Conduct a public wetland education effort through seminars, workshops, field days, and media

Wetland Type

- Depressional wetlands

Assemblages

- Macroinvertebrates
- Vascular plants

Status

Since 1997, the project has operated every summer. In 2002, the program consisted of 15 monitoring teams representing 19 communities in Dakota and Hennepin Counties.

Project Description

The Dakota County Wetland Health Evaluation Project (WHEP) uses sampling methods and evaluation metrics developed by the Minnesota Pollution Control Agency (MPCA). The project started in 1997 and was conducted by MPCA and Minnesota Audubon. A total of 30 wetlands were monitored by 5 citizen teams representing the Minnesota Zoo, Dakota County, and the cities of Burnsville, Eagan, and Lakeville. MPCA staff trained volunteers in sampling protocols, quality assurance, and plant and macroinvertebrate identification. The sampling methods are a scaled-back version of the Minnesota Pollution Control Agency Wetland Bioassessment Program (see "Minnesota: Developing Wetland Biocriteria" in this module). Each citizen team worked under the direction of a local teacher or nature center staff. The time commitment for volunteers was approximately between 40-50 hours per year, including training, field work, and analysis.

In 1998, the project expanded to eight citizen teams, and a technical consultant was hired to conduct MPCA's full sampling methods to facilitate comparisons. Results of the comparison indicate that the volunteer assessments, although not as rigorous as the professional assessments, provide repeatable results that are consistent with the more detailed assessments. However, the volunteers tend to score the high-quality wetlands too low because they are unable to identify as many organisms (e.g., species of *Carex*) resulting in lower scores for the richness metrics. A detailed description of this project is provided on the web site listed under the contact information (http://www.extension.umn.edu/county/dakota/Environment/wetlands/wetld.html).

In 1999, the funding source changed from EPA grants to funding from the Minnesota State Legislature through the Minnesota Environment and Natural Resources Trust Fund. The Minnesota State Legislature approved continued funding for this project for the period of 1999 to June 2002. For the 1999 season, the project expanded to a total of 10 cities. An additional 11 wetlands were assessed for the first time and 24 previously sampled wetlands were resampled for trend analysis. The invertebrate and vegetation IBI scores were generally consistent for each wetland, although the invertebrate score was slightly lower on average than the vegetation scores. Each team performed a crossheck on a wetland monitored by another team. In addition, the technical consultant field checked 4 of the 35 wetlands sampled by the volunteer teams. The volunteer results were compared to MPCA standard sampling method results and were consistent for most samples.

In 2000, 10 city teams monitored 38 wetlands. Invertebrate scores were generally lower than vegetation scores, a more significant difference than the previous year. Below-normal precipitation for the previous fall, winter, and spring may account for the poor showing of invertebrate populations. Volunteers continued to use MPCA protocols and be trained by the MPCA scientists who developed the protocols. Eight of the 10 teams performed a cross-check on a wetland monitored by another team. Of these, six of the eight sites showed consistent scores for the invertebrate index and seven of the eight sites showed consistent scores for the vegetation index. The technical consultant field-checked 4 of the 38 wetlands sampled by the volunteer teams. In general, the citizen data were consistent with consultants' findings.

In 2001, 10 teams representing 11 cities monitored 41 wetlands. A wet spring may have contributed to higher invertebrate scores than the 2000 monitoring season. Vegetation scores were consistent with previous seasons' scores. Twenty-eight of 39 wetlands sampled for both vegetation and invertebrates were considered to have consistent scores between the 2 indexes. Seven city cross-checks were performed using the invertebrate metrics. Of these seven, five resulted in similar scores. Six city cross-checks were performed using the vegetation metrics. Of these six, four resulted in similar scores. The technical consultant field-checked 3 of the 41 wetlands sampled by the volunteer teams. The consultant's check resulted in identical scores for two of the three volunteer teams and was very similar for the third for the invertebrate metrics and the vegetation metrics. This showed higher consistency between the citizen teams and the professional cross-check than in previous years. In addition to the 10 Dakota County teams, 3 teams within Hennepin County monitored 14 wetlands with funding from U.S. EPA and the Minnehaha Creek Watershed District.

In addition to wetland monitoring, a wetland remediation project was begun in 2001 at one of the wetlands previously monitored by the project. Cedar Pond in Eagan, MN, had scored among the lowest of all county wetlands for both vegetation and invertebrates when sampled by volunteers in 2000. In cooperation with the City of Eagan, a retaining wall surrounding the pond was removed, the slope was regarded, and three zones of native wetland vegetation, emergent, wet meadow, and upland buffer, were planted around the wetland. Three rainwater gardens were constructed and planted to receive and filter runoff from an adjacent city street. WHEP volunteers participated in planting the rainwater gardens and trees along the upland buffer.

Complete reports of the 1999, 2000, and 2001 monitoring season results can be found on the web site given above. Information on the 2001 monitoring season within Hennepin County can be found at http://www.hcd.hennepin.mn.us/whep.html.

In 2002, communities sponsoring teams are providing funding for the project. Nine teams representing 11 cities in Dakota County and 6 teams representing 4 cities and 1 watershed district in Hennepin County are participating in the Project. Results from the 2002 monitoring season for both counties will be available on the web in February 2003. The project is expected to continue.

Minnesota: University of Minnesota's IBI Development

Contact Sue Galatowitsch

Organization University of Minnesota
Department of Horticultural Science
305 Alderman Hall, 1970 Folwell Ave.
St. Paul, MN 55108

Phone (612) 624-3242

E-mail galat001@maroon.tc.umn.edu

Website http://www.hort.agri.umn.edu/mnwet/begin.htm

Purpose

Develop assessment methods to evaluate ecological condition of wetlands

Wetland Type

- Variety

Assemblages

- Amphibians
- Birds
- Fish
- Macroinvertebrates
- Vascular plants

Status

- Analyzing data and writing reports

Project Description

Developing indices of biological integrity (IBIs) for Minnesota was pursued by researchers at the University of Minnesota to enable quality assessments of existing and restored wetlands. Eight series of 15 wetlands (120 sites) were used to develop wetland IBIs. Each series covers a major wetland type in the State and is composed of reference sites (unaltered wetlands in an unimpaired setting), sites surrounded by land use typical of the region, and sites that are highly altered. Plants, birds, fish, invertebrates, and amphibians were surveyed to select the best IBIs for each series.

To identify possible patterns in biological communities that may relate to land use differences, each organismal data set (except amphibians-few organisms encountered during surveys) was explored with TWINSPAN. TWINSPAN organizes data so that the most similar sites (as described by their species) are grouped together as columns on a table, and so that the species with similar habitat affinities (as described by sites where they occur) are grouped together as rows. This TWINSPAN table was used to generate a list of potential indicators for analysis with land-use data. Species or groups of species that appeared preferential to sites with similar land use characteristics were deemed to be potential indicators. Other common ecological measures, such as richness (number of species), were routinely included in the list of potential indicators, as well. Twenty-eight potential indicators identified for this series are listed below. Proportional indicators for animals are calculated as a total of all organisms observed (not a proportion of taxa) unless noted. Absolute abundances for plant species (vegetation) cannot be reliably estimated from cover class data. Importance values, an approximate measure of abundance, were calculated by summing cover class scores (r=0.1, +=0.5, and classes 1-5).

Potential indicators

Amphibians: None

Birds (12): Species richness (BSR); total abundance of all birds (BABU); number of wetland taxa (BWR); proportion of wetland birds (PWB); abundance of brown-headed cowbirds (ABHC); proportion of forest-nesting birds (PFB); number of taxa with large territories (BLTR); proportion of insectivorous birds (PBIN); abundance of marsh and sedge wrens (CIS); abundance of yellow throats, swamp sparrows, and LeConte's sparrows (SSYT); number of open ground nesting species (BOGR); proportion of open ground nesting species (PBOG).

Fish: None

Invertebrates (4): Total abundance (IABU), taxa richness (ISRI), number of snail species (GASR), proportion of snails (PGAS).

Vegetation (12): Species richness (VSR), invasive perennial species importance (IPI), number of Carex species (CAR), importance of Carex species (CAI), number of native herbaceous perennials (HPNR), importance of native herbaceous perennials (HPNI), number of native perennial graminoids (GPNR), importance of native perennial graminoids (GPNI), number of introduced species (INR), importance of introduced species (INI), ratio of graminoids to herbaceous species (VGH), ratio of annuals to perennials (VAP).

Relationship of potential indicators to land-use measures

Values for each potential indicator (PI) were calculated for each site in the series. PI values were correlated with land use data: site alteration score, land use cover at 500 m, 1,000 m, 2,500 m, and 5,000 m radii. For the radii data, six correlations were calculated, one for each land cover category (agriculture, urban, disturbed, forest, grassland, and wetland). For this series a total of 700 relationships were tested. Relationships with Pearson correlation coefficients greater than 0.53 ($p < 0.1$) are worthy of further consideration as indicators of wetland quality. Each of these relationships were plotted to detect if the high coefficients were based on outliers. Those with outliers were not considered significant.

Eighteen of the original 28 potential indicators (64%) were found to have a high correlation to land use. Seven of the bird PIs show a land-use relationship. Birds with large territories (BLTR) are more common to sites with less agriculture and disturbance at the regional scale (2,500-5,000 m). Likewise, regional patterns of urbanization are negatively associated with wetland bird richness (2,500 m). Wetland bird richness is greater on sites with more wetlands at this same scale. Overall bird richness is lower with more surrounding urbanization at most scales. In contrast, most of the seven promising vegetation PIs show stronger land-use relationships at local scales. Introduced species (INI, INR) and annuals (VAP) are more common on sites that are immediately impacted by agriculture and urbanization (site). Four invertebrate PIs will be further considered for indicator development. The richness of snail taxa (GASR) is positively associated with forest and wetland cover from 1,000 to 5,000 m.

Montana: Developing Wetland Bioassessment Protocols To Support Aquatic Life Beneficial Use-Support Determinations

Contact Randall S. Apfelbeck

Organization Montana Department of Environmental Quality
2209 Phoenix Avenue
P.O. Box 200901
Helena, MT 59620-0901

Phone (406) 444-2709

E-mail rapfelbeck@state.mt.us

Website http://www.deq.state.mt.us/ppa/mdm/Wetlands/Lakes&Wetlands_Index.asp

Purpose

Montana's wetland research objectives are to:

- Determine the status and trends in wetland water quality.
- Acquire an understanding of how climate, hydrologic controls, and geomorphic settings influence wetland biological communities for the development of successful biocriteria.
- Develop biological measurements that could be used in developing biocriteria to define the extent and degree of anthropogenic impacts to wetland water quality.
- Develop an integrated assessment of wetland, streams and lakes within a watershed.
- Report on aquatic health at the watershed level through the development of landscape assessment tools.
- Develop rapid field assessment protocols to evaluate aquatic ecological conditions by using indicators and best professional judgement.

Wetland Type

- Variety of wetland types

Assemblages

- Algae
- Macroinvertebrates
- Vegetation
- Amphibians and aquatic reptiles

Status

- Ongoing, revising analytical methods

Project Descriptions

1992

Montana Department of Environmental Quality (DEQ) began developing wetland biological criteria. At that time, there was little information concerning the status or trends of the water quality of Montana's wetlands. Furthermore, Montana's water quality standards were developed to protect the beneficial uses (e.g., aquatic life) of lakes; river and stream wetlands were not considered State waters when Montana's water quality standards were developed. Since 1992, Montana has had an ongoing program to develop bioassessment protocols and water quality standards that will more adequately evaluate and protect the aquatic life that live in wetlands.

In 1998, as a result of a TMDL lawsuit, Montana DEQ shifted focus from development of biological criteria to the development of guidelines for making beneficial use decisions that apply to all Montana waterbodies. Montana's guidelines for making beneficial use decisions can be found at http://nris.state.mt.us/wis/tmdlapp/pdf2002/Appendix_A.pdf .

1998

In conjunction with Montana DEQ's research program, Montana State University (MSU) designed a study that focused on development of vegetation biocriteria for western Montana depressional wetlands (Borth 1998). The focus on vegetation biocriteria is key in Montana because wetland vegetation is easier to assess than macroinvertebrates or diatoms for depressional wetlands that are seasonally dry. The MSU study sampled vegetation and also macroinvertebrates and diatoms for 24 depressional wetlands with similar climate, hydrology, and water chemistry. The research included sampling across three levels of human disturbances—minimally impacted, slightly impacted, and moderately impacted. The study also involved two anthropogenic impairments—dryland agriculture and grazing.

2000

Researchers from the University of Montana conducted a study to determine the effects of natural variability on the use of macroinvertebrates as bioindicators of disturbance in intermontane depressional wetlands in northwestern Montana (Ludden 2000). Their study design included collection of macroinvertebrate samples and physiochemical data from 15 pristine and 6 disturbed intermontane prairie potholes. Their study also included analysis of macroinvertebrate samples that were previously collected by Borth (1998). The researchers collected macroinvertebrate samples from across three seasons and from three wetland zones. They used multivariate detrended correspondence analysis to ordinate the raw macroinvertebrate data and physiochemical variables as secondary matrices to establish vectored biplots of correlation. Candidate metrics were analyzed using univariate analysis. They determined that no environmental variables were strongly correlated with the macroinvertebrate data, and many of the metrics varied across wetland zones and across seasons. Nevertheless, 35 candidate metrics were able to discriminate between minimally and highly disturbed sites. These wetlands were also sampled intensively to develop and test a model for assessing depressional wetlands using the hydrogeomorphic (HGM) functional assessment approach.

2002

Montana DEQ initiated the development of a comprehensive watershed monitoring and assessment program. The comprehensive program will be developed to determine the causes, effects, and extent of pollution to aquatic resources (including wetlands) and for developing pollution prevention, reduction, and elimination strategies.

The comprehensive program has three components: landscape, site-specific, and rapid assessment. First, a landscape-level process to evaluate and rank wetlands and watersheds for protection and restoration is critical to maximize the use of limited financial and management resources. This process will use existing digital data and evaluate a range of landscape impacts using a Geographic Information System. Second, Montana will continue to develop site-specific assessment protocols such as biocriteria (i.e., for assessing amphibians, vegetation, algae, and macroinvertebrate assemblages). This information is important for managers of Montana's water resources for making informed watershed management decisions. Development of site-specific wetland biological, physical, and chemical assessment tools will be used to evaluate aquatic life beneficial use support to determine impairment as per section 303(d) of the Clean Water Act. Third, like most States, Montana has very limited resources for assessing wetland water quality and aquatic conditions for 305(b) purposes. For this reason, Montana will develop an assessment approach that is truly rapid and cost effective. A rapid assessment component will be developed based upon field data collected, tested and refined during this study, best professional judgment and information gathered from a literature search.

The comprehensive watershed monitoring and assessment program will include a probabilistic study design to assess the ecological condition of depressional and riverine wetlands and streams. Three pilot subbasins (fourth code HUCs) will be selected. The pilot subbasins will represent Rocky Mountain, Intermountain Valleys and Prairie Foothills, and Plains ecoregions (Figure 11).

Within each pilot subbasin, three sixth-code HUCs (fifth-code HUCs for the Middle Milk subbasin) will be randomly selected from each of three disturbance strata (high, medium, and low) determined from landscape-scale assessments. For vegetation, soils, water chemistry, macroinvertebrates, diatoms, and land use, a total of six wetlands (three riverine and three depressional) will be monitored in each watershed. A total of 27 watersheds (9 in each subbasin) and 162 wetlands (3 riverine and three depressional in each watershed) will be studied. For the determination of the detection/nondetection of amphibians and aquatic reptiles, all standing water bodies identified in each watershed will be surveyed.

