United States Environmental Protection Agency
Office of Water
Washington, DC 20460
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METHODS FOR EVALUATING WETLAND CONDITION

#6 Developing Metrics and Indexes of Biological Integrity

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Notice

The material in this document has been subjected to U.S. Environmental Protection Agency (EPA) technical review and has been approved for publication as an EPA document. The information contained herein is offered to the reader as a review of the “state of the science” concerning wetland bioassessment and nutrient enrichment and is not intended to be prescriptive guidance or firm advice. Mention of trade names, products or services does not convey, and should not be interpreted as conveying official EPA approval, endorsement, or recommendation.

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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

Summary

Multimetric indexes, such as Indexes of Biological Integrity (IBIs) are powerful tools for informed management decisions related to wetlands and wetland health. A number of States are currently developing wetland bioassessments by adapting bioassessment frameworks originally developed for streams. Although many aspects of stream bioassessment may apply, wetland floral and faunal assemblages are unique, and specific data from those assemblages are required to construct an IBI for wetlands. The information in this module is designed to provide a framework for the development of IBIs using specific examples from wetlands. The module describes a step-by-step process to propose, evaluate, and ultimately select metrics into the IBI that will best reflect the biological condition of wetlands.

Purpose

The purpose of this module is to provide a framework to help wetland and water quality professionals develop metrics and IBIs for wetlands.

Introduction

As discussed in Module 1: Introduction to Wetland Biological Assessment, biological assessments (bioassessments) are designed to evaluate the health or biological integrity of wetlands. Many of the States currently developing wetland bioassessments are adapting bioassessment frameworks originally developed for streams. This reliance on stream information is in part due to the comparatively abundant body of knowledge and literature that has accompanied the development of stream IBIs over the past two decades. However, the floral and faunal assemblages of wetlands as well as the ecological processes that occur within them (Wissinger 1999) are unique. Therefore, although many aspects of stream bioassessment may apply to wetland bioassessment, specific information from wetland biological assemblages is required to construct an IBI for wetlands.

Of the 10 States currently developing bioassessment methods for wetlands, 9 are attempting to use IBIs (Table 1). Maine and Montana use advanced statistical tests (e.g., canonical correspondence analysis) in addition to IBI (multimetric) analyses to analyze algal data (Danielson 1998, Apfelbeck 1999). Multimetric and statistical methods are both valid, and the framework provided by this module can benefit the development of both methods. This module, however, focuses on the development of IBIs. Consult Chapter 9 of the *Rapid Bioassessment Protocols for Use in Streams and Wadeable Rivers: Periphyton, Benthic Macroinvertebrates, and Fish* (Barbour et al. 1999) for a detailed description of statistical analysis.

Multimetric indexes are numbers that integrate several biological metrics to indicate a site's condition. They can be designed to be sensitive to a range of factors (physical, chemical, and biological) that stress biological systems, and they are relatively easy to measure and interpret (Karr and Chu 1999). Through a multimetric approach, each metric is given a rating according to whether its value approximates, deviates somewhat from, or deviates strongly from values measured in least-disturbed ecosystems of a particular type within a region. These ratings (e.g., excellent, moderate, fair, and poor) can be used to make decisions about whether the wetland condition indicates that aquatic life is being supported.

Because wetlands vary so widely geographically, hydrologically, biologically, and by wetland class, it is unrealistic to expect a single "off-the-shelf" multimetric index to be applicable everywhere. Even though techniques may vary, those that adhere to some basic biological, sampling, and statistical principles maintain the strengths of a multimetric

Table 1: Current pilot projects developing wetland bioassessment methods and assemblages

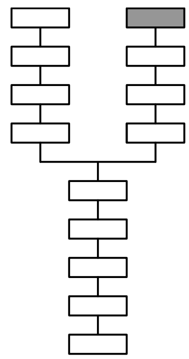
Sponsoring Organization	Type(s) of Wetlands	Assemblage(s)
Minnesota Pollution Control Agency	Depressional Starting riparian and would like to do Vernal Pools next.	Macroinvertebrates Plants
Ohio EPA	Depressional Riparian	Macroinvertebrates Plants Amphibians
North Dakota Department of Health	Depressional Riparian	Plants Macroinvertebrates
Montana Department of Environmental Quality	Depressional	Macroinvertebrates Algae Some plant work
Florida Department of Environmental Protection	Depressional & Others	Macroinvertebrates Plants
Massachusetts Coastal Zone Management	Coastal Marshes (salt and fresh)	Macroinvertebrates Plants Birds
Maine Department of Environmental Protection	Casco Bay Watershed (depressional, fringe, riparian)	Macroinvertebrates Algae (some plant work)
Vermont DEC	Vernal Pools White Cedar Swamps	Macroinvertebrates Amphibians Plants
Wisconsin DNR	Great Lake Fringe Wetlands	Macroinvertebrates Fish
Duke University	Southeast forested swamps and Everglades	Macroinvertebrates
Michigan State University	Great Lake Fringe Wetlands	Fish Macroinvertebrates
USGS Patuxent Wildlife Research Center	Restored depressional wetlands on Delmarva Peninsula (MD & DE)	Macroinvertebrates Plants Birds Amphibians
Penn State Cooperative Wetlands Center	All types focusing first in Juniata Watershed and then expanding to whole state.	Macroinvertebrates Plants Amphibians Birds
Volunteer Project - Mass Bays	Salt marshes	Plants Macroinvertebrates Birds
Volunteer Project - King County, WA	Depressional Wetlands	Amphibians
Volunteer Project - MPCA,Minnesota Audubon, and Dakota County, MN	Depressional Wetlands	Plants Macroinvertebrates

Note: Data compiled from information contained in EPA, Biological Assessment of Wetlands Workgroup website, www.epa.gov/owow/wetlands/bawwg/.

assessment approach as well as reflect the reality of regional variation in biological condition (Miller et al. 1988). The goal of a multimetric index is not to measure every biological attribute; indeed, doing so is impossible. Rather, the goal is, first, to identify those biological attributes that respond reliably to human activities, are minimally affected by natural variability, and are cost-effective to measure and, second, to formulate them as "metrics" and combine them into a regionally appropriate index (Karr and Chu 1999).

Figure 1 provides a framework that describes how attributes are derived, evaluated, and ultimately incorporated into an IBI as metrics. However, the first and most important step is to define the objectives for developing an IBI, which may influence several steps in the development process (see Module 5: Administrative Framework for Implementation of a Wetland Bioassessment Program). Do not expect all the steps in IBI development to follow this sequence exactly. Undoubtedly, each project will deviate from the path and will require revisiting steps on the basis of individual circumstances. However, this framework provides a useful way to organize actions and ensure that all major steps in developing an IBI are addressed. In Figure 1, the upper left portion shows the steps related to the establishment of a gradient of human disturbance (the dose), and the upper right shows the steps for developing attributes that will, at least predictably, respond to that gradient (the response). The column of steps in the lower middle shows the process by which the dose-response relationship is analyzed to select and score the best performing metrics for the IBI. The remainder of this report follows the framework provided in Figure 1 and includes small icons to help track progress through the sequence of activities.

Identify Regional Wetland Flora and Fauna



The success of multimetric indexes is largely dependent on choosing metrics that reflect diverse responses of biological systems to human actions (Karr and Chu 1999). Therefore, the best multimetric indexes combine measures of condition in individuals, populations, communities, ecosystems, and landscapes. Work with stream bioassessments indicates that valuable multimetric indexes typically use one or more assemblages for evaluating stream condition, such as macroinvertebrates, fish, and/or periphyton (Barbour et al. 1999). Professionals implementing stream bioassessments have found that monitoring more than one assemblage greatly increases the power of their assessment methods. Assemblages sometimes differ in their sensitivity to environmental stressors, such as sedimentation and eutrophication. Having more than one assemblage provides sensitivity to a wider range of stressors and increases confidence in management decisions. Although more investigations are needed to confirm the relationship, preliminary results from wetland IBI development indicate that monitoring a combination of assemblages (e.g., plants and macro-invertebrates) may similarly increase the power of bioassessments in wetlands (Gernes and Helgen 1999).

Current wetland bioassessment projects are exploring the use of several assemblages, including algae, amphibians, birds, fish, macroinvertebrates, and vascular plants (Table 1). It is not yet clear which assemblages will work best in wetland bioassessments. It is entirely likely that specific as-

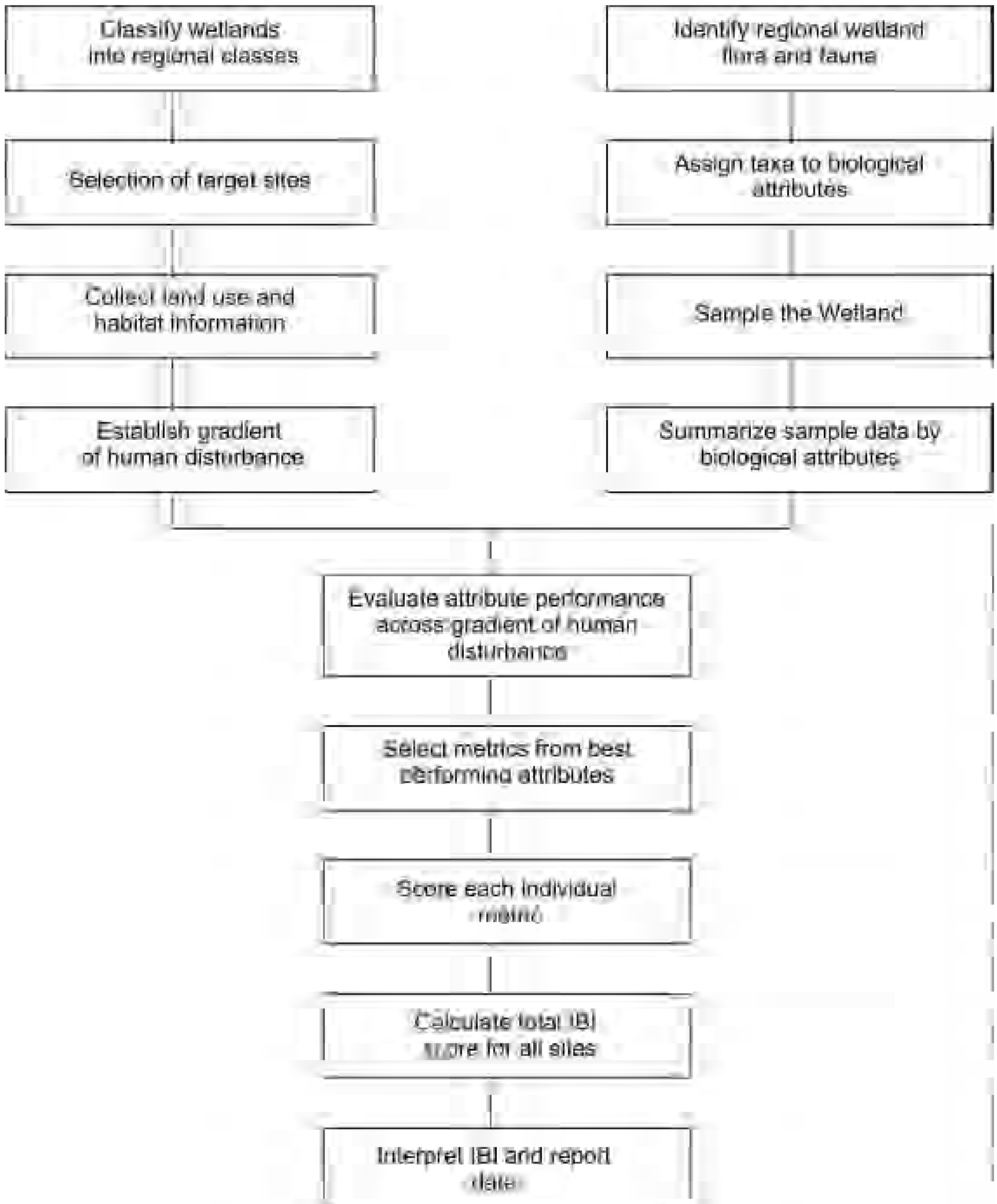
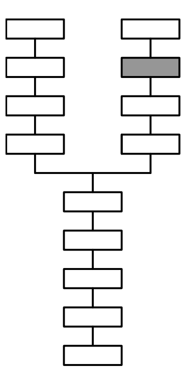