Note: the remainder of this case study will describe in more detail the study initiated in 1992.

Study Design

The original Montana DEQ study was designed in 1992 and involved sampling 80 wetlands throughout Montana during 1993 and 1994. The bioassessment project included collection of macroinvertebrate and diatom samples from wetlands in all ecoregions of Montana (Apfelbeck 2001).

Montana DEQ's approach to developing biocriteria involved several study designs aimed at developing tools to help detect human influence on wetland water quality. The original study was designed in 1992 and involved sampling 80 wetlands

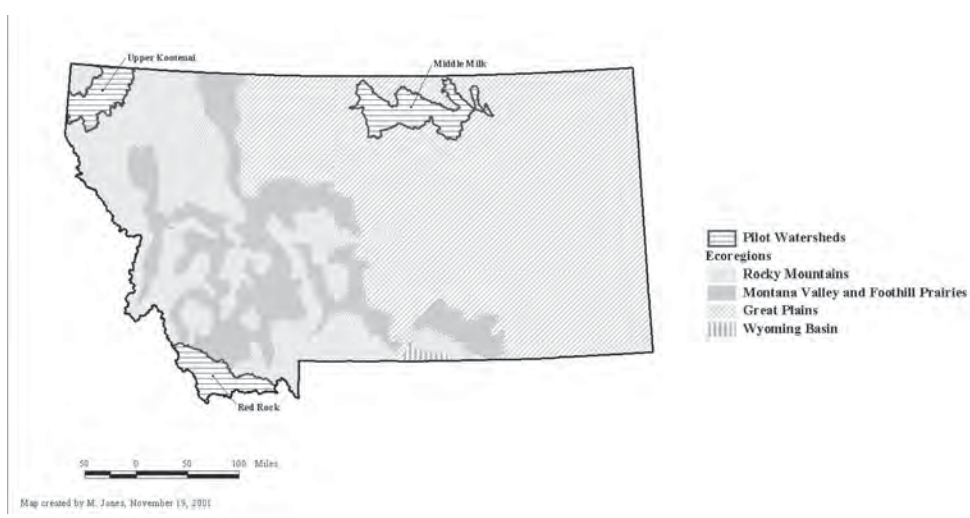


Figure 11: Montana ecoregions and pilot watersheds.

throughout Montana during 1993 and 1994 (Figure 12). The study design included collection of samples that represent the wetland's macroinvertebrate (e.g., aquatic insects) and diatom (algae) communities. Sampling methods were designed so that 1-2 hours was sufficient for the data to be collected in the field for each wetland. Samples of each wetland's water column, sediment, and macroinvertebrate and diatom communities were collected. Water-column and sediment samples were collected to document impairments and for classification purposes.

Ecoregions of Montana

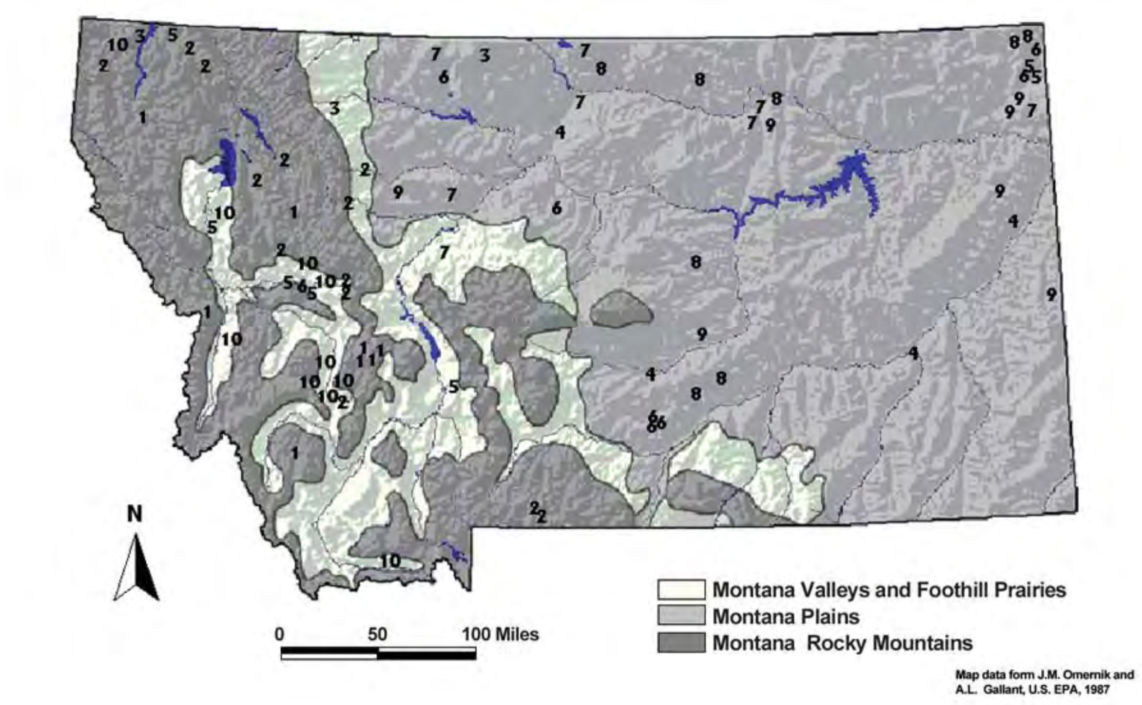


Figure 12: Ecoregions and sampling locations by wetland class.

Wetland Classes: (referred to by number in the above figure)

Class 1 Dilute Closed Basins and Headwater Wetlands of the Rocky Mountain Ecoregion
Class 2 Riparian Wetlands of the Rocky Mountain amd Intermountain Valley Ecoregions
Class 3 Groundwater Recharge Closed Basin Wetlands
Class 4 Riparian Wetlands of the Plains Ecoregion
Class 5 Alkaline Closed Basin Wetlands
Class 6 Saline Wetlands
Class 7 Surface Water Supported Closed Basin Wetlands
Class 8 Ephemeral Wetlands
Class 9 Open Lake Wetlands of the Plains Ecoregion
Class 10 Open Lake Wetlands of the Rocky Mountain and Intermountain Valley Ecoregions

The sites were classified using ecoregions and hydrogeomorphology, and several of the wetland classes were further delineated using water-column chemistry variables. A representative number of wetlands from the following Omernik ecoregions were sampled: Rocky Mountains, Intermountain Valleys and Prairie Foothills, Glaciated Plains, and Unglaciated Plains Ecoregions. To reduce seasonality, all wetlands within the same ecoregion were sampled during similar time periods. Wetlands in the Glaciated Plains Ecoregion were sampled from early April through mid-June, wetlands of the Intermountain Valleys and Prairie Foothills Ecoregion from mid-June until early August, and wetlands of the Rocky Mountain Ecoregion from early July through September.

The classification framework was developed by sampling the full spectrum of wetland types in Montana. The study was designed such that approximately 75% of the sites were reference and 25% were impaired. This approach was useful because it allowed Montana DEQ to determine the reference condition of a wide variety of wetland types. Also, the design provided the opportunity to test the ability of the biological measurements to detect water quality impairment.

Anthropogenic impacts such as irrigation or logging were included in the study design. If anthropogenic activities such as dryland agriculture, irrigation, feedlots, grazing, silviculture, road construction, hydrologic manipulation, urban runoff, wastewater, mining, and oil and natural gas production occurred in the wetland's watershed, the wetland was considered impaired. Wetlands for sampling were selected on the basis of many variables, including availability of historical data, special interests by other entities, cooperation by landowners, and accessibility.

In order to classify or document impairment, a hydrogeologist for the Montana Natural Heritage Program (MNHP) assisted Montana DEQ in developing a wetland classification system through summarizing and interpreting the physical and chemical data. Using topographic maps, field observations, and information gathered from land management agencies, geomorphic characteristics were interpreted and a hydrogeomorphic database developed. Maps for each wetland were completed using a Geographic Information System (GIS). Map features included hydrologic delineations, land management areas, counties, cities, major transportation corridors, wetland watershed boundaries, and sampling locations. Visit Montana DEQ's website to get more detail on the types of wetland classes and for photographs of each type: http://www.deq.state.mt.us/ppa/mdm/Wetlands/classification.asp.

Sampling Methods: Diatoms

Montana DEQ collected diatoms as composite grab samples. The algae were identified to the lowest taxonomic level possible. Samples were collected using a 250-mL plastic container and then preserved with Lugol's solution. Samples were collected from a location determined to best represent the wetland. These locations were restricted to areas that were easily accessible when wearing hip boots. Sampling was done from April through September. Each site was sampled once.

Analytical Methods: Diatoms

The multivariate approach was used to analyze wetland diatom communities. Multivariate analysis is a statistical approach used by biologists to determine relationships among biota such as diatoms or macroinvertebrates, and environmental variables such as water-column chemistry. The multivariate approach to investigate relationships between Montana wetland diatom assemblages and environmental variables (mostly water-column chemistry) was detrended canonical correspondence analysis (DCCA) and two-way indicator analysis

(TWINSPAN). Clusters of diatoms with similar composition were graphically displayed using DCCA. Vectors were displayed and labeled to illustrate the relationship between diatom assemblages and environmental variables. Longer vectors show us a stronger correlation among diatom assemblages and environmental variables (Figure 13). Envelopes were used to graphically enclose all reference sites using the wetland class delineations (Figure 14). The Academy of Natural Sciences of Philadelphia (ANSP) performed the subsampling, digestion, and mounting of the diatoms (see Charles et al. 1996).

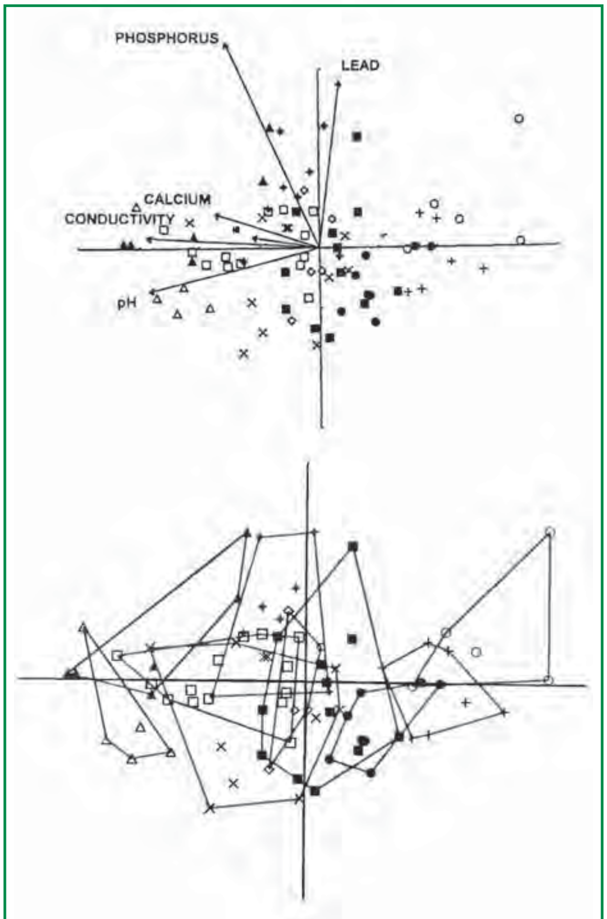


FIGURE 13: DIATOM DATA RELATED TO ABIOTIC FACTORS USING DETRENDED CANONICAL CORRESPONDENCE ANALYSIS.

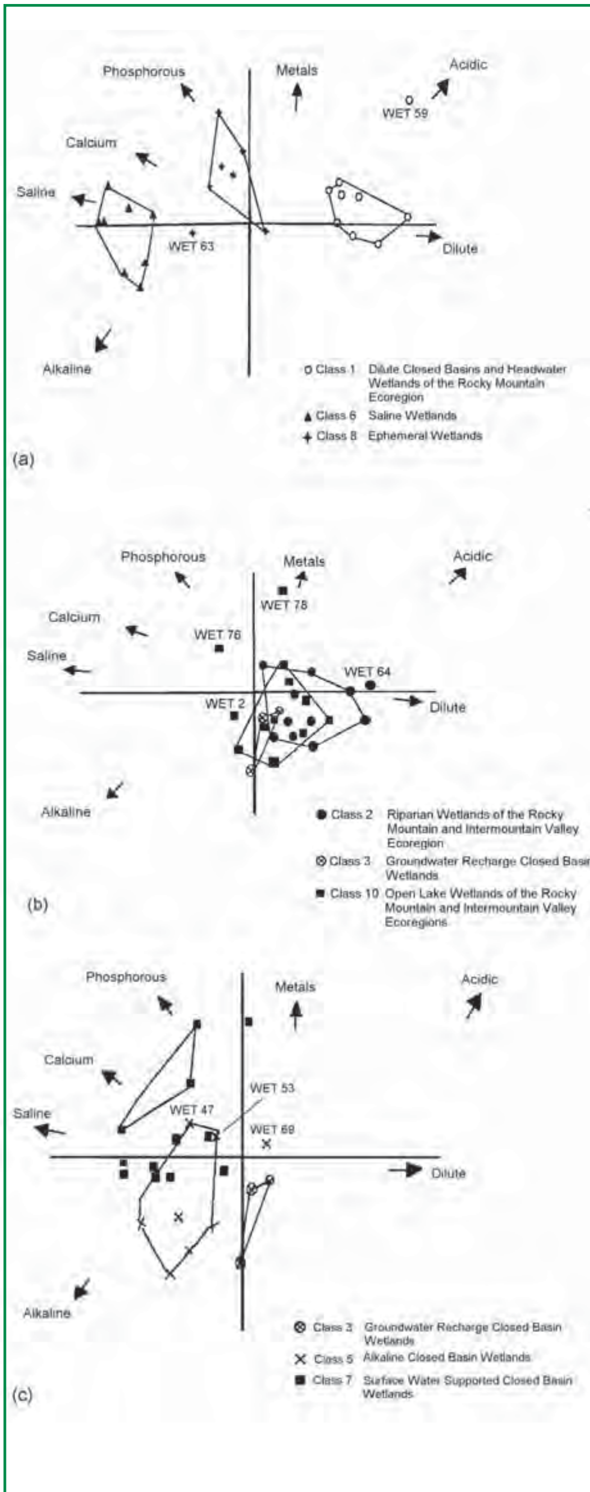


FIGURE 14: CLUSTERS OF WETLANDS BASED ON DIATOM DATA.

Sampling Methods: Macroinvertebrates

Montana DEQ collected macroinvertebrates using a 1-mm mesh D-net in a sweeping motion. Macroinvertebrates were collected from all microenvironments in a sampling location. These locations were restricted to areas that were easily accessible when wearing hip boots and that best represented the wetland. Samples were composited with associated materials such as vegetation and sediment and then preserved with ethanol in a 1-L plastic container. An effort was made to collect 300 organisms from each location using a consistent method of collection. To ensure preservation, sample bottles were refreshed with ethanol several days after collection. Sampling was done once per site from April through September.

Analytical Methods: Macroinvertebrates

The multimetric approach was used to evaluate wetland macroinvertebrate communities. The multimetric approach incorporates many attributes into the assessment process and has the ability to integrate information from the biological communities to provide an overall indication of biological condition or ecological health.

The project contractor assisted Montana DEQ in developing wetland macroinvertebrate multimetric indices. The proposed metrics and associated environmental data were evaluated in an attempt to develop an understanding of ecological relationships, to test each proposed metric's ability to predict various anthropogenic stressors, and to test redundancy. Table 3 lists some of the proposed metrics used for macroinvertebrates and Figure 15 shows macroinvertebrate index values for several classes of wetlands.

The macroinvertebrate samples are subsampled and sorted by contractors using a gridded sorting pan. A subsampling of 200 organisms is performed for analysis. All individuals are counted when there are less than 200 organisms in the sample. Organisms are identified to the lowest taxonomic level (at least genus if possible). The taxonomic level of identification is standardized using the Montana Stream when possible. Amphipoda are identified by species. Common, easily identified midge taxa are identified using a dissecting microscope equipped with 25× oculars. Midge taxa that require slide mounting are cleaned with warm water solution of 10% KOX, rinsed in distilled water followed by 95% ethanol, and mounted on slides in Euperal. If fewer than 30 individuals are to be mounted, all individuals are mounted. If there are more than 30 midge larvae, at least 10% of each morphotype are mounted. Mounted midges are identified using a Zeiss Axiolab phase contrast compound microscope or equivalent.

The contractor identified the organisms in the wetland samples and standardized the taxonomic level of identification based on Montana Stream Protocols. Several taxa were eliminated from consideration for metric development, as they were determined to be nonbenthic taxa or semiaquatic surface dwellers and considered uninformative for reflecting water quality. These taxa included Gerridae, Collembola, Dytiscidae, Hydrophilidae, Ostracoda, Anostraca, Copepoda, Cladocera, Notonectidae, and Corixidae.

Other Parameters: Water Chemistry and Sediment

Water-column and sediment samples were collected to document impairments and for classification purposes. Each sample was collected from a location determined to best represent the wetland. These locations were restricted to areas that were easily accessible when wearing hip boots. Field chemical measurements, observations, and photographs were recorded at each location.

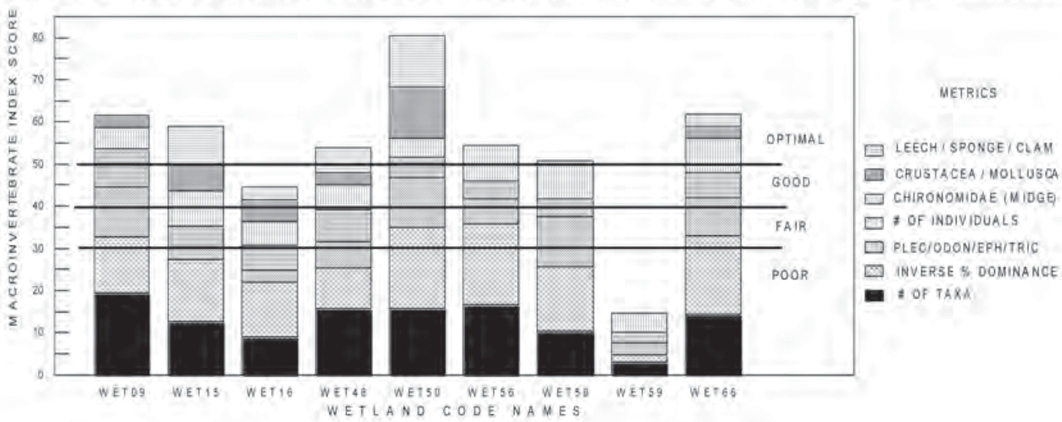
Table 3: Proposed metrics, proposed metric calculations, and score calculations used for developing wetland macroinvertebrate indices

Proposed Metrics	Theorized Direction of Change in Presence of Stressor	Proposed Metric Calculation	Score Calculation
Number of taxa	Decrease	Count taxa	(number of taxa) × 0.75
Percent dominance Percent 1 dominant taxon Percent 2 dominant taxa Percent 5 dominant taxa	Increase Increase Increase Increase	Average of percent 1, 2, and 5 most dominant taxa	(100% dominance) × 0.36
POET	Decrease	Count Plecoptera, Odonata, Ephemeroptera, and Tricoptera taxa	(POET) × 3
Number of individuals	Decrease	Count individuals in total sample (maximum count of 300)	(number of individuals) ÷ 33
Chironomidae Number of chironomidae taxa Percent chironomidae taxa Percent Orthocladiinae/ Chironomidae	Decrease Decrease Increase Decrease	Number of Chir taxa × (100% chir + 50) × (((% orthocladiinae to total Chir) ÷ 100) +0.5)	(Chironomidae) ÷ 83
Crustacea/Mollusca Number of crustacea & mollusca taxa Percent crustacea & mollusca taxa	Decrease Decrease Increase	Number of crustacea and mollusca taxa × (100% crustacea/mollusca taxa + 50)	(crustacea/mollusca) ÷ 33
Leech/Sponge/Clam	Decrease	Count Hirudinea, Porifera, and Sphaeriidea taxa	(leech/sponge/clam) × 3

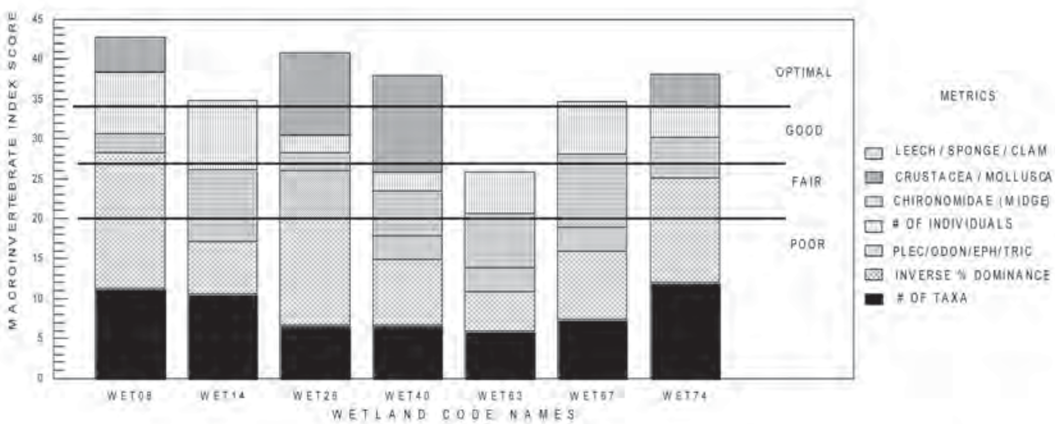
Lessons Learned

- We found that diatoms and macroinvertebrates were most useful for evaluating the biological integrity of perennial wetlands with open-water environments that had relatively stable water levels and were not excessively alkaline or saline.
- We concluded that multivariate analysis was a useful tool for developing a wetland classification system and that hydrogeomorphology and ecoregions were practical approaches to classifying wetlands for the development of biocriteria.
- We determined that both the multimetric and multivariate techniques were valuable for developing wetland biocriteria.
- In most cases, the multimetric and multivariate approaches that we used to assess the macroinvertebrate and diatom communities both identified the same wetlands as impaired.
- Two wetland types in the arid west (including Montana) are difficult to classify. Wetlands such as potholes are highly complex and difficult to classify because of both spatial and temporal variability. For these wetlands, the hydrology, water chemistry, and biology can change dra-

Class 1. Dilute closed basins and headwater wetlands of the Rocky Mountain Ecoregion



Class 8. Ephemeral wetlands



Class 10. Open lake wetlands of the Rocky Mountain and Intermountain Valley ecoregions

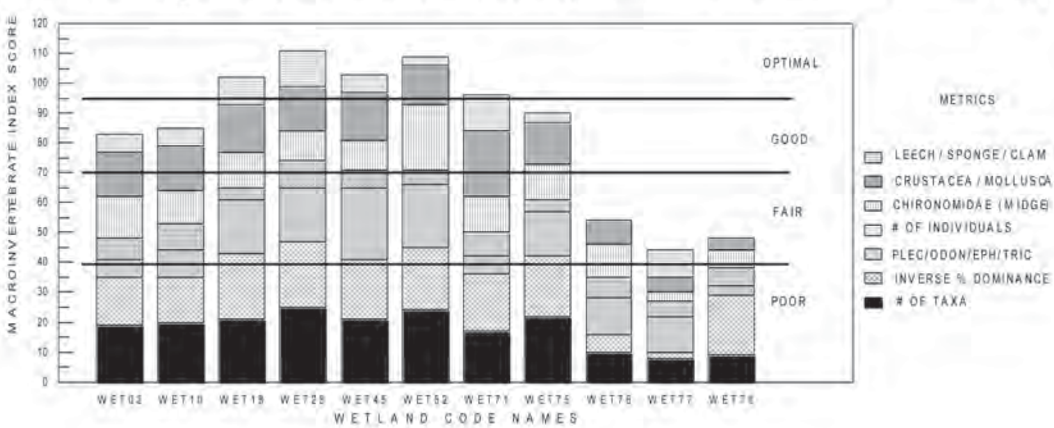


Figure 15: Macroinvertebrate index scores for wetlands in several classes.

matically throughout a season or from year to year as a result of climatic change. For example, the biological community of a wetland often changes through an increase in salinity or a decrease in water content caused by drought.

- Aquatic macroinvertebrate and diatom biocriteria did not appear to be very useful for detecting impairment in wetlands that usually lack surface water. Vegetation biocriteria are likely to be the most appropriate for assessing the biological conditions of these wetland types.

- A more quantitative approach is needed for the measurement of wetland physical disturbances if we are to link physical disturbances to changes in biological communities.

Note: Montana DEQ has developed a set of proposed metrics, proposed metrics calculations, and score calculations used for developing wetland macroinvertebrate indices. These are included in Table 3.

North Dakota: Wetland Bioassessment Protocols for Making Aquatic Life Beneficial Use-Support Determinations

Contact Mike Ell

Organization North Dakota Department of Health
Division of Water Quality
1200 Missouri Avenue
P.O. Box 5520
Bismarck, ND 04333

Phone (701) 328–5214

E-mail mell@state.nd.us

Purpose

The primary purpose of North Dakota's wetland bioassessment program is to develop wetland water quality standards for North Dakota. This currently involves developing biological community metrics and an Index of Biological Integrity (IBI) for temporary and seasonal depressional wetlands. A secondary project goal is to compare the IBI with hydrogeomorphic (HGM) functional assessments.

Wetland Type

- Depressional wetlands

Assemblages

- Algae
- Macroinvertebrates
- Vascular plants

Status

- Analyzing data, developing IBIs, and expanding project

Project Description

North Dakota Department of Health (NDDH) initiated its wetland bioassessment program in 1993 as a component of the State's strategy to develop wetland water quality standards. NDDH began field sampling in 1995 and is currently analyzing data collected from 1995 through 1999 to develop assemblage metrics, IBIs for the assemblages, and ultimately biocriteria. The NDDH wetland sampling plan includes vascular plants, macroinvertebrates, and algal assemblages.

Development of a North Dakota sampling protocol for the State's wetland types is ongoing through cooperative work agreements with North Dakota State University, Departments of Animal and Range Sciences and Zoology.

Study Design

North Dakota's study design has continued to expand in scope since the project was initiated. Initial field sampling began in 1995 and 1996 with 13 "least disturbed" temporary and seasonal wetlands. In 1997, the number and range of wetlands was expanded to 20 temporary and seasonal wetlands. Four of the original 13 "reference" wetlands sampled in 1995 and 1996 were sampled in 1997 along with 16 new wetlands. These wetlands captured a larger range of disturbance categories.

In 1998, the number of wetlands again expanded. An additional 16 temporary and seasonal wetlands were added, bringing the total number of wetlands sampled to 36 in 1998 and the total number of reference wetlands to 6. The 16 wetlands added in 1998 were part of an HGM classification project. These wetlands were added to facilitate a comparison of the two assessment methods. In addition to the 16 HGM wetlands, NRCS personnel conducted an HGM assessment for the 20 wetlands selected by the Department.

During the 1999 sampling season, a second set of 30 temporary and seasonal depressional wetlands, not sampled previously, were sampled for vascular plants. Macroinvertebrates and algae were not sampled in 1999.

Since the inception of the project, temporary and seasonal wetlands have been the focus of IBI development efforts. Further, all wetland study sites have been within the Northern Glaciated Plains and Northwestern Glaciated Plains ecoregions (Figure 16), a region commonly referred to as the "Prairie Pothole Region."

Sampling Methods: Macroinvertebrates

NDDH currently uses only the "sweep" or jab net method for macroinvertebrate sampling; however, in the past, activity traps have also been used. Sampling consists of two site visits in June. During each site visit two samples are collected with the emergent vegetative zone.

Sweep net

The method employs a "D" frame net (0.6-mm mesh size). At each site within the wetland, the substrate (i.e., wetland vegetation and benthos) is "jabbed" for a distance of 1 m. The disturbed area is then swept two more times to ensure a representative sample of macroinvertebrates is collected. Each sample collected within the wetland will be placed in a shallow pan where any excess debris or water can be removed. The "cleaned" sample is placed in a jar a preserved with buffered formalin to a concentration of 10% by volume.

Analytical Methods: Macroinvertebrates

In the laboratory each sample is washed through a 0.6-mm sieve to remove the preservative and cleaned to remove excess debris. The cleaned sample is placed in a shallow white pan divided in quadrants of equal size. Because each sample typically contains a large number of organisms, it is necessary to subsample. A subsample is obtained by randomly selecting a sample quadrant and counting and identifying the organisms in that quadrant. Additional quadrants are selected at random and organisms counted and identified until a subsample of 300 organisms is counted. For samples with less than 300 organisms, every quadrant is sampled. All organisms are identified to the lowest taxonomic level practical.

Sampling Methods: Algae (Phytoplankton)

Phytoplankton samples are collected just below the water's surface in the middle or deepest area of the wetland basin. Each wetland is sampled two times in June at the same time macroinvertebrate samples are collected. The sample is collected by submerging a clean 200-mL sample bottle just below the water's surface and filling it. The sample is preserved in the field with M3 fixative to a concentration of 2% by volume.

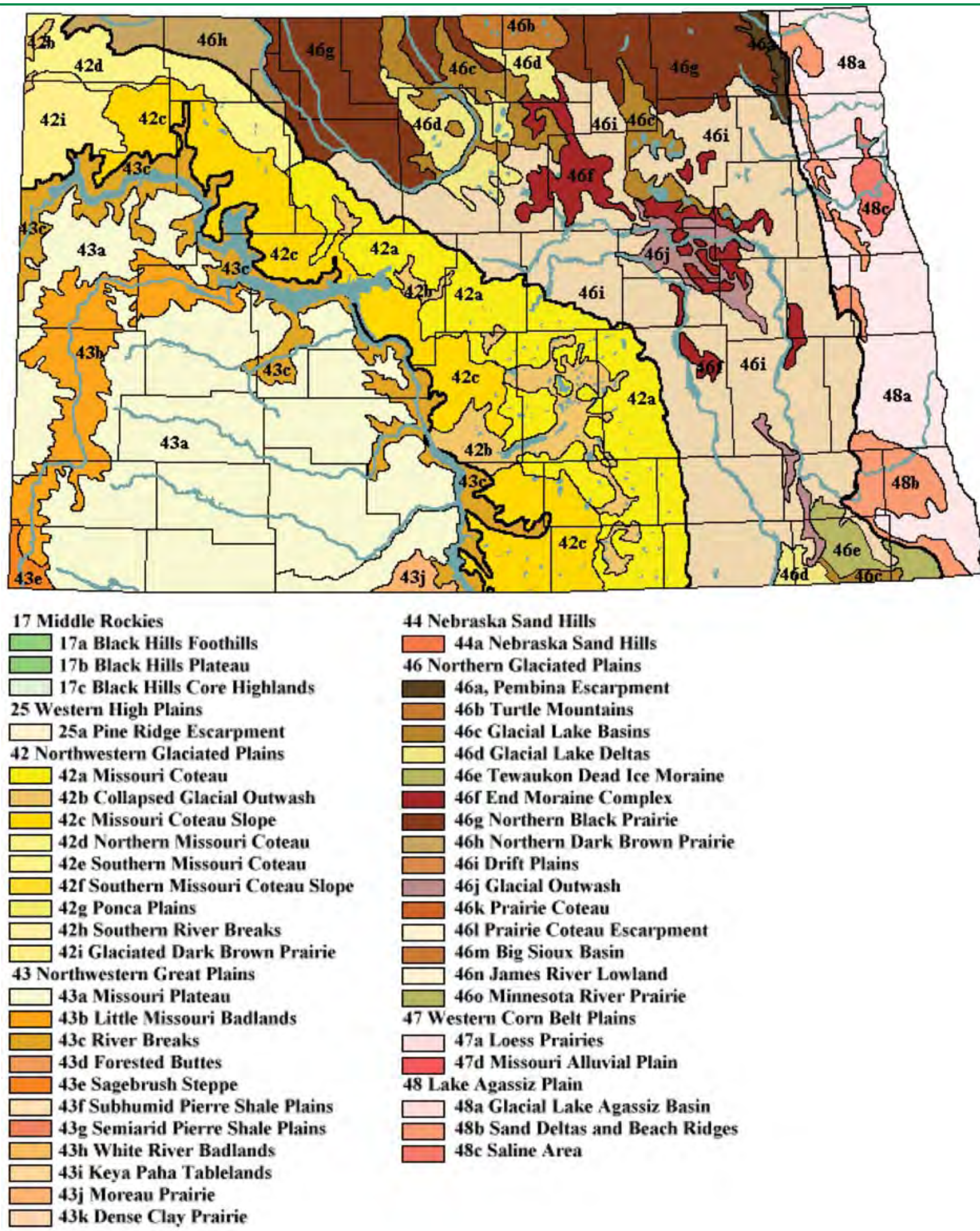


Figure 16: Level IV ecoregions of North Dakota.