Figure 1: Sequence of activities in developing metrics and IBI, adapted from Karr et al. 1986.

semblages may be appropriate in some wetlands and not in others because of the wide variety of wetland types (e.g., salt marsh, bog, and forested swamp). For example, using wetland fish communities in bioassessments may be effective in only a small number of wetlands, such as wetlands on the fringes of large bodies of water. It is also likely that some assemblages will work in some parts of the country and not in others. For example, wetland amphibian communities are naturally less diverse in western states, and there simply may be too few species to develop a typical IBI. One of the first steps in developing an IBI, however, is carefully considering the advantages and disadvantages of sampling any particular assemblage (See other modules in this series to assist in the appropriate choice.) After selecting an assemblage, it is very helpful to make a list of the taxa (e.g., species, genera, and families) in that assemblage that are expected to occur in the study area. Such lists may be based on pre-project sampling or on previous studies from a particular regional wetland class (Sparling et al. 1996). This list will help with identifying organisms and with the next step of assigning taxa to biological attributes. Such lists should accommodate new species findings, which will result from additional sampling.

Assign Taxa into Biological Attributes



Attributes, in the context of bioassessments, are defined as measurable components of a biological system (Karr and Chu 1999). They include the ecological processes or characteristics of an individual or assemblage of species that may or may not provide useful information regarding response to human disturbances. Attributes for most IBIs are commonly placed in the following four general categories: species richness and composition, tolerance and intolerance to human disturbances, trophic composition, and population characteristics (including health and condition of individuals). After defining the list of taxa, list the attributes within these categories that will change in value along a gradient of human disturbance from least impaired to severely damaged wetlands. Also, predict whether each attribute will increase or decrease in value as wetlands become more damaged. This list of predictions will be tested later to identify metrics, that is, attributes empirically shown to change in value along a gradient of human disturbance. Examples include a change in species richness or a change in the relative abundance of tolerant organisms. (For an example, see Module 9: Developing an Invertebrate Index of Biological Integrity for Wetlands.) The list of useful attributes will differ for any wetland type depending on the assemblage monitored. Consult other modules for more detailed discussions of potential wetland attributes for algae, birds, macroinvertebrates, amphibians, and vascular plants. The ultimate goal in the selection process is to test the many attributes and from these identify 8 to 12 metrics that show strong relationships across a gradient of human disturbance. Through the process of evaluation, many of the predicted attributes will display limited value for detecting human disturbance. Expect only about one-third of the initial predictions to actually be good metrics.

Each attribute considered for an IBI should be based on sound ecological theory. Although theory can be a good guide for selecting metrics, it must be tested with real-world data before a metric is used. Even if the underlying theory is sound, many variables control an attribute's response to human disturbance, which in turn affects its utility. For example, an attribute that works in one type of wetland may not work in another type because of differences in ecological function and the prevailing human disturbance. In stream fish IBIs, for example, the "anomalies metric" (percentage of individuals with lesions, tumors, or eroded fins) may function in only extremely degraded conditions. It provides valuable information to a region if at least some of the streams are severely degraded but little information if all streams are only moderately degraded or unimpaired. There may even be inherent differences in how an attribute responds biologically to human disturbances. In the case of stream fishes, the number of species typically declines with added human disturbance; however, in some coldwater streams, the effect may be reversed because increased nutrients and temperatures may result in increased species numbers. Thus, it is necessary to test attributes and their underlying assumptions not only to validate the existence of an empirical dose-response relationship, but also to be able to understand and predict the nature of that relationship. The primary underlying assumptions that have been used in stream IBIs are presented in Table 2. Although not all of those assumptions have been extensively tested in wetlands, at least some appear to be applicable to wetlands.

Attribute Categories

Because development of multimetric indexes for wetlands is relatively new, no one group of organisms has proven superior to another for bioassessment. In fact, several taxa or taxa groups show promise. Regardless of the taxonomic groups sampled, as previously stated, several studies suggest that attributes and metrics can be conveniently grouped into the following categories:

- Species richness and composition
- Tolerance and intolerance to human disturbances
- Trophic composition
- Population characteristics (including health and condition of individuals)

Because most multimetric studies have been conducted in streams, there may be metric categories that are yet to be considered in wetlands. Therefore, this categorization is meant as a general guide to the development of attributes and metrics. A properly constructed IBI, as defined by Karr and Chu (1999), will have some metrics from each of these metric categories.