Analytical Methods: Algae (Phytoplankton)

Identification and enumeration of phytoplankton is made using the phase-contrast inverted microscope method (Utermohl 1958). Sample analysis is conducted from a sample aliquot ranging from 2 to 15 mL, depending on turbidity. Phytoplankton greater than 20 micrometers in diameter are enumerated at ×125 magnification. (Note: At ×125 the entire bottom of the chamber is in view.) Following enumeration at ×125 smaller algae are counted at ×1,250. At least 250 cells of the most numerous algae are counted using the strip count method at ×1,250 magnification (APHA 1989). Cell volumes are estimated for dominant taxa by measuring cell dimensions of 50 to 100 individuals using the closest geometric formulas of Wetzel and Likens (1991) and Tikkanen (1986). For rare taxa, volume estimates are made from fewer than 50 cell measurements. Diatoms will be identified after clearing in 30% hydrogen peroxide and mounting in Hyrax Mounting Medium.

Sampling Methods: Vascular Plants

Working in cooperation with NDSU, the Department is evaluating three methods to sample the vascular plant community. The first method is more qualitative in nature. It involves a simple inventory of plant species present within each wetland zone, low prairie, wet meadow, and shallow marsh. The other two methods, termed the point and quadrat methods, are quantitative. The inventory is conducted each time the wetland is visited. The point and quadrat methods are done once each, usually in July or August.

In the point method, 200 points are evenly stratified in each wetland zone and the nearest plant species recorded. The quadrat method involves placing 15 1-m^2 quadrats evenly throughout each wetland zone. Each species is recorded within the quadrat and a Daubenmire cover class is recorded. With both methods, a secondary species list is made for species encountered, but not sampled.

Note: For specific details on abiotic sampling methods for nutrients, trace elements, general water parameters, and sediment chemistry, directly refer to North Dakota Department of Health.

Lessons Learned

NDDH has made a great deal of progress within the past 5 years. Our experience, however, has not been without problems and mistakes. As mentioned previously, the first 2 years of the program focused solely on "least impaired" or reference condition wetlands. Although beneficial in testing sampling methods, the lack of a disturbance gradient in the study design did allow for the testing of attributes and the selection of metrics. Therefore, beginning in 1997 the NDDH chose wetlands across a disturbance gradient, including both reference wetlands and degraded wetlands.

NDDH has also found it beneficial to stratify wetlands based on ecoregion and wetland class. This minimizes the amount of variation in the biological assemblage and allows more sensitivity in the response of the metrics to the disturbance gradient. Current IBI development efforts are focusing on temporary and seasonal depressional wetlands within the Northern Glaciated Plains and Northwestern Glaciated Plains ecoregions. In 2000 the NDDH will be cooperating in two projects to develop wetland IBIs for semipermanent depressional wetlands and for floodplain wetlands along the Missouri River.

Ohio: Developing IBI Assessment Methods for Water Quality Standards and Regulatory Decisions

Contact John J. Mack

Organization Ohio EPA
Division of Surface Water
122 South Front Street
P.O. Box 1049
Columbus, OH 43216-1049

Phone (614) 644-3076

E-mail john.mack@epa.state.oh.us

Purpose

- Test and develop biological criteria for wetlands using vascular plants, macroinvertebrates, and amphibians as indices of biological integrity (IBIs) for eventual adoption into the State's water quality standards
- Use results from IBIs to calibrate the Ohio Rapid Assessment Method for Wetlands to support regulatory decisionmaking under the State's wetland antidegradation rule, which requires that wetlands be assigned to one of three categories based on the wetland's quality and functionality.

Wetland Type

- Depressional wetlands

Assemblages

- Algae
- Macroinvertebrates
- Vascular plants

Status

- Refining metrics and developing IBIs

Project Description

The initial objective of this study is to provide the reference data needed to implement the wetland water quality standards and wetland antidegradation rule. The pilot metrics developed from this study should enable Ohio wetlands to be assigned to one of the three regulatory categories. Generally, the study objectives are as follows:

1. Develop pilot biological metrics that may be used to evaluate the function and ecological integrity of a wetland. These metrics will be based on the vegetation, macroinvertebrate, and amphibian data, and will form the basis for wetland biocriteria.
2. Identify and describe reference wetlands in Ohio's four main ecoregions; Eastern Cornbelt Plains, Erie/Ontario Drift and Lake Plain, Huron-Erie Lake Plain, and Western Allegheny Plateau. These reference wetlands will be used to develop biocriteria and also as "goals" for wetland mitigation projects.

3. Continue to assess whether the Ohio Rapid Assessment Method correlates well with the more in-depth measures of wetland quality, and to test and refine breakpoints between the wetland categories.
4. Begin to assess the sensitivity of different methods in evaluating the relationship between wetland quality and the degree of disturbance

The State of Ohio has well-developed biological criteria (or biocriteria) for streams, such as the Invertebrate Community Index (macroinvertebrates), the Index of Biological Integrity (fish), and the Modified Index of Well Being (fish) (Ohio EPA 1988a,b and 1989). These indices are codified in Ohio Administrative Code Chapter 3745-1. Until recently, however, surface waters of the State that are jurisdictional wetlands were only generically protected under Ohio's water quality standards.

On May 1, 1998, the State of Ohio adopted wetland water quality standards and a wetland antidegradation rule. These wetland quality standards developed narrative criteria for wetlands and created the "wetland designated use." All jurisdictional wetlands are assigned the "wetland designated

Ecoregions of Ohio and Indiana and Neighboring States**

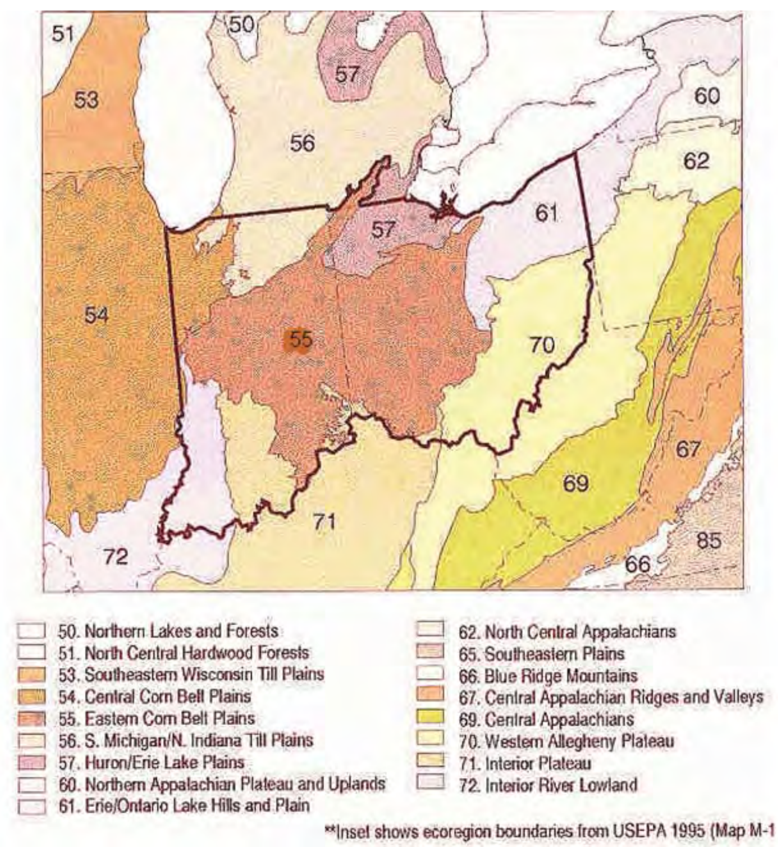


Figure 17: Ecological regions of Ohio and Indiana (from U.S. EPA 1995).

use." The State of Ohio did not attempt at this time to identify multiple wetland *functions* as wetland *uses*, because of the lack of data to develop quantitative water quality criteria for wetlands. However, the development of such biocriteria is the ultimate goal and the primary thrust of this project.

The key part of Ohio's current regulatory program for wetlands is found in the wetland antidegradation rule. The wetland antidegradation rule categorizes wetlands based on their functions, sensitivity to disturbance, rarity, and irreplaceability, and scales the strictness of avoidance, minimization, and mitigation to a wetland's category. Three categories were established: Category 1 wetlands with minimal wetland function and/or integrity; Category 2 wetlands with moderate wetland function and/or integrity; and Category 3 wetlands with superior wetland function and/or integrity. In order to implement the wetland standards and antidegradation policy, wetlands must be assessed on their relative quality. Ohio EPA has developed a draft Ohio Rapid Assessment Method. The Ohio Rapid Assessment Method has proved to be a fast, easy-to-use procedure for distinguishing between wetlands of differing quality. However, it does not and was not intended to substitute for direct, quantitative measures of wetland function (i.e., biocriteria).

Ohio began development of sampling methodologies and began sampling reference wetlands for biocriteria development in 1996. To date, Ohio has sampled 56 wetlands located primarily in the Eastern Cornbelt Plains Ecoregion located in central and western Ohio. These wetlands have included depressional emergent, forested, and scrub-shrub wetlands; floodplain wetlands; fens; kettle lakes; and seep wetlands. The wetlands being studied span the range of condition from "impacted" (i.e., those that have sustained a relatively high level of disturbance) to "least-impaired" (i.e., the best quality sites available).

On the basis of results to date (see Fennessy et al. 1998a,b; Mack et al. unpublished data), Ohio's research supports the use of vascular plants, macroinvertebrates and/or amphibians as biological metrics in wetlands, and also the continued use and development of the Ohio Rapid Assessment Method as a rapid assessment tool.

This work has been funded since 1996 by several EPA Region 5 Wetland Program Development Grants.

Study Design

Fifty-seven wetlands were sampled during the 1996, 1997, 1998, and 1999 field seasons. The first 2 years of data laid the groundwork for standardizing sampling methodologies, classifying wetlands, identifying potential attributes, and developing metrics using vascular plants, amphibians, and macroinvertebrates.

In 1996, Ohio EPA monitored a series of riparian forested across a gradient of disturbance (i.e., least impacted to impaired) (Fennessy et al. 1998b). Estimates of the relative level of disturbance were made on a scale of 1 (most disturbed) to 10 (least disturbed) based on visual evidence of disturbances, review of aerial photographs of the wetland and the surrounding area, and interviews with staff from the Natural Resource Conservation Service and/or the landowner. In 1996 and 1997, Ohio EPA monitored 21 forested and emergent depressional wetlands. Relative disturbance was evaluated using a tiered flow chart to assign a relative disturbance score and also with the score from the Ohio Rapid Assessment Method (Fennessy et al. 1998a, Figure 2.2).

Ohio EPA found a good correlation between the scores of the Ohio Rapid Assessment Method score and level of disturbance a wetland site has experienced. Higher ORAM scores correlate well with

Plate 7: Impaired wetland.
Photo: Mick Micacchion

Plate 8: Least-disturbed wetland.
Photo: Mick Micacchion

lower levels of disturbance based on our model, as do lower ORAM scores with disturbed sites. In 1999, the ORAM score of the site was used as a measure of the level of disturbance. So far, this appears to be a highly effective "x-axis" disturbance gradient for the development of IBIs for wetland plants.

Reference wetlands are sites or data sets from sites that typify a class of wetlands within a relatively homogeneous physiographic region. Reference sites should include wetlands that have been degraded or disturbed. Site selection in this study is made using an ecoregional approach and to reflect a gradient of disturbance (i.e., least-impacted to impaired).

Reference sites are selected such that relatively similar proportions of low-, medium-, and high-disturbance sites are sampled. To date, almost all the wetlands studied by Ohio EPA have been located in the Eastern Cornbelt Plains (ECBP) ecoregion. For the years 2000 and 2001 field seasons, Ohio EPA will be studying reference sites in the Erie Ontario Lake Plain (EOLP) ecoregion of northeastern Ohio.

Sampling Methods and Analysis: Macroinvertebrates and Amphibians

Below is a detailed description of the sampling methodology and analysis process used by Ohio EPA for macroinvertebrates and amphibians. Also included are the lessons learned for sampling these assemblages.

Amphibian and macroinvertebrate taxa were selected as potential indicators of wetland condition. On the recommendation of many field professionals, experienced in amphibian monitoring, funnel traps were used. The funnel traps also proved to be extremely effective at sampling wetland macroinvertebrate communities. Therefore, the same sampling protocols are used for both amphibians and macroinvertebrates in Ohio EPA's study.

Funnel trap

The funnel traps used in this project have cylinders constructed of aluminum window screen and funnel ends made from fiberglass window screen. The funnel traps are similar in design to commercially available minnow traps. The cylinders are 18 inches long and 8 inches in diameter. The two funnel ends are attached to the cylinders and begin 8 inches in diameter and taper inward 5 inches to a 1¾-inch opening. A string handle that runs from end to end is attached to the two seams where the cylinder and funnels ends join.

To date, Ohio sampling data have come from depressional wetland systems in the Eastern Corn Belt Plains (ECBP) ecoregion. The Eastern Corn Belt Plains ecoregion spans most of the western half of Ohio and accounts for about 40% of Ohio's land mass. Within the ECBP, depressional study wetlands that demonstrated a gradient of disturbance levels, from least impacted to greatly disturbed, were selected.

Selected wetlands are sampled for amphibians and macroinvertebrates three times during the year (early, middle, and late spring). An early spring sample is collected in the period from late February to late March. This early spring sample run allows sampling of adult Ambystomatid salamanders and early spring macroinvertebrates (e.g., fairy shrimp) that are only present in wetlands for a limited time. Adult salamanders enter wetlands to breed in early spring following the first few warm, rainy nights of late winter and early spring. The actual timing of their arrival in Ohio is highly weather dependent and varies greatly by year and location. A second sample is collected during the month of April in order to collect adult frogs entering wetlands to breed, as well as amphibian larvae already present, and to sample for macroinvertebrates. Mosquito larvae, an important prey item for many predators, are abundant in April in Ohio wetlands. The final sampling is conducted between May 15 and June 15. Salamander larvae and frog tadpoles are collected, in addition to the resident macroinvertebrates. This last sampling run occurs late enough in the breeding cycle to allow collection of larvae from all breeding amphibians. However, it is still early enough that even in drought years temporary wetlands will not have dried up.

Generally, 10 funnel traps are installed at each wetland. Prior to installing the first funnel trap, the perimeter of the area where standing water is present is measured using a hip chain or by pacing. The total perimeter length is then divided by 10, and 10 funnel traps are installed uniformly around the perimeter of the wetland at intervals of 10% of the total perimeter distance. Each funnel trap location is permanently marked with flagging for use throughout the sampling season. The funnel traps are installed on the bottom at a location deep enough to submerge the trap. The traps are left in the wetland 24 hours to ensure unbiased sampling for animals with diurnal and nocturnal activity patterns. The design of the traps allows them to collect any amphibians and macroinvertebrates that swim or crawl into the funnel openings.

Upon retrieval, the traps are emptied by inverting one of the funnels and dumping and shaking the contents into a white sorting pan. Organisms that can be readily identified in the field are counted and recorded in the field notebook and released. The remaining organisms are transferred to a 1-L plastic bottle and preserved with 70% ethanol. The contents of each trap are kept in separate, clearly marked bottles for individual analysis in the laboratory. If large numbers of amphibians are kept for identification in the lab, the samples are transferred to formalin for long-term storage. All organisms collected are identified in the lab using appropriate keys and the results are recorded.

Dipnet

Qualitative collections are made concurrently with funnel trapping at each wetland once during each of the three sampling periods. Qualitative sampling involves the collection of amphibians and macroinvertebrates from all available natural wetland habitat features. This is achieved by using triangular ring frame 30-mesh dipnets and manual picking of substrates with field forceps. The goal is to compile a comprehensive species/taxa list of macroinvertebrates and amphibians at the site. A minimum of 30 minutes is spent collecting the qualitative sample. Sampling continues until the field crew determines that further sampling effort will not produce new taxa. At least one specimen of all taxa collected during the qualitative sampling is preserved in a jar of ethanol for positive identification in the laboratory.

Hester-Dendy artificial substrate sampler

Five Hester-Dendy (HD) artificial substrate samplers were tied to the top of a concrete block and placed in wetlands where they remained submerged for 6 weeks. The samplers were collected and preserved in formalin. All the macroinvertebrates colonizing the samplers were counted and identified.

Analytical Methods: Macroinvertebrates and Amphibians

Macroinvertebrates were identified to genus or species. Amphibians were identified to species except for some small salamander larvae identified to genus. Each funnel trap collection was analyzed individually so that location-specific information was not lost by pooling all samples from a site.

The number of individuals collected in the traps was divided by the number of hours trapped to give a relative abundance consisting of number of individuals per trap hour. Results from the different wetland study sites were examined for faunal differences in distribution and abundance. Analysis of the data for potential biological indicators of human disturbance is under way.

Lessons Learned for Macroinvertebrates and Amphibians

- Funnel traps consistently collected an average of 10 more macroinvertebrate taxa than qualitative sampling using dipnets. Funnel traps were much more effective in sampling amphibians and fish than sampling with dipnets.
- Qualitative sampling collected somewhat more Mollusca and Chironomidae taxa than did funnel traps.
- Funnel traps collected more leech taxa, Hemiptera taxa, Coleoptera taxa, Odonata taxa, and Crustacea taxa than did qualitative sampling.
- Hester-Dendy artificial substrate samplers were ineffective for sampling most wetland macroinvertebrates, except oligochaetes, Chironomidae, and Mollusca.
- A 24-hour sampling period for funnel traps is preferred as it allows for the collection of nocturnal species that are infrequently collected by daytime sampling methods.

A single wetland often has several vegetation classes. Even if only three main classes are identified (forested, scrub-shrub, and emergent), the wetlands included for study can exhibit multiple combinations. For example, of the 20 wetlands studied in Fennessy et al. (1998a), 6 combinations of vegetation classes were found: emergent, emergent/scrub-shrub, forested, forested/emergent, forested/emergent/scrub-shrub, and forested/scrub-shrub. Thus, a sampling method should be flexible enough to account for horizontal and vertical variation in vegetation.

After testing a transect-quadrat method, Ohio EPA has adapted the method used by the North Carolina Vegetation Survey as its standard vegetation sampling method (Peet et al. 1998). This is a flexible, multipurpose sampling method that can be used to sample such diverse communities as grass- and forb-dominated savannahs, dense shrub thickets, forest, and sparsely vegetated rock outcrops, and has been used at more than 3,000 sites over 10 years as part of the North Carolina Vegetation Survey. This method is appropriate for most types of vegetation, flexible in intensity and time commitment, compatible with other data types from other methods, and provides information on species composition across spatial scales. It also addresses the problem that processes affecting vegetation composition differ as spatial scales increase or decrease and that vegetation typically exhibits strong autocorrelation (Peet et al. 1998). Peet et al. (1998) state, “Our solution to the problems of scale and spatial auto-correlation is to adopt a modular approach to plot layout, wherein all measurements are made in plots comprised of one or more 10x10-m quadrats or “modules” (100 m^2 = 1 are = 0.01 hectare). The module size and shape were chosen to provide a convenient building block for larger pots, and because a body of data already exists for plots of some multiple of this size. The square shape is efficient to lay out, ensures the observation is typical for species interactions at that scale of observation, and avoids biases built into methods with distributed quadrats or high perimeter-to-area ratios” (Peet et al. 1998, p. 264). Basically, the method employs a set of 10 modules in a 20×50-m layout. Within the site to be surveyed, these 20×50-m grids are located such that the long axis of the plot is oriented to minimize the environmental heterogeneity within the plot.

Once the plot is laid out, all species within the plot are identified, an aggregate wood stem count is made, and cover is estimated at the 0.1-hectare scale. In addition, four 10×10-m modules are intensively sampled in a series of nested quadrats. Within these “intensive” modules, species cover class values and woody stem tallies are recorded for each module separately and for each nested quadrat separately. In effect then, the method proposed by Peet et al. incorporates use of reléves found in the Braun-Blanquet methodology inasmuch as the length, width, orientation, and location of the modules are qualitatively selected by the investigator on the basis of site characteristics; however, within the modules, standard quantitative floristic and forestry information is recorded, such as density, basal area, cover, etc.

Once the location of the plot or plots has been selected, the primary purpose of the vegetation survey is to obtain a comprehensive list of all vascular plant species growing at a particular wetland at the time of sampling and to characterize the relative dominance of these species at several levels of scale (basically herbaceous, shrub, small tree, and large tree scales, or at 1 m^2, 10 m^2, 100 m^2, and 0.1 ha (1,000 m^2 or 1 are).

All vascular species within the modules are identified to species. Immature plants or plants missing structures (e.g., fruiting bodies, etc.) that cannot be identified to species are identified to genus or family or noted as unknown. Within the intensively sampled modules, percent cover is recorded for each species within modules and nested quadrats. Cover classes suggested by Peet et al. (1998) are used as a faster and more repeatable method for assigning cover values: Class 1 (solitary/few), Class 2 (0 to 1%), Class 3 (1% to 2%), Class 4 (2%-5%), Class 5 (5%-10%), Class 6 (10%-25%), Class 7 (25%-50%), Class 8 (50%-75%), Class 9 (75%-95%), Class 10 (95%-99%). The midpoints of the cover classes are used to calculate percent cover, relative cover, etc.

Woody stem data (trees, shrubs, and woody lianas reaching breast height or 1.4 m) are collected as counts of individuals in diameter classes. Peet et al. (1998) suggest the following diameter classes (in cm): 0-1, 1-2.5, 2.5-5, 5-10, 10-15, 15-20, 20-25, 25-30, 30-35, and 35-40, with stems greater than 40 cm counted individually and measured to the nearest centimeter. Multiple stems arising from a common root system are recorded separately if they branch below 0.5 m from the ground. Peet et al. (1998) recommend that the area surveyed by stem count be adjusted based on conditions at the site, e.g., reduced to 20% of the modules for dense shrubland or increased by 200% for oak savannahs. This is easily implemented by reducing the width of the modules for woody species only.

An important part of vegetation surveys is collection, preparation, and depositing of voucher specimens in major herbariums in order to document a permanent record of that plant at that location. Although staff resources make collecting vouchers of every vascular plant infeasible, a voucher specimen of at least 10% of the vascular plant species at any given site is collected; however, in every instance in which the identity of any species cannot be confirmed in the field, or where field personnel disagree as to the identity of a species, a voucher specimen is collected for identification in the office. In particular, difficult genuses and families (e.g., Cyperaceae, Poaceae, Ranunculaceae, *Viola, Aster, Potamogeton*), as well as endangered, threatened, rare, or otherwise unusual plants, are almost always collected for confirmation.

Finally, data on standing biomass for emergent wetlands are collected. These data can be used in several ways. Biomass production in emergent wetlands dominated by herbaceous vegetation is estimated by harvesting 900-cm^2 quadrats in each wetland. The quadrats are located within the intensive modules of each plot. The plants within each quadrat are cut at the soil surface and placed into paper bags. In the lab, plants are oven dried at 105^{o}C for at least 24 hours and then weighed.

Lessons Learned for Vascular Plants

Floristic Quality Assessment Indexes

Ohio EPA has found that the FQAI score and subscores of the FQAI, e.g., percent coverage of plants with coefficients of conservatism of 0, 1, or 2, are very successful attributes and metrics for detecting disturbance in wetlands. See the following references:

Andreas B, Lichvar R. 1995. A Floristic Quality Assessment System for Northern Ohio. Wetlands Research Program Technical Report WRP-DE-8. U.S. Army Corps of Engineers, Waterways Experiment Station.

Herman KD, Masters LA, Penskar MR, Reznicek AA, Wilhelm GS, Brodowicz WW. 1996. Floristic Quality Assessment With Wetland Categories and Computer Application Programs for the State of Michigan. Michigan Department of Natural Resources, Wildlife Division, Natural Heritage Program.

Wilhelm GS, Ladd D. 1988. Natural area assessment in the Chicago region. Transactions 53rd North American Wildlife and Natural Resources Conference, pp. 361- 375.

Wilhelm GS, Masters LA. 1995. Floristic Quality Assessment in the Chicago Region and Application Computer Programs. Lisle, IL: Morton Arboretum.

Semiquantitative disturbance/integrity scales

Ohio EPA has had good success in developing a semiquantitative disturbance/biological integrity scale called the Ohio Rapid Assessment Method for Wetlands v. 5.0. Until more quantitative variables such as percent impervious surface are found, this type of tool is a good candidate for the problematic x-axis in wetland biocriteria development. See also "Plants and Aquatic Invertebrates as Indicators of Wetland Biological Integrity in Waquoit Bay Watershed, Cape Cod," Carlisle BK, Hicks AL, Smith JP, Garcia SR, Largay BG. Environ Cape Cod 1999; 2(2):30-60, where a similar system was used to rank levels of disturbance.

Classification

Classification is definitely an iterative process. Investigators should consider a hydrogeomorphic (HGM) classification scheme if one has been developed for their region of interest, at least as a starting point. However, the experience in Ohio suggests that grosser classes based on dominant vegetation (emergent, scrub-shrub, forested, etc.) may work also. A goal of a cost-effective biocriteria program is to have the fewest classes that provide the most cost-effective feedback. With vegetation, data from Ohio are suggesting that somewhat diverse wetland types may be "clumpable," because even though their flora are different at the species level, the quality/responsiveness of their unique flora to human disturbance is equivalent. This is also a concern in States with high degrees of wetland loss where too few wetlands of a particular HGM class remain to analyze as a separate class.

Field and lab methods

After experimenting with both transect/quadrat and releve-style plot methods, Ohio has adopted a plot-based method that allows for a qualitative stratification of wetland by dominant vegetation communities. This method is flexible and adaptable to unique site conditions, provides dominance data for all species in all strata, provides data that are intercomparable with other common methods, is relatively easy to learn, and is relatively fast and cost-effective (up to two to three plots can be completed in a day).

Whatever sampling method is adopted, it is essential that dominance and density information (cover, basal area of trees, stems per unit area, relative cover, relative density, importance values, etc.) be collected. Many of the most successful attributes Ohio has found in developing a vegetation IBI are based on cover data of the herb and shrub layers and density data of the shrub and tree layers.

Definitely consider using cover classes in general and a class scheme that works on a doubling principle to aid in consistent inter-investigator usage (see Peet et al. 1998). Then use the midpoints of the class for your analysis. This seems to help with consistent usage and smoothing out the roughness in cover data.

Finally, it is recommended that initially the sampling method should "overstratify" in both the vertical and horizontal dimensions until it can be determined which strata and communities are responding best to human disturbance. Ohio has found that the herb and shrub (subcanopy layers) seem to respond the best, although some intermediate tree size classes (e.g. 10- to 25-cm dbh) also appear responsive.

Overstratifying horizontally may also make sense at the reference development stage; however, ultimately the decision whether to split or clump communities depends on whether this is necessary to detect the disturbances. "Homogenizing" community types by placing a releve or transect across them (e.g., aquatic bed to emergent to shrub zone) can be appropriate if splitting does not matter to detect the disturbance. The caveat, of course, is that you cannot separate the data set later if you detect something of interest in one of the clumped communities.

Vouchers and QAQC

On the basis of Ohio's experience, voucher as much as you can for later confirmation in the lab and deposit vouchers in local and regional herbariums. Definitely collect all Cyperaceae, Poaceae, and Juncaceae, and also consider collecting shrubs genuses and families (Salix, Viburnum, Vaccinium, Rosa, Alder, etc.), *Polygonum spp*., *Aster spp*., *Viola spp*., and Cryptograms.

Oregon: Simultaneous Development, Calibration, and Testing of Hydrogeomorphic-Based (HGM) Assessment Procedures and Biological Assessment Procedures

Contact Paul Adamus, Ph.D

Organization Adamus Resource Assessment, Inc.
6028 NW Burgundy Drive
Corvallis, OR 97330

Phone (541) 745-7092

E-mail adamus7@attbi.com

Contact Dana Field

Organization Oregon Division of State Lands
775 Summer St. NE
Salem, OR 97310

Phone (541) 378-3805, ext. 238

E-mail Dana.Field@dsl.state.or.us

Purpose

- Collect and analyze field data on wetland and riparian plant communities and hydrogeomorphic (HGM) features to define reference standard conditions and performance criteria for four subclasses of wetlands in Oregon, two in the Willamette Valley in western Oregon and two on the Oregon coast
- Use that information to develop quantitative but rapid, HGM-based assessment models and procedures for those subclasses, as well as to identify new wetland plant community metrics that have demonstrated relationships with landscape condition and with internal alterations of wetlands in this region
- Compare results of using HGM models for functions with metrics that describe floristic characteristics, to verify the results on independent data sets, and demonstrate implementation of the methods for assessing progress of restored wetlands and for helping recommend compensation ratios in a wetland mitigation bank context

Wetland Type

- Slope, flats, and estuarine fringe wetlands

Assemblages

- Vascular plants

Status

Completed reports for the two Willamette Valley subclasses, and currently initiating development of the estuarine fringe project. Information on purchasing the reports can be found at: http://statelands.dsl.state.or.us/hgm_guidebook.htm.