Species richness and composition

Attributes in this category are usually the most common feature of IBIs. In general, these attributes display a declining response to added human disturbance (Karr 1981). Normally, a population must be viable at a site for some period of time before one can consistently detect a species presence (Karr and Chu 1999). The absence of a species at a site (especially a species with low dispersal abilities) may suggest that viable populations are not being maintained. Over time, species assemblages have evolved that are capable of withstanding or rapidly recovering from most natural perturbations. For example, invertebrates often adapt to tolerate the frequent drying that is typical in many wetland habitats (Sharitz and Batzer 1999). However, changes in the chemical, physical, and biological environment caused by humans often cannot be tolerated; thus one or more species declines in abundance or becomes extirpated (Karr et al. 1986, Euliss and Mushet 1999, King and Brazner 1999). This observation is true particularly for the most intolerant taxa of an assemblage. For example, in a Minnesota study, impairment to wetlands resulted in a reduction in the number of kinds of dragonflies, damselflies, mayflies, caddisflies, and chironomids (midges), with very few intolerant taxa being observed at the most impaired sites (Gernes and Helgen 1999).

Attributes within this category generally include overall taxa richness as well as richness for taxa that are particularly sensitive to specific kinds of degradation (e.g., impairments to benthic habitats) (Kerans and Karr 1994, Fore et al. 1996). Groupings may include taxon (or taxa) (e.g., number of dragonfly and damselfly genera), specialized habitat (e.g., submerged aquatic plant species), or a combination of both. Attributes within the category have often been refined by restricting the groupings

Table 2: Assumed effects of environmental degradation on biological assemblages in streams

Number of native species, and those in specialized taxa or guilds, declines*

- Number of sensitive species declines
- Percent of trophic and habitat specialists declines
- Total number of individuals declines*
- Percent of large individuals and the number of size classes decrease
- Percent of alien or nonnative species or individuals increases
- Percent of tolerant individuals increases
- Percent of trophic and habitat generalists increases
- Percent individuals with anomalies increases

* In some instances, particularly in oligotrophic environments, reverse relationships may be observed.

Source: Modified from Hughes and Oberdorff 1999.

Note: Effects on wetland biological assemblages may be different.

to native species. However, the taxonomy and geographic origin of many wetland organisms is poorly known, which may limit the use of certain taxa in this regard.

Tolerance and intolerance to human disturbances

Tolerance, as it relates to IBI development, implies a general tolerance of a species, or group of species, to several human disturbances, rather than tolerance to a specific variable. Therefore, in attribute development, a concept can be applied in several ways to any organism, or group, that displays these tendencies. Metrics such as percentage of exotic species, number of intolerant species, and percentage of dominant species are ways of expressing characteristics that sometimes are related to tolerance. Some taxa are very intolerant (i.e., are very sensitive) to a variety of perturbations, whereas others are adept at exploiting particular types of disturbances. For example, in Minnesota wetlands, increasing levels of impairment resulted in fewer intolerant plant species, such as iris (*Iris* sp.), slender riccia (*Riccia fluitans*), and common bladderwort (*Utricularia macrorhiza*), and increased coverage of tolerant species, such as reed canarygrass (*Phalaris arundinacea*), duckweed (*Lemna* sp.), and cattail (*Typha* sp.) (Gernes and Helgen 1999).

Intolerant species may be among the first to be decimated after perturbation and the last to recolonize after normal conditions have returned (Karr et al. 1986). Endangered or threatened species should not automatically be considered intolerants, because their low numbers may be due to factors other than human disturbance. They might, for example, be glacial relics (Karr et al. 1986). Trends (increases or decreases) in distribution or abundance from historical data can be examined to help assign taxa to these attributes. Tolerance rankings may also be based on factors that indicate the ecological conservatism of taxa (those taxa adapted to a specific narrow range of biotic and abiotic factors) (Wilhelm and Ladd 1988, Andreas and Lichvar 1995). However, because of a lack of information for many wetland taxa, empirical, rather than theoretical, approaches may be necessary or preferred to establish tolerance rankings. For example, taxa that are represented in the least impaired sites and tend to disappear in the most impaired sites would be empirically defined as intolerant. Similarly, taxa that tend to increase in disturbed sites would be defined as tolerant (Gernes and Helgen 1999).

The mere presence of intolerant taxa is a strong indicator of good biological condition. The relative abundance of these taxa, in contrast, is often difficult to estimate accurately without extensive and costly sampling efforts (Karr and Chu 1999). Therefore, intolerant taxa should be represented simply as the number of intolerant species per unit sample effort. In contrast to intolerant taxa, the presence alone of tolerant taxa says little about biological condition, because tolerant groups inhabit a wide range of places and conditions. However, note that many wetland organisms can tolerate the stressful levels produced by a variety of natural environmental disturbances (Wissinger 1997, Euliss et al. 1999, Higgins and Merritt 1999), and care should be taken to base tolerance designations on human disturbances and not natural ones.

Tolerance attributes should be expressed as the percentage of tolerant individuals from either a single species or a grouping of highly tolerant species. Note that if a high number of tolerant or intolerant species is included in the composition of attributes, the usefulness of those attributes will be diminished. In general, it is recommended that only about 10% (no fewer than 5% or no more than 15%) of taxa in a region should be classed as intolerant or tolerant. The point of these metrics is to highlight the strong signal coming from the lowest and highest ends of the biological integrity continuum, without being swamped by the weak or intermediate signals from in between (Karr and Chu 1999).