Project Description

These projects are being done cooperatively with EPA Region 10 and the Oregon Division of State Lands, which in 1997 identified a need, in the context of Section 404 and 401 responsibilities, for a more quantitative assessment method tailored specifically to these wetland subclasses. The procedures have been designed to be applicable to nonjurisdictional riparian sites as well as to wetlands. The project began in 1998 with formation of an interagency oversight committee, intensive review of regional literature, and reconnaissance and preliminary selection of 124 reference sites throughout the Willamette Valley ecoregion. In 1999, the list of sites was narrowed, workshops of local experts were held to develop function models and field procedures, 38+ volunteers were recruited and trained to assist with field work, and 2 assessment teams ("A-teams") of scientists with help from the volunteers collected data from the selected sites. A three-volume final report was published in early 2001 (see above). The report for the estuarine fringe wetlands is scheduled for completion in 2004. Under administrative rules recently reissued by the Oregon Division of State Lands, applicants for most State-authorized wetland removal-fill permits are now required to use the regionalized HGM classification that was developed by this project, and are encouraged to use the reference-based function assessment procedures when available for a particular subclass and region of the State.

Study Design

For the Willamette Valley project, plant and hydrogeomorphic data were collected from 109 wetland and riparian sites (54 riverine impounding subclass, 55 slope/flats subclass). The "riverine impounding" sites include all wetland and riparian areas within a 2-year river floodplain, e.g., sloughs, oxbows, cut-off channels, beaver impoundments, stream-fed ponds with water control structures. "Slope/Flats" sites include most ash swales, wet prairies, springs, and foothill seeps. From visual inspection alone, most sites in the "slope" HGM class could not be reliably separated from most sites in the "flats" HGM class, due to the flat topography and varied geology of the region, so the two were combined into a "slope/flats" subclass.

Within the subclasses, assessment sites were selected nonrandomly, in a manner intended to span gradients of human disturbance, size, and plant community succession. Restored/created sites of various ages were also included. Nearly all sites were on public lands, and ranged in size from 0.1 to 233 acres. Most sites were nominated by local wetland experts. From the collected data, five least-disturbed riverine sites were selected as reference standards, as were six least-disturbed slope/flat sites. Use of cluster analysis and other statistical analysis methods verified that sites belonging to the two main subclasses were significantly different based on landscape position and mapped soil characteristics important to ecosystem function.

For defining the disturbance gradient (the "x axis"), information specific to each candidate site was collected during the reconnaissance phase. This information pertained to past management practices, surrounding land use, and recent physical alterations. Physical alterations were noted visually during reconnaissance visits. At each site each type of alteration was categorized as (a) absent, (b) physically affects <10% of site, (c) affects >10% of site but not all, or (d) affects entire site. The categories that were used for alterations were:

- Flow-impounding – berms, dikes, dams
- Flow-impounding – excavations, pits
- Flow-redirecting
- Water subsidy (e.g., stormwater pipes)
- Drainage-inducing (ditches, tile)
- Soil compacting (e.g., fill, machinery, cows)
- Soil mixing (e.g., plowing)
- Soil grading (e.g., flattening)
- Vegetation removal (e.g., extreme grazing, logging)

Sampling Methods: Plants

At each site, the A-teams identified plants in each of potentially three hydrologic zones: permanent water zone, seasonally inundated zone, saturated-only zone. For woody plants, the team walked the entire site and made an overall estimate of relative percent of the area of each zone occupied by each shrub species (understory and open) and tree species. For herbaceous plants, the team assessed relative cover of each species in 1x1-m quadrats. No more than nine quadrats were used at any site (and fewer at smaller sites and sites with fewer hydrologic zones). Larger numbers of quadrats were not used because of time and resource constraints. Within zones, quadrats were located so as to maximize the cumulative number of species found. This less formal approach was used because our goal was to develop metrics based mainly on community composition rather than quantitative measures of abundance or cover. Two teams assessed all 109 sites in about 50 workdays.

Analytical Methods: Plants

From the collected data, the following plant metrics were compiled for each site:

- Number of native herb species, relative to the intensity of sampling (# of plots at each site) (based on analysis using species-area curves and regression)
- Percent of herb species that are native
- Percent of the herb species that are "remnant-dependent" (i.e., reputedly most characteristic of unaltered sites)
- Percent of the dominant herb species in any plot that are natives
- Percent of the dominant herb species in any plot that are remnant-dependent
- Percent of the true wetland species (facultative or wetter) that are natives
- Percent of shrub species that are natives (when shrubs present)
- Percent of native shrub species that are at least moderately dominant
- Percent cover of non-native shrubs

Preliminary analysis suggests many of the above metrics had a statistically significant association with categorical observations of partial physical degradation of sites (see Study Design, above) and/or with amount and proximity of surrounding land cover categories (agriculture, urban, natural) that were assessed visually during fieldwork as well as by a GIS analysis of existing digital imagery. Analyses of the HGM and plant data sets, each containing 4,000+ records, were performed on a PC using Excel, PC-ORD, and NCSS.

Lessons Learned

- Random or systematic sampling, whether within a region or within a site, is not always appropriate for use in identifying good biological indicators or developing rapid models for assessing wetland condition and function.
- Systematic, repeatable, rapid procedures can be developed for assessing some of the disturbance gradients. This is a necessary precursor to selecting reference sites that will yield the most useful data.
- The biological metrics investigated or used should be appropriate to the study design and measurement protocols.
- Data suitable for identifying biological indicators of wetland condition sometimes can be collected simultaneously with data collected for calibration of HGM models. This does not necessarily require a great deal of additional training or field time.

Shared field experiences are a good forum for sharing wetland knowledge among agencies, and among agencies and consultants and citizens. Shared field experiences lead to participants feeling more vested in the process of developing models and multimetric indexes. This informal "buy-in" can lead to greater willingness of participants to use the methods that ultimately result.

Pennsylvania: Assessing Wetland Condition at a Watershed Scale Using Hydrogeomorphic Models and Measures of Biological Integrity

Contact	Rob Brooks
Organization	Penn State University Penn State Cooperative Wetlands Center 301 Forest Resources Laboratory University Park, PA 16802
Phone	(814) 863-1596
E-mail	rpb2@psu.edu
Website	http://www.wetlands.cas.psu.edu/

Contact	Denice Heller-Wardrop
Organization	Penn State University Penn State Cooperative Wetlands Center 301 Forest Resources Laboratory University Park, PA 16802
Phone	(814) 863-1005
E-mail	dhw110@psu.edu
Website	http://www.wetlands.cas.psu.edu/

Purpose

- Develop biological and functional assessment methodologies for many wetland types in Pennsylvania

Wetland Type

- Variety of wetland types

Assemblages

- Amphibians
- Birds
- Macroinvertebrates
- Vascular plants

Project Description

The Penn State Cooperative Wetlands Center (CWC) has numerous ongoing projects that involve bioassessment methodology for assessing the condition of wetlands. These projects include:

- Bird community index of biological integrity for the Mid-Atlantic Highlands
- Verified suitability index for the Louisiana waterthrush
- Amphibian indicator landscape study
- Macroinvertebrates indicator pilot study
- Otter and beaver interactions in the Delaware Water Gap
- Using bioindicators to develop a calibrated index of regional ecological integrity for forested headwater systems

In addition to these projects, CWC is conducting a 2-year pilot study to assess the ecological condition of wetlands in the Juniata River watershed of central Pennsylvania. The Juniata River is a major tributary of the Susquehanna River and lies within the headwaters region of the Chesapeake Bay ecosystem. The objective of the Juniata Wetland Monitoring Project is to define the ecological health of wetland resources, thereby providing a scientific context for resource managers to plan future protection and restoration activities.

CWC is using HGM functional assessment models that incorporate a floristic quality assessment index (FQAI) similar to the one used by Ohio EPA (see Ohio EPA's case study). They use a three-step process. The first step is to develop and calibrate HGM functional assessment models that are sensitive to environmental disturbance. The second step is to randomly sample wetlands within the watershed that represent a range of HGM classes and disturbance levels. The final step is to apply the HGM models to the data collected in the field to determine the condition or health of wetlands within the Juniata River watershed.

Preliminary HGM functional assessment models have been developed by the CWC. These models are currently being calibrated using a set of 102 reference wetlands that span a variety of HGM subclasses (mainstem floodplain, headwater floodplain, riparian depression, slope, etc.) and disturbance levels (severe, moderate, or pristine). Disturbance levels are assigned based on the surrounding landscape, the type of buffer present, and potential stressors identified at the site. It is essential to characterize wetlands across a disturbance gradient to determine not only the level of function a given wetland type may achieve, but also the level of functioning that is attainable in an impacted landscape.

The second step of the process, data collection, is being undertaken by the CWC and trained interns from Penn State University, the University of Pittsburgh at Johnstown, and the Department of Environmental Protection. Two types of data are collected for each wetland sampled: landscape-level data and site-level data. Landscape-level data are obtained at the CWC by characterizing land uses within a 1-km radius circle surrounding the wetland using aerial photographs. This gives an indication of the type and magnitude of potential wetland stressors. For example, activities such as land clearing in the surrounding watershed may stress wetland systems by increasing sediment loads.

In addition to land uses, the buffer type and width is also determined for each site. It is important to look at buffers when studying disturbance because buffers can ameliorate or magnify the affects of negative landscape activities. A wetland surrounded by a forested buffer may not be as stressed by activities in the landscape as one surrounded by an urban or agricultural buffer.

Site-specific data are collected at each wetland site in the field. Site-level responses of wetlands to stressors are many and may involve both abiotic and biotic indicators. At each wetland sampled, data are gathered on microtopography, soils, plants, and animals. Although not all plant species are highly sensitive to disturbance, the immobility of the plant community, its amenity to remote sensing techniques, and easily recognized signs of stress make it a good indicator of wetland condition. Previous studies by the CWC have investigated the potential utility of plant community measures as indicators of wetland health. Plant community measures that may prove to be good indicators of disturbance include the FQAI score, the number or dominance of introduced or aggressive native species, and the number of different species of Carex present. The CWC method contains a comprehensive plant sampling methodology that has been used in a variety of project types. Three sizes of plots are used to record various measures of the plant community including percent cover and richness of herbaceous species, shrub volume, and tree dbh.

Vermont: Classification, Biological Characterization, and Biometric Development for Northern White Cedar Swamps and Vernal Pools

Contact Doug Burnham

Organization Vermont Department of Environmental Conservation
WQD
103 S. Main St. – 10N
Waterbury, VT 05676

Phone (802) 241-3784 or 244-4520

E-mail DOUGB@dec.anr.state.vt.us

Purpose

The Vermont Wetlands Bioassessment Project is a collaboration between the Vermont Department of Environmental Conservation and the Vermont Nongame and Natural Heritage Program, with a 3-year time frame. The primary objectives of the first phase of the Vermont Wetlands Bioassessment Program are:

- Gather chemical, physical, and biological data from seasonal pools that will facilitate an ecologically based classification of minimally disturbed (reference) seasonal pools in Vermont
- Use both previously and newly collected Nongame and Natural Heritage Program data to try to identify specific biological attributes that can serve as indicators of ecological integrity in northern white cedar swamps.

Wetland Type

- Vernal pools
- White cedar swamps

Assemblages

- Algae
- Amphibians
- Birds
- Macroinvertebrates
- Vascular plants

Status

Analyzing data and writing final report. A final report is expected in the near future.

Project Description

The Vermont Wetlands Bioassessment project currently focuses on two types of wetlands: (1) vernal (seasonal/ephemeral) pools and (2) Atlantic northern white cedar swamps.

Vernal pools
We sampled a total of 28 vernal pools for breeding amphibians, macroinvertebrates, algae (diatoms), soils, vegetation, and water chemistry in April, May, and June of 1999 and 2000. A variety of other physical and riparian observations were also made.

Pools were distributed throughout Vermont across seven biophysical regions. A range of condition, from "reference" or least disturbed, to highly disturbed, was represented in the selection of pools. Upon completion of the field phase of the project and assessment of relative hydroperiod, it is likely that 5 of the 28 pools may be less "seasonal" than originally thought. Data are being analyzed for biological signals that may identify pools with more-or-less permanent standing water. Each pool was visited twice, the two visits approximately 4 to 6 weeks apart, for aquatic macroinvertebrate and water chemistry sampling. Algae samples were collected on the second pool visit. Amphibian surveys either preceded or coincided with the first macroinvertebrate sampling visit. Five of the 28 pools were dry on the second sampling visit.

Aquatic macroinvertebrates. Macroinvertebrates were sampled using three different methods: funnel traps to sample the actively swimming invertebrates (i.e., beetles, bugs, mosquitoes, crustaceans), a D-net to sample benthic invertebrates in the leaf litter and muck (i.e., snails, bivalves, chironomids, oligochaetes, caddisflies), and a qualitative search for any taxa we might have missed with the previous two methods. Funnel traps were made of window screen and designed to function like minnow traps. The traps were placed approximately 10 m apart and were left in place for approximately 24 hours. When the traps were emptied, any amphibians were returned to the pool and the macroinvertebrates were collected and preserved. The contents of each trap were stored separately. The D-net scoop and qualitative samples were preserved in the field, and later picked and sorted into taxonomic orders according to standard protocol. Except for zooplankton, all taxa were identified to the lowest practicable level. Scoop sampling along transects often created more of a disturbance in the pool than was comfortable for those conducting the sampling. The presence of egg masses and/or large concentrations of larval amphibians greatly restricted the efficiency of the scoop sampling at many pools. Trap sampling was less disruptive, but care had to be taken to not completely submerge the traps when adult amphibians were present so that entrapped adults would have air space to breathe. High concentrations of mosquito larvae tended to affect the efficiency of the traps. Qualitative sampling was less disruptive (although excessive wading in the pools often created an uncomfortable level of disturbance) but provided an inconsistent sampling effort. The majority (70%) of macroinvertebrate taxa collected were represented in trap samples. Two orders represented a large proportion of all taxa collected: Diptera (35%) and Coleoptera (23%).

Water. Water temperature, pH, and apparent color were recorded in the field. The remainder of the water sample was preserved and later analyzed for alkalinity, conductivity, anions, cations, and aluminum, and again for pH. Field pH values ranged from a minimum of 4.41 to a maximum of 7.75, alkalinity values ranged from 0 mg/L to 173.0 mg/L, conductivity readings ranged from 14.9 umhos/cm to 335 umhos/cm, and apparent color ranged from 4.5 to 489 over the sampling season. Apparent color, alkalinity, and cation concentrations show a general increasing trend within each site over the sampling season, whereas pH and aluminum and anion concentrations exhibited no consistent trend.

Algae. Algae were collected and analyzed for diatoms from 18 pools sampled in 1999. Algae sampling primarily targeted diatoms; however, filamentous algae were collected when present. We attempted to collect both benthic samples (scraping algae from leaves, sticks, and rocks) and planktonic diatom samples from each pool. Unfortunately, five of the pools were already dry in May, so we were only able to collect planktonic samples in the remaining 13 pools. Benthic samples were collected from 17 of the 18 pools. We froze all samples upon return to the lab, and samples were sent to Dr. Jan Stephenson for identification.

Amphibians. We observed amphibians initially during the amphibian survey, and continued to observe and collect specimens during both rounds of macroinvertebrate sampling. At the beginning of the field season, we visually surveyed each pool for egg masses and spermatophores, identified each egg mass, recorded an approximate number of eggs per mass, counted and identified breeding adults, and described physical parameters of the habitat. The timing of this first visit did not necessarily coincide with amphibian emergence; however, the funnel traps used during invertebrate sampling effectively caught active amphibians. Breeding adults were typically captured during the first round of sampling, whereas tadpoles and larvae were present in the traps on the second visit. All amphibians caught by the funnel traps, including adults, larvae, and egg masses, were identified in the field, counted, and returned to the pool. We continued to survey egg masses and record physical parameters throughout the field season. All 28 pools sampled showed signs of use by breeding amphibians. Amphibian species commonly observed included wood frogs (27/28 sites), yellow-spotted salamanders (27/28 sites), Jefferson (hybrid) salamanders (7/28 sites), red-spotted newts (18/28 sites), and green frogs 15/28 sites).

Ongoing and upcoming work. Data are currently being evaluated using a variety of procedures in order to identify and describe efficient and least disruptive sampling methods, and evaluate biological, chemical, and physical indicators of natural variability and disturbance. Methods of data analysis include two-way indicator species analysis (TWINSPAN) and detrended correspondence analysis (DCA) as well as standard descriptive and comparative statistical methods. A detailed site report is being developed for each vernal pool site.

Northern white cedar swamps

Seven northern white cedar swamps were surveyed in June for breeding birds and vegetation. Two of these cedar swamps were considered to be of reference quality, whereas the other five had some degree of impairment associated with them. Disturbances at the impaired sites included logging, roads, and storm water and agricultural discharge. We visited three of the sites early in summer to assess the feasibility of sampling aquatic macroinvertebrates for bioassessment and monitoring purposes.

Breeding birds/vegetation. Vegetation and biophysical data were collected at each site and species lists are being constructed. Listening stations were established and a bird census was taken twice during the breeding season. The data collected from the vegetative, biophysical, and bird sampling will be compiled and compared to the existing data from the statewide inventory of northern white cedar swamps.

Aquatic macroinvertebrates. Sampling for aquatic macroinvertebrates had been scheduled for May, but because of a busy vernal pool sampling season, we were unable to visit the cedar swamps until June. Unfortunately, a very dry spring may have caused the cedar swamps to be drier than usual this year. We visited two impaired sites and one reference site during the month of June. We were unable to find any standing or flowing water at either the reference site or at one of the impaired sites, so we could not effectively sample. However, we found evidence that suggested the swamps had contained water earlier in the season. The second impaired site contained many braided, slow-flowing channels and some standing water. We qualitatively sampled three of the channels and a small hollow at the base of the boulder. The samples were preserved in the field, picked, and sorted, and will be identified according to standard protocol. It is possible that aquatic microhabitats in cedar swamps are not available consistently enough to sample for aquatic macroinvertebrates; however, our limited sampling efforts did not conclusively elucidate the feasibility of using aquatic macroinvertebrates as biological indicators in cedar swamps.

Ongoing and upcoming work. Using the data we have collected, we will work to identify attributes associated with vegetation and bird assemblages that can serve as indicators of ecological integrity and anthropogenic disturbance.

Washington: King County Wetland-Breeding Amphibian Monitoring Program

Contact Klaus O. Richter

Organization King County Department of Natural Resources & Parks
Water and Land Resources Division
201 S Jackson Street, Suite 600
Seattle, WA 98104-3855

Phone (206) 205-5622

E-mail klaus.richter@metrokc.gov

Organization Viewpoint Services
11040 104th Ave. NE
Kirkland, WA 98033-4423

Phone 425-822-4016

E-mail korichter @msn.com

Purpose

Provide yearly data regarding wetland condition and the health of breeding amphibians for environmental review, to identify threats to wetlands and amphibians, to evaluate current wetland protection measures, and to improve local-land use decisions. Specifically, to stem amphibian extinctions in urban landscapes by first identifying cause-and-effect relationships among amphibian distribution, abundance, and health and anthropogenic impacts to wetlands and uplands, and second, using this information to provide amphibian conservation recommendations.

Wetland Type

- Depressional wetlands

Assemblages

- Amphibians

Status

- Implementing methods

Project Description

The King County Wetland-Breeding Amphibian Monitoring Program was originally designed to provide the county with long-term wetland, amphibian, and landscape information for planning and regulatory purposes. From 1993 to the present, a minimum of 150 volunteers were trained to census amphibian eggs, juveniles, and adults in 90 freshwater wetlands of 26 watersheds in King County. Analysis of amphibian distribution and health for all wetlands was completed. A targeted subsample of 21 wetlands in 3 rapidly urbanizing watersheds in which amphibians were declining was additionally monitored for wetland hydrology, predators, and watershed condition to determine the causes of population declines, in hope of stemming declines through better wetland conservation practices.

Today, a skeleton program totally run by volunteers continues to monitor a few of the initially surveyed wetlands without county support. A more intensive monitoring program targeting amphibians as bioindicators of wetland condition has been initiated with the cooperation and partnership of the University of Washington's Certificate Program in

Wetland Science and Management, in which several students research and monitor an important wetland and amphibian issue.

The project's monitoring goals include the following:

- Identify the occurrence of the State-endangered Oregon spotted frog
- Determine land uses compatible with wetland and amphibian conservation objectives
- Provide data to help develop and implement regulations for the protection of amphibians and their habitats
- Identify population distribution status of other county declining species
- Obtain standardized baseline inventory data on the distribution, abundance, and health of amphibians in King County wetlands
- Provide information to King County, Washington State Department of Fish and Wildlife, Washington State Department of Ecology, and Federal resource agencies for developing regional wetland and wildlife management programs
- Develop an effective public outreach and education program to train citizens to monitor amphibians and wetland conditions to foster wetland stewardship
- Educate potential wetland scientists to the diversity of wetland issues and train them in developing monitoring programs, assessing wetland condition, and crafting management plans for better wetland conservation
- Investigate local urban amphibian decline and extinction issues
- Develop methods for utilizing amphibians as bioindicators of wetland condition by establishing cause-and-effect relationships between amphibian declines and wetland hydrology, water quality, predators and pathogens, and watershed land use.

Wisconsin: Developing Biological Indexes for Wisconsin's Palustrine Wetlands

Contact Jennifer Hauxwell

Organization Wisconsin Department of Natural Resources
Bureau of Integrated Science Services
1350 Femrite Drive
Monona, WI 53716

Phone (608) 221-6338

E-mail hauxw@dnr.state.wi.us

Purpose

- Develop a biological index for Wisconsin's palustrine wetlands
- Compare performance of one plant and two macroinvertebrate multimetric indices
- Develop biological integrity rating system for classifying wetlands

Wetland Type

- Palustrine wetlands

Assemblages

- Macroinvertebrates
- Vascular plants

Status

Findings representing preliminary investigations are presented in Final Report to EPA Region V, Wetland Grant #CD985491-01-0 prepared by Wisconsin DNR (January 2000).

Project Description

Field studies for this project were conducted during the spring and summer of 1998, with laboratory analysis and data synthesis completed the following year. Funding was provided by a grant from EPA Region 5. The findings formed the basis for a second EPA-funded grant to refine and further evaluate the preliminary indices and expand communities covered to include amphibians, small mammals, diatoms, and zooplankton.

Study Design

We sampled 104 palustrine depression wetlands distributed across the major ecoregions of Wisconsin during early spring and summer of 1998. Study sites included a mixture of least-disturbed reference basins and degraded or restored wetlands, representing a range in vegetative cover types, water chemistries, and water duration.

Sampling Methods: Macroinvertebrates

We sampled macroinvertebrates early in the spring to minimize influences of immigration-emigration. Three different field collection procedures were evaluated and two laboratory approaches were used. On all 104 wetlands, we collected two sets of three standard D-frame net sweeps of approximately 1-m length each. Sampling stations (< 60 cm deep) were established at equally spaced points around the wetland perimeter that approximately trisected the basin (and ensured coverage of the major plant communities present). The first set of net sweeps was concentrated (large coarse materials were rinsed, examined, and removed) into a 1-quart container; the second set of net sweeps was not field-concentrated, but rather was placed directly into a 1-gallon plastic bag. Both sets were preserved in ethanol and returned to the laboratory for processing. The A third set (used on a subset of 17 wetlands) of D-frame net sweep samples was placed on a coarse wire screen over a collection basin for a period of approximately 10 minutes. Organisms falling (or crawling) through the screen and entering the collection container were collected and preserved as above. This last set represented a "clean" sample that was much easier to sort and process than the standard samples.

Laboratory Methods: Macroinvertebrates

Macroinvertebrates were processed using a two-tiered approach. The first stage consisted of a fixed 100-count (sensu Hilsenhoff Biotic Index procedures) using a grid-marked tray with 24 cells. Organisms were picked and sorted at a coarse taxonomic level, usually order or family only. Following completion of the 100-count sample, we processed the balance of the sample in its entirety (except for subsampling dominant taxa). The unconcentrated "bag" samples proved to be too large to process in an economical fashion, so only the complete set of 104 "field-concentrated" samples were processed. The 17 "screened" samples generally contained less than 100 total organisms and were processed completely.

Analytical Methods: Macroinvertebrates

We used SYSTAT to perform all statistical and graphical analyses. Percentage data were transformed using the arc-sine square-root transformation, and abundance data were either log-transformed or power-transformed as applicable. Metric development was based on a series of visual comparisons of community attribute responses to suspected measures of disturbance using box plots and jittered dot density plots. Those attributes that exhibited evidence of separation between reference wetland conditions and wetlands suspected to be impacted by human disturbance were selected as potential metrics. Attributes that exhibited inconsistent or overlapping responses between impacted and reference systems were eliminated from further consideration.

Sampling Methods: Plants

We conducted simplified plant surveys during July 1998 using a combination of techniques. This included a subjective estimate of cover and an objective survey of percent cover and frequency of occurrence within six equidistantly spaced 20 by 50 cm rectangular quadrats positioned along each of three transects that trisected the wetland basin (total of 18 quadrats per wetland).

Laboratory Methods: Plants

No biomass or stem counts were performed. Voucher specimens were pressed and identified to species when possible, but most plant metrics were based on a coarser taxonomic level.

Analytical Methods: Plants

We developed the plant biological index using the same procedures described for the two macroinvertebrate-based indices. Because we wanted to develop a practical tool for managers with limited botanical expertise, we lumped taxa at various taxonomic levels (e.g., family, genus) or structural groups (e.g., grass-like, emergent) for analysis. Importance values (average of percent cover and frequency of occurrence) were used as the attribute of concern for family-genus-species levels, and percent cover was used for emergent, submergent, floating-leafed, and open-water attributes.

Other Data Collected

We also collected associated physical and chemical data on each wetland, including pH, alkalinity, conductivity, color, temperature, clarity, and depth. Riparian cover type within a 100-foot buffer area surrounding each wetland was subjectively estimated and recorded, as was shade canopy cover.

Preliminary Findings

Three multimetric indices (two macroinvertebrate and one plant index) were developed. The Wisconsin Wetland Macroinvertebrate Index (WWMI) is a multimetric index based on 15 metrics derived from a total count of organisms in three composited net sweeps. A total of 69 community attributes were evaluated. The WWMI is composed of 12 abundance metrics, two richness metrics, and one percentage metric. Abundance metrics include mollusks, annelids, fairy shrimp, damselflies, pigmy backswimmers, water boatmen, limnephild caddisflies, total caddisflies, phantom midges, mosquitoes, soldier flies, and total invertebrates. Richness metrics are noninsects and total taxa. The percent caddisflies is the only percentage metric. Apparent redundancies (e.g., caddisflies) in the metric may or may not be an issue; differences in taxonomic rate of development in wetlands due to thermal dynamics may require a certain amount of redundancy to ensure that important taxonomic groups are accounted for. The WWMI is used to rate, rank, or compare wetland biological condition.

The second macroinvertebrate index, termed the 100-count macroinvertebrate biotic index (100-count MBI), is based on 10 metrics derived from a random pick of 100 organisms found in the three composited net sweeps. The 100-count MBI is composed of 9 percentage metrics and 1 richness metric (noninsect taxa). Percentage metrics include pigmy backswimmers, water boatmen, total "bugs," limnephilid caddisflies, total caddisflies, chironomids, soldier flies, and the sum of Ephemeroptera-Odonata-Trichoptera (EOT) taxa. Noninsects represent the only richness metric in the 100-count MBI. The ninth percentage metric, mollusks, may be only useful in prairie-type wetlands. The 100-count MBI is intended to be applied in the field by experienced staff as a means of rapid bioassessment.

The third index is the Wisconsin Wetland Plant Biotic Index (WWPBI) is based on eight (or nine) plant metrics derived from transect data (representing 18 quadrats) and is intended to serve as a supplementary index to the WWMBI to rate, rank, and compare wetland biological condition. Of 24 plant community attributes tested, only one richness (count) metric, one percent metric, and seven importance value-based metrics demonstrated consistent and predictable response. The single richness metric, total taxa, may require further modification after reaching some consistency regarding taxonomic resolution (currently mixed family-genus-species). Importance value-based metrics included Carex, reed canary grass, cattail, duckweed, bluejoint grass, and "good" plants (the sum of a group of plants including all Carex, Utricularia, Potamogeton, Calamogrostis, Sagittaria, Polygonum, and Equisetum). An additional importance value-based metric, "pondweeds," would only be applied to wetlands with water duration exceeding 7 months per year. The only percentage-based metric, floating-leafed plants, would likewise only be applied to wetlands with water durations exceeding 7 months.

Lessons Learned

Macroinvertebrates

- Water duration is an important factor shaping macroinvertebrate community composition and derived metrics that must be accounted for in metric scoring.
- A coarse level of taxonomic resolution (order and family) appears to be satisfactory in developing wetland macroinvertebrate metrics.
- Issues relating to redundancy among metrics, influences of water chemistry, differences among ecoregions, and seasonal variations need to be addressed in more detail. Undoubtedly, these factors need to be accounted for in establishing rating scores and/or in refining metrics for use in different areas or habitats.
- Basic differences exist in macroinvertebrate communities between wetlands representing wooded kettle depressions and open-prairie type depressions in Wisconsin.
- Our greatest difficulty was in selecting and assigning some measure of "human impact" to the study site wetlands. Further research will be required to quantify the degree of human impact in order to refine biological response metrics and indices.
- The WWMBI was not stable across dates (and not designed to be); consequently, its use is restricted to early spring.
- Each macroinvertebrate index has its own set of advantages and disadvantages; further refinement is required to enable their successful application in the field.

Plants

- Some biases were apparent in the WWPBI, as reference kettles scored consistently higher than prairie wetlands.
- WWPBI scores in restored prairie wetlands were better than in many natural wetlands, suggesting that wetland restorations in Wisconsin may be adequate in terms of "restoring" the vegetative community (not true for macroinvertebrate response).
- The WWPBI shows some promise in its performance and, because of its taxonomic simplicity, it could be applied by nonbotanists.

Followup: Continuing Work

With the assistance of a second EPA wetland grant, we have refined the macroinvertebrate and plant multimetric indices and explored expanding the community components to include zooplankton (in cooperation with Dr. Stanley Dodson, University of Wisconsin–Madison), diatoms (Paul Garrison, Wisconsin DNR), amphibians, and small mammals. Findings associated with the second grant are reported in the second Wisconsin case study below.

Wisconsin: Refinement and Expansion of the Wisconsin Wetland Biological Index for Assessment of Depressional Palustrine Wetlands

Contact Jennifer Hauxwell

Organization Wisconsin Department of Natural Resources
Bureau of Integrated Science Services
1350 Femrite Drive
Monona, WI 53716

Phone (608)-221-6373

E-mail jennifer.hauxwell@dnr.state.wi.us

Purpose

- Test and refine a Biotic Index for Wisconsin's palustrine wetlands
- Expand list of assemblages to include macroinvertebrates, zooplankton, diatoms, amphibians, plants, and small mammals
- Establish a biological integrity rating system for classifying wetlands based on the response of selected biological attributes (metrics) of the above communities to surrogate measures of human disturbance

Wetland Type

- Palustrine wetlands

Assemblages

- Macroinvertebrates
- Aquatic plants
- Zooplankton
- Diatoms
- Amphibians
- Small mammals

Status

Findings presented in Final Report to EPA Region V prepared by Wisconsin DNR dated April 2002.