Trophic composition

Because the food base is central to the maintenance of a community, information about trophic composition may be important to an IBI. All organisms require a reliable source of energy. The dominance of trophic generalists occurs as specific components of the food base become less reliable, and the opportunistic foraging habits of the generalists make them more successful than trophic specialists (Karr et al. 1986). Sometimes entire groups of specialized organisms have been reduced or extirpated from ecosystems as a result of human disturbances. For example, top carnivores such as ospreys and bald eagles have disappeared from regions as a result of eggshell thinning and reproductive failure associated with the bioaccumulation of chlorinated hydrocarbon pesticides from aquatic food webs (Carson 1962, Rudd 1964, Thomann 1989). Depending on the type of disturbance, primary producers and invertebrates of wetlands may also respond as the ecosystem begins to function improperly. Some wetland invertebrates will respond to both bottom-up (e.g., enrichment, exotic plants) and top-down (e.g., heavy metal biomagnification, exotic fish) alterations in the food web (Rader 1999). For example, the benthic communities of contaminated Louisiana bayous supported a "poor trophic mix" of macrobenthic invertebrates, and fewer predatory invertebrates and suspension feeders were found at those sites than elsewhere (Gaston 1999). Thus, the trophic structure of a community can provide information on patterns of consuming and producing organisms that are affected by impairment.

In general, attributes in this category can be designed to assess trophic balance and structure. For example, the biota of stream ecosystems in good condition usually will not be overly dominated by omnivores or trophic generalists and will normally contain at least some trophic specialists, including top carnivores that form the apex of many aquatic food pyramids (Karr et al. 1986). However, manipulations of top carnivores have been shown to have little effect in some ecosystems, such as wetlands (Batzer 1998), whereas such manipulations have had a significant effect in others, such as lakes (Carpenter et al. 1987). Furthermore, surface water levels in most natural wetlands fluctuate widely. Such variation also affects water and substrate chemistry, nutrient availability, vegetation, primary production, detritus processing, and the presence of vertebrate predators (Wissinger 1999). Therefore, caution should be exercised in developing trophic attributes for wetlands, and particular attention should be paid to those attributes involving functional feeding groups. Karr and Chu (1999) observe that despite widely accepted theory, metrics pertaining to functional feeding groups among benthic macroinvertebrates may or may not be good indicators; their dose-response relationship to human influences must be carefully tested and established for multiple data sets and circumstances before they are used in a multimetric index.

Population attributes

The attributes in this category assess characteristics of populations such as reproduction, growth, and condition of individual organisms within the populations (Fore et al. 1996). Ecosystems can maintain themselves only if the populations of organisms that characterize the ecosystems are able to compensate for loss of members by reproducing. Human disturbances that negatively affect reproduction are ordinarily indicated by an accompanying reduction in the proportion of reproductive specialists. In addition, conditions must be favorable for the young of a population to survive, disperse, and grow to sexual maturity (Pickett and White 1985, Wissinger and Gallagher 1999). For example, the eggs of some species, such as fairy shrimp, require aerated soil for development; therefore, these species rely on the drying up of temporary ponds for reproduction. Any alterations of the frequency and duration of ponding in wetlands that contain these species would greatly influence the viability of populations. In another example, certain invertebrates have become extremely specialized in their dependence on the fluid of pitcher plants (e.g., *Sarrecinia purpurea*) to reproduce and carry out their life cycle. At least some of these inquilines (organisms that live within the abode of another species) are poor dispersers and may be slow to colonize isolated wetland habitats that have become fragmented as a result of human disturbance (Giberson and Hardwick 1999). Therefore, attributes that characterize population structure and patch dynamics can be effective indicators of human disturbance.

Certain structural and functional attributes of wetland vegetation and algae are also included in this category. Many of these attributes have been used in and out of the context of IBI to assess a wide variety of human disturbances (e.g., hydrologic changes, nutrient enrichment, sediment loading, and accumulation of heavy metals). For example, increased algae biomass is one of the most widely used indicators of eutrophication in aquatic ecosystems (see Module 11: Using Algae To Assess Environmental Conditions in Wetlands). Likewise, the percent coverage of submerged aquatic vegetation has been a useful indicator of nutrient enrichment in many estuarine environments (see Module 16: Vegetation-Based Indicators of Nutrient Enrichment in Freshwater and Estuarine Wetlands). Minnesota has used increased persistent plant litter, resulting from human-induced changes in the composition of the plant community, as an IBI attribute to indicate reduced detrital energy to drive wetland ecosystems (Gernes and Helgen 1999).

Individual abundance is a common surrogate for system productivity, and some types of highly degraded sites are expected to support fewer individuals than are high-quality sites (Karr 1981). However, Karr and Chu (1999) suggest that abundance may be a poor candidate for a multimetric index because it varies too much, even when human disturbance is minimal, and it is also difficult to measure and score. Recognizing the tendency for moderate levels of nutrient and thermal enrichment to elevate fish abundance, Oberdorff and Hughes (1992) scored this metric so that very high abundances received lower metric scores than moderate numbers did; only very low abundances received the lowest score. This scoring adaptation is an example of the need to evaluate metric performance along disturbance gradients before applying the IBI in resource assessments (Hughes and Oberdorff 1999).

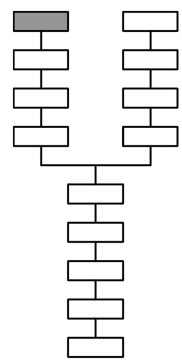
Sites with especially severe degradation often yield a high number of individuals in poor health (Mills et al. 1966, Brown et al. 1973, Baumann et al. 1982, Sanders et al. 1999). Parasitism has been shown to reflect both poor environmental condition and reduction in reproductive capacity (sterility) in fish (Mahon 1976). Indications of poor health include individuals with tumors; limb, mouthpart, or other structural malformations; heavy infestations of parasites; and discoloration, excessive mucus, edema, rash, or hemorrhaging. For example, malformation in chironomid mouthparts has been used to indicate impairments to water from sedimentation, eutrophication, and contamination (Warwick 1980). Recently, increasing numbers of amphibian populations with unusually high frequencies of morphological abnormalities (>5%) have been reported throughout North America; however, the ecological significance of these observations remain poorly understood (Ouellet 2000, Helgen et al. 2000). Leonard and Orth (1986) found increases in the incidence of disease and anomalies in stream fish only after substantial degradation was evident, indicating that this metric may be sensitive at only the most severely impaired sites. In cases in which only low to moderate levels of impairment occur, the metric has been dropped; however, it should be considered wherever the possibility exists for changes in the incidence of diseased or deformed organisms (Hughes and Oberdorff 1999).

Lessons Learned from the Biological Assessment of Wetlands Working Group

These lessons should be treated as general rules of thumb and not absolutes. Nature keeps reminding us that when dealing with biology and ecology, there are always exceptions to the rules. The following lessons on attributes and metrics are based on the experience of the Biological Assessment of Wetlands Working Group (BAWWG) members:

- Explicitly define attributes. To aid in testing attributes and to improve communication with others, it is very important to explicitly define attributes. There are many ways to examine an attribute. For example, tolerance can be described in the following ways:
    - Number of tolerant individuals
    - Percentage of tolerant individuals (number of tolerant individuals/total number of individuals)
    - Number of tolerant species or taxa
    - Percentage of tolerant species, or taxa (number of tolerant taxa/total number of tolerant taxa)
- Be careful in using measures of abundance. Although Karr and Chu (1999) suggest that abundance may be a poor candidate for a multimetric index, BAWWG members have found some exceptions to this rule. For example, the abundance, or biomass, of algae may be a useful attribute, but further testing is needed.
- Avoid combining attributes. In general, restrict ratios to relative abundance or proportions in which the total number of individuals or taxa is used as the denominator. Avoid using attributes such as the number of water boatmen taxa divided by the number of midge taxa. Also be careful about adding attributes together, such as the number of sedge taxa plus the number of rush taxa plus the number of grass taxa. Ratios and sums of attributes can hide valuable signals, can be difficult to interpret, and are often more variable than the individual attributes (Karr and Chu 1999). It may be more effective to test each attribute independently. However, BAWWG members have found some exceptions to this rule. For example, Minnesota uses a metric that combines the number of mayfly and caddisfly genera plus the presence of dragonflies and fingernail clams (Table 3).

Classify Wetlands into Regional Classes



Successful biological monitoring begins with a judicious classification of sites. Classification is an essential step in developing bioassessment methods. Biological assemblages are influenced by natural variations in wetland types (e.g., landscape position, source of water) and by variations in the amount and type of human disturbances. Classification is a way to account for the effects of natural environmental influences on wetlands and helps avoid comparing wetlands of unlike classes. Yet, excessive emphasis on classification, or inappropriate classification, can impede development of cost-effective and sensible monitoring programs. Using too few classes fails to recognize important distinctions among places and can produce insensitive metrics; using too many adds unnecessary costs to the development of biocriteria. The challenge is to create a system with only as many classes as needed to represent the range of relevant biological variation in a region and the level appropriate for detecting and defining the biological effects of human activity in that place (Karr and Chu 1999).

It is important to remember that for the purpose of developing bioassessment methods, classification is a way to help minimize "noise" and make it easier to identify signals of human disturbance. Clas-

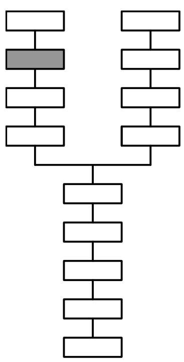
Table 3: Invertebrate metrics and scoring for a wetlands invertebrate index of biological Integrity (IBI)

Metric	Description	Range	Score	Ref	Ag	SW
Richness metrics						
Total taxa	# Genera chironomids, caddisflies, mayflies, damselflies, dragonflies, leeches, snails, macrocrustaceans, presence of Chaoborus and clams	28 - 46	5	5	3	
		20 - 27	3	1	3	6
		12 - 19	1		2	4
Chironomid	# of genera of Chironomidae larvae	13 - >20	5	3		
		6 - 12	3	2	5	6
		> 70%	1		4	5
ETSD	# Genera of Ephemeroptera, Trichoptera, plus presence of Sphaeriidae and Dragonflies	4 or more	5	6		
		2 - 3	3		3	6
		> 70%	1		5	5
Leech taxa	# Genera of Hirudinidae found in BT and DN samples	4 - 5	5	4	2	2
		2 - 3	3	2	5	8
		0 - 1	1		1	1
Odonata	# Genera of Dragonfly & Damselfly larvae	4 - 6	5	5		
		3	3	1	4	3
		0 - 2	1		4	8
Snails	# Taxa of snails to species most cases	5 - 7	5	4	1	1
		3 - 4	3	1	5	6
		0 - 2	1	1	2	4
Tolerance/Intolerance metrics						
Intolerant Taxa	# Intolerant taxa: Leucorrhinia, Libellula, Tanytarsus, Procladius, Triaenodes, Oecetis	4 or more	5	5		
		2 - 3	3	1		
		0 - 1	1		8	11
Proportion metrics						
Corixidae	% Corixidae of Hemiptera plus Coleoptera from BT samples.	< 30%	5	5	2	1
		30 - 70%	3	1	2	5
		> 70%	1		3	4
Erpobdella	% Erpobdella in BT and DN samples of the total abundance in DN samples.	0 - 11%	5	6	3	6
		>11 - 22%	3		2	3
		> 70%	1		3	2
3 Dominants	% top 3 dominants of total abundance in DN samples	34 - 55%	5	5	1	
		> 55 - 80%	3		6	9
		> 70%	1	1	1	2

Abbreviations: BT, bottletrap; DN, dipnet; number of sites scoring in range is given for Reference sites (Ref), agricultural (Ag) and stormwater (SW) influenced wetlands (Gernes and Helgen 1999).

sification is not the end product, and there is no perfect classification system. Also, classification is an iterative process. Try applying an existing classification system (e.g., Cowardin et al. 1979, USDA 1981, Brinson 1993, Omernik 1995, Omernik and Bailey 1997) and then lump and split as needed to produce groups of biologically similar wetlands that respond in similar ways to anthropogenic influences. For further discussion of classification issues and approaches, including some wetland bioassessment examples, see Module 7: Wetlands Classification.

Target Selection of Sample Sites



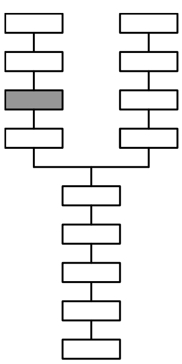
After wetlands are classified, sample sites should be selected. Module 4: Study Design for Monitoring Wetlands describes the options for developing sampling schemes for a wetlands monitoring program, including random sampling, targeted sampling, and before/after or control/impact designs. The members of BAWWG recommend the use of a targeted sampling design when developing bioassessment methods for wetlands. They have found that random sampling often fails to capture enough least impaired reference wetlands or enough of the severely degraded wetlands. Also, random sampling may not consider important factors such as accessibility to sites and the selection of relatively permanent reference sites that will not be subjected to a great deal of future human disturbance. The process of testing attributes and selecting metrics depends on plotting data on a graph with an attribute value on the y-axis and a gradient of human disturbance on the x-axis. It is very important to select enough reference wetlands and severely damaged wetlands to anchor both ends of the disturbance gradient. It is also important to select enough wetlands with varying degrees of human disturbance in order to reveal a signal in the attribute if there is one. Based on the experience of the BAWWG members, targeted sampling is probably the best way to ensure that this is accomplished. The development of human disturbance gradients is discussed further in this module in the sections "Collect Land Use and Habitat Information" and "Establish Gradient of Human Disturbance."

One challenge to the characterization of reference conditions is that there are few, if any, places left that have not been influenced by human actions. In many regions, what are described as least impaired wetlands may in fact be very degraded, because accessible less-degraded sites cannot be found. This may complicate the development of an IBI but does not prohibit it. A common pitfall is the failure to canvass a wide enough area for sites that may be less impaired. It may be advantageous to survey neighboring areas and expand the geographic range to find similar wetlands in better condition. Another failure is to not include an adequate number of most impaired sites. Sampling only from the least impaired end of the gradient creates problems because it does not represent the full range of site degradation and it prohibits analysis of the relationship between biological attributes and highly negative human influences.

Biological communities in wetlands with a high proportion of degrading land uses (e.g., commercial, residential, agricultural) in their vicinity or watersheds can sometimes be assumed, for purposes of developing an IBI, to be potentially stressed. However, forested wetlands, and in particular, forested wetlands with dense canopies, should not automatically be considered the least impaired. This is because in some regions, some wetlands are normally dominated by herbaceous vegetation due to persistently high water table levels and/or other natural influences. Some of these herbaceous vegetation sites may in fact be in better condition than are forested wetlands. However, if herbaceous wetlands historically prevailed in an area, their invasion by woody vegetation could be a sign of stress, not an indication of good health.

As mentioned previously, it is important to select sites that are minimally impaired reference sites and severely damaged sites as well as some in between to represent the full gradient of human disturbance. The number of sites that are needed depends on the number of wetland types within a given class, the ecological variability of the wetland types, the kinds and amount of human disturbances, and the size of the region. The number of sites should be sufficient to show a gradient of human disturbance for each wetland class in the region. See Module 7: Wetlands Classification for more detail on classifying wetland types. Sampling an adequate number of sites will provide confidence in the sensitivity of the metrics showing a response.

Collect Land-Use and Habitat Information

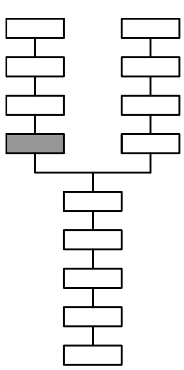


Before starting the field sampling, gather information about the study sites through published sources and field reconnaissance. The overall goal of this step is to collect information to help verify that wetlands are classified correctly, provide data for constructing human disturbance gradients, and provide insights into why biological communities are damaged during the IBI interpretation phase. A wealth of information can be collected about sites without even leaving the office. Information sources include the following:

- U.S. Geological Survey quadrangle topographic maps. These maps can provide baseline information on slope, elevation, land use, and the hydrological network in the vicinity of the study wetland.
- National Resource Conservation Service soil surveys. These surveys are an invaluable source of information on wetland soils and their characteristics. Hydric soils are mapped and descriptions of soil characteristics and how they vary by soil horizons are provided.
- National Wetland Inventory maps. These maps are useful for identifying the presence of wetlands and estimating wetland size. They also include the Cowardin et al. (1979) classification.
- U.S. Department of Agriculture aerial photos. If available, these photos are very useful for gathering information on the landscape surrounding wetlands. They can also be used to reconstruct historical changes in land use by analyzing a series of photos taken over past years.
- Geographic Information System (GIS) databases. Many state and local units of government have digital land-use and other information available on GIS databases. Often, this information can be linked to other available data (e.g., percent impervious surface and point source discharges) to locate and display geographically referenced sources of impairment. Such information is also increasingly available from many online websites. The layering and interactive features of GIS databases greatly enhance landscape-level assessments (see Module 17: Land-Use Characterization for Nutrient and Sediment Risk Assessment). However, the scale and quality of GIS information should be closely examined in view of the intended use. Other information, such as low-altitude photographs, may be needed to supplement GIS information in order to derive a meaningful estimate of the gradient of human disturbance.

In addition to landscape features, onsite wetland characteristics are important in establishing gradients of human disturbance. Onsite investigations should provide general information on landscape setting, hydrologic features, and vegetative cover of the wetland. Such observations can generally be made from visual assessments from the wetland's edge. Obvious stressors, such as hydrologic alterations (e.g., ditches and dikes), should be identified. The extent of vegetation cover and the characteristics of buffer areas should be noted. Buffer areas should also be clearly defined (e.g., the 100 m of land surrounding the wetland boundary at full pool), and land uses within those buffers should be described. Examples of other data that should be recorded at each site include the following:

- Topography and landscape features that surround the site. Many characteristics should be recorded, including the shape of the wetland; adjacent land use; the general distribution of wetland vegetation and open water; the interspersion of plant communities; the type of buffer; hydrological features, including surface water inflows and outflows; and human-made water control structures. This information should be included on a site sketch map.
- Patterns in the vegetation community, such as the number of community types and their location in relation to each other, the vegetation strata present, the dominant species, and presence of nonnative or invasive species.
- Evidence of human disturbance (if any). One way to approach this question is to prepare a stressor checklist that can be used to survey each site (see "Establish Gradient of Human Disturbance" below for potential stressors).
- An estimate of the size of the wetland.
- Photographs of the site.



Establish Gradient of Human Disturbance

Once sites have been classified by natural factors, it is essential to categorize the same sites according to degrees of human disturbance. This step is important to ensure that metrics are sensitive. Human disturbance serves as the gradient along the x-axis to which biological attribute data along the y-axis are compared. Determining the human factor and the likely range of the gradient must be done before sampling begins, not as an afterthought, because post hoc categorization may reveal that the full range of human disturbance was not captured, thus requiring additional sampling to develop a useful IBI.

To help establish sampling sites that reflect the full range of human disturbance, it can be useful to first identify and perhaps prioritize the main types of disturbances in a region, such as hydrologic modification, eutrophication, sedimentation, and chemical contamination. U.S. Environmental Protection Agency (EPA) and State reports (305b, 303d) can be used with other sources, particularly interviews with local conservation groups and resource agencies, to obtain an initial indication of watersheds whose wetlands might be candidates for designation as least or most impaired sites. In rare instances, detailed descriptions may exist of a wetland's biological communities before its alteration and development of the surrounding watershed. In such instances, the historic condition can be used to define the least impaired reference condition.

In some cases, human disturbance may comprise a single impairment, such as vegetation removal by grazing. For example, Montana used a simple discrete disturbance gradient based on grazing and farming for wetlands in a rural landscape (Apfelbeck 1999). Three classes were identified: wetlands that are currently grazed or farmed, wetlands that were grazed or farmed but not within the recent past, and wetlands that have either never been grazed or never been farmed or at least not within recent history. In most circumstances, diverse and often subtle human activities interact to affect conditions in watersheds, bodies of water, or wetlands. As a result, it may be advantageous to develop a gradient that integrates several human disturbances to account for multiple stressors.

Where there is adequate information, the development and use of an index may greatly enhance the establishment of the gradient of human disturbance. Such an index incorporates values that represent the various degrees and combinations of prevailing human disturbances for all sites, not just the least and most impaired, as illustrated in the Minnesota Wetland Disturbance Analysis (Figure 2). Although there is no standard protocol for constructing such an index, human disturbance should not be represented simply by a single score from a single source of disturbance. Instead, it should be developed by combining scores from several prevailing disturbances from both the landscape and the wetland. Possible landscape-level disturbances in an index include the following:

- Percentage of the watershed within impairing land uses (e.g., cropland, pastureland, and urban land)
- Presence, condition, and width of wetland buffer
- Proximity of wetland to other natural habitats (e.g., wetlands, natural grasslands, and forest)
- Percentage of other natural habitats in close proximity (e.g., a 1-km radius) of the wetland
- Impoundment of runoff or flow by dams or berms located upstream or upslope

Possible local human disturbance factors in an index include the following:

- Impoundment of runoff or flow by excavations (pits) within wetlands
- Redirection of runoff or flow within wetlands
- Extraction of water from the wetland (e.g., for irrigation)
- Removal of vegetation (e.g., through logging, grazing, mowing, and controlled burning)
- Flattening of wetland microtopography
- Steepening of wetland topography (especially shorelines)
- Persistent disturbance of wetland soils or vegetation through compaction, tillage, or trampling
- Drainage of wetlands through ditching or tiling
- Water or atmospheric deposition
- Simplification of shoreline complexity
- Pollution (e.g., thermal and chemical)

The type of land cover that surrounds a wetland is generally one of the most dominant influences on wetland condition (see Module 17: Land-Use Characterization for Nutrient and Sediment Risk Assessment). GIS information can be useful in mapping and analyzing land-use patterns and other spatial relationships that broadly affect wetlands. For example, several recent GIS studies have found significant negative correlations between watershed-wide agricultural or urban land uses and stream IBIs (Lenat and Crawford 1994, Richards et al. 1996, Roth et al. 1996, Wang et al. 1997). When considering the influence of particular land uses, it is important to consider their distance from a wetland, the intervening slope, and the time period (current or recent or distant past) at which they occurred. Land use of the surrounding landscape should be assessed within a meaningful