Project Description

This project represents the evaluation and expansion phase of an earlier study that resulted in the preliminary development of a Wisconsin Wetland Biological Index based on plant and macroinvertebrate metrics (please see final report to EPA -Wetland Grant #CD985491-01-0). Data from this second study were used to refine and further evaluate the preliminary indices and expand communities covered to include zooplankton, diatoms, amphibians, and small mammals. Field studies for this project were conducted during the spring and summer of 2000, with laboratory analysis and data synthesis completed in 2001. Funding was provided by a grant from EPA Region 5.

We sampled 75 palustrine depression wetlands in southeast Wisconsin during early spring and summer of 2000. Study sites included a mixture of least-disturbed reference basins (17 prairie and 19 wooded kettles) and impacted wetlands (18 urban and 20 agriculture), representing a range in vegetative cover types and water chemistries. A severe drought, which began the previous year, caused most of the smaller wetlands with short hydroperiods (seasonal and temporary) to dry out, and consequently only long-duration wetlands (semipermanent and permanent) were sampled.

We sampled macroinvertebrates early in the spring to minimize influences of immigration-emigration. On each wetland, we collected three standard D-frame net sweeps of approximately 1-m length each. Sampling stations (less than 60 cm deep) were established at equally spaced points around the wetland perimeter that approximately trisected the basin and assured coverage of the major plant communities present. The contents of the three net sweeps were concentrated (large coarse materials were rinsed, examined, and removed) into a 1-quart container, preserved in ethanol, and returned to the laboratory for processing. Seven field replicates were collected.

Laboratory Methods: Macroinvertebrates

Each macroinvertebrate sample was processed entirely, using a two-phase approach with subsampling employed only for extremely abundant organisms. The first stage involved using a grid-marked tray with 24 cells. Cells were selected randomly, and organisms were picked and sorted at a coarse taxonomic level, usually to order or family level. The abundance of organisms present in the first two or three randomly selected cells was used to project the abundance of the most common taxa in the sample. Taxa whose abundance was estimated to exceed 300 in the total sample were then overlooked while the balance of the sample was processed (the second phase of processing). All specimens were vouchered (preserved in 70% ethanol) for possible further evaluation.

Sampling Methods: Plants

We conducted simplified plant surveys during July 2000 using a combination of techniques. This included a subjective estimate of cover and an objective survey of percent cover and frequency of occurrence within six equidistantly spaced 20x50-cm rectangular quadrats positioned along each of three transects that trisected the wetland basin (total of 18 quadrats per wetland).

Laboratory Methods: Plants

No biomass or stem counts were performed. Voucher specimens were pressed and identified to species when possible, but most plant metrics are based on a coarser taxonomic level.

Sampling Methods: Zooplankton

Note: zooplankton studies were conducted by Dr. Stanley Dodson, University of Wisconsin-Madison, Zoology Department, Madison, Wisconsin. E-mail: sidodson@facstaff.wis.edu.

We collected one zooplankton sample from a central basin location in each wetland during June 2000 using a 5-L plastic bucket. We filtered a known volume of water through a No. 10 (60-micron mesh) net to capture zooplankton within. Seven field replicates were collected. Samples were preserved in 70% ethanol until processed.

Laboratory Methods: Zooplankton

Each sample was scanned at moderate magnification for species of cladocera, copepods, ostracods, and aquatic insects. Slides and dissections were made where necessary; for example, to aid in the identification of copepod species. Total number of male and female Daphnia was counted in each sample. More than 200 slides were prepared, and identification of organisms is in progress. Organisms will be identified to species where possible.

Sampling Methods: Diatoms

Diatom studies were conducted under the direction of Paul Garrison, WDNR, Bureau of Integrated Science Services, Environmental Contaminants Section, 1350 Femrite Drive, Monona, WI 53716. E-mail: garrip@dnr.state.wi.us

Using a 1-dram vial as a sample collection device, we collected and composited surficial (upper 0-1 cm) sediments from five sites in each wetland. Seven field replicates were collected. Samples were kept on ice or refrigerated until processed.

Laboratory Methods: Diatoms

Each diatom sample will be thoroughly mixed, and a small amount will be placed into a tall beaker. Hydrogen peroxide will be added and the sample will be allowed to steep for about 5 minutes. Potassium dichromate will be added (under a ventilated hood and handled with safety gloves) to facilitate reduction of organic matter. The sample will be washed at least four times with deionized water by centrifuging for 10 minutes. Two portions of the cleaned sample will be dried on separate No. 1 cover-slips and mounted with Naphraxâ, and labeled accordingly. Specimens from both cover slips will be identified and counted under oil immersion objective (1,400X) until a total of 250 frustules are counted. Identification of difficult taxa will be made using a scanning electron microscope. Undetermined specimens representing a significant portion of a sample will be sent to Dr. Rex Lowe at Bowling Green University, Dr. Jan Stevenson at Louisville University, and/or Dr. Gene Stormer at the University of Michigan.

Sampling Methods: Amphibians

Because amphibians are extremely sensitive to weather and temperature, we assessed amphibian communities during two separate sampling periods. We conducted standardized frog-toad calling surveys (using WDNR protocols) during the first two phenologies (early spring and late spring) between the hours of 8 and 10:30 pm for 10-15 minutes when water temperatures were above 50°F during the first phenology or above 60°F during the second phenology. We recorded all calling to permit verification of questionable identifications. In addition to calling, we added to the database any personal observations of amphibians made during any of the daylight visits and any specimens captured during the macroinvertebrate surveys.

Laboratory Methods: Amphibians

No laboratory methods were used.

Sampling Methods: Small Mammals

Small mammal studies were conducted by R. Bautz, WDNR, Bureau of Integrated Science Services, Ecological Inventory & Monitoring Section, 1350 Femrite Drive, Monona, WI 53716. E-mail: bautzr@dnr.state.wi.us.

We assessed small mammal communities by trapping during August-September 2000. On each wetland, we set 46 baited traps (mixture of 40 museum special grade and 6 larger tomahawk traps) along transects (zigzag scattered routes) in the riparian zone (variable dimensions, depending on setting) for one night. A one-day/night trapping period was used to minimize disturbance by raccoons and other predators. Bait consisted of a mixture of peanut butter and rolled oats. Traps were cleaned and rebaited each morning. Specimens were placed in labeled freezer bags and returned to the laboratory for identification.

Laboratory Methods: Small Mammals

Identifications of rare taxa and species of special concern were verified by local experts from the University of Wisconsin.

Other Data Collected

We also collected associated physical and chemical data on each wetland. The water chemistry measures included pH, alkalinity, conductivity, color, chloride, calcium, silica, nitrate-nitrite, organic-nitrogen, and total phosphorus. Chemical analyses were performed at the Wisconsin State Laboratory of Hygiene following standard EPA-approved procedures. Physical data collected included water depth, apparent color, temperature, and size. Riparian cover type within a 100-foot buffer area surrounding each wetland was subjectively estimated and recorded, as well as shade canopy cover.

Analytical Methods: Data Analysis and Development of Biological Indices

Data were entered into Excel spreadsheet and Access databases and examined for entry errors (univariate checks) and anomalies (e.g., bivariate plots) prior to analysis. Various community or species attributes (e.g., taxa or species richness, diversity, presence or absence of selected functional feeding guilds, trophic structure, percentages) were evaluated and scored as potential metrics based on their responsiveness to measures of human disturbance. Community attributes were examined using a combination of procedures (outlined below) to select promising metrics for index development. Attributes that exhibited strong positive or negative correlation with selected human response variables were selected as metrics. We compared the sensitivity and correspondence among the selected community metrics, and developed a single multimetric index of ecosystem integrity that related to overall wetland condition.

Because different forms of human disturbance elicit different responses among the various wetland biological communities, it is difficult if not impossible to choose one measure that represents “the” single best measure of human disturbance. For example, the amphibian community may respond more directly to woodland impacts (distance to nearest woodland, patch size, corridor dimensions, etc.) than to nutrient or pesticide inputs, whereas zooplankton and diatom communities may be more responsive to percent row crops in the watershed. Consequently, we evaluated biotic responses to a combination of a priori classes of human disturbance and a composite chemical index that incorporated nitrogen, phosphorus, and chloride concentrations. The a priori classes represented agricultural and urban impacts as well as subclasses of each, including three intensity levels of agricultural impact and two forms of urban impact. Biotic responses occurring within these classes were measured against the responses in least disturbed kettles and prairie depression wetlands. Statistical procedures and approaches used in the analysis varied among the communities assessed because of inherent differences in responses and the type of data collected. Canonical correspondence analysis was used to explore unimodal distributions of the diatoms along the various environmental gradients. We used SYSTAT and a combination of other available statistical software programs to perform statistical and graphical analyses. Metric development was based on a series of visual comparisons of community attribute responses to measures of disturbance (combination of selected water chemistry and land use characteristics as discussed above) using box plots and jittered dot density plots. Attributes that exhibited evidence of separation between reference and impacted wetlands were selected as metrics (or incorporated into the existing list of metrics). Attributes that exhibited inconsistent or overlapping responses between impacted and reference systems were eliminated from further consideration.

Lessons Learned

- Finding willing and cooperative private property landowners for access to potentially impacted wetlands is difficult!
- Determining what attribute or attributes (e.g., land use, chemical contaminant concentration, distance to nearest road) to use as surrogate measures of human disturbance is critical to the successful development of reliable multimetric biological indices.
- Drought (or floods) can seriously hamper sampling plans and interfere with the best of plans!
- The list of suitable metrics among macroinvertebrate communities of permanent wetlands is different and much shorter than the list of metrics suitable for wetlands with shorter water duration periods.

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Glossary

Aquatic life use A type of designated use pertaining to the support and maintenance of healthy biological communities.

Assemblage An association of interacting populations of organisms that belong to the same major taxonomic groups. Examples of assemblages used for bioassessments include: algae, amphibians, birds, fish, amphibians, macroinvertebrates (insects, crayfish, clams, snails, etc.), and vascular plants.

Attribute A measurable component of a biological system. In the context of bioassessments, attributes include the ecological processes or characteristics of an individual or assemblage of species that are expected, but not empirically shown, to respond to a gradient of human disturbance.

Benthos The bottom fauna of waterbodies.

Biological assessment (bioassessment) Using biomonitoring data of samples of living organisms to evaluate the condition or health of a place (e.g., a stream, wetland, or woodlot).

Biological integrity "the ability of an aquatic ecosystem to support and maintain a balanced, adaptive community of organisms having a species composition, diversity, and functional organization comparable to that of natural habitats within a region" (Karr and Dudley 1981).

Biological monitoring Sampling the biota of a place (e.g., a stream, a woodlot, or a wetland).

Biota All the plants and animals inhabiting an area.

Composition (structure) The composition of the taxonomic grouping such as fish, algae, or macroinvertebrates relating primarily to the kinds and number of organisms in the group.

Community All the groups of organisms living together in the same area, usually interacting or depending on each other for existence.

Competition Utilization by different species of limited resources of food or nutrients, refugia, space, ovipositioning sites, or other resources necessary for reproduction, growth, and survival.

Criteria A part of water quality standards. Criteria are the narrative and numeric definitions conditions that must be protected and maintained to support a designated use.

Continuum A gradient of change.

Designated use A part of water quality standards. A designated use is the ecological goal that policymakers set for a waterbody, such as aquatic life use support, fishing, swimming, or drinking water.

Disturbance "Any discrete event in time that disrupts ecosystems, communities, or population structure and changes resources, substrate availability or the physical environment" (Picket and White 1985). Examples of natural disturbances are fire, drought, and floods. Human-caused disturbances are referred to as "human disturbance" and tend to be more persistent over time, e.g., plowing, clearcutting of forests, conducting urban stormwater into wetlands.

Diversity A combination of the number of taxa (see taxa richness) and the relative abundance of those taxa. A variety of diversity indexes have been developed to calculate diversity.

Dominance The relative increase in the abundance of one or more species in relation to the abundance of other species in samples from a habitat.

Ecological risk assessment An evaluation of the potential adverse effects that human activities have on the plants and animals that make up ecosystems.

Ecosystem Any unit that includes all the organisms that function together in a given area interacting with the physical environment so that a flow of energy leads to clearly defined biotic structure and cycling of materials between living and nonliving parts (Odum 1983).

Ecoregion A region defined by similarity of climate, landform, soil, potential natural vegetation, hydrology, and other ecologically relevant variables.

Gradient of human disturbance The relative ranking of sample sites within a regional wetland class based on degrees of human disturbance (e.g., pollution, physical alteration of habitats, etc.)

Habitat The sum of the physical, chemical, and biological environment occupied by individuals of a particular species, population, or community.

Hydrology The science of dealing with the properties, distribution, and circulation of water both on the surface and under the earth.

Impact A change in the chemical, physical (including habitat), or biological quality or condition of a waterbody caused by external forces.

Impairment Adverse changes occurring to an ecosystem or habitat. An impaired wetland has some degree of human disturbance affecting it.

Index of biologic integrity (IBI) An integrative expression of the biological condition that is composed of multiple metrics. Similar to economic indexes used for expressing the condition of the economy.

Intolerant taxa Taxa that tend to decrease in wetlands or other habitats that have higher levels of human disturbances, such as chemical pollution or siltation.

Macroinvertebrates Animals without backbones (insects, crayfish, clams, snails, etc.) that are caught with a 500-800 micron mesh net. Macroinvertebrates do not include zooplankton or ostracods, which are generally smaller than 200 microns in size.

Metric An attribute with empirical change in value along a gradient of human disturbance.

Minimally impaired site Sample sites within a regional wetland class that exhibit the least degree of detrimental effect. Such sites help anchor gradients of human disturbance and are commonly referred to as reference sites.

Most-impaired site Sample sites within a regional wetland class that exhibit the greatest degree of detrimental effect. Such sites help anchor gradients of human disturbance and serve as important references, although they are not typically referred to as reference sites.

Population A set of organisms belonging to the same species and occupying a particular area at the same time.

Reference site (as used with an index of biological integrity) A minimally impaired site that is representative of the expected ecological conditions and integrity of other sites of the same type and region.

Stressor Any physical, chemical, or biological entity that can induce an adverse response.

Taxa A grouping of organisms given a formal taxonomic name such as species, genus, family, etc. The singular form is taxon.

Taxa richness The number of distinct species or taxa that are found in an assemblage, community, or sample.

Tolerance The biological ability of different species or populations to survive successfully within a certain range of environmental conditions.

Trophic Feeding, thus pertaining to energy transfers.

Wetland(s) (1) Those areas that are inundated or saturated by surface or groundwater at a frequency and duration sufficient to support, and that under normal circumstances do support, a prevalence of vegetation typically adapted for life in saturated soil conditions [EPA, 40 C.F.R.§ 230.3 (t) / USACE, 33 C.F.R. § 328.3 (b)]. (2) Wetlands are lands transitional between terrestrial and aquatic systems where the water table is usually at or near the surface or the land is covered by shallow water. For the purposes of this classification, wetlands must have one or more of the following three attributes: (a) at least periodically, the land supports predominantly hydrophytes, (b) the substrate is predominantly undrained hydric soil, and (c) the substrate is nonsoil and is saturated with water or covered by shallow water at some time during the growing season of each year (Cowardin et al. 1979). (3) The term "wetland" except when such term is part of the term "converted wetland," means land that (a) has a predominance of hydric soils, (b) is inundated or saturated by surface or ground water at a frequency and duration sufficient to support a prevalence of hydrophytic vegetation typically adapted for life in saturated soil conditions, and (c) under normal circumstances does support a prevalence of such vegetation. For purposes of this Act and any other Act, this term shall not include lands in Alaska identified as having a high potential for agricultural development which have a predominance of permafrost soils [Food Security Act, 16 U.S.C. 801(a)(16)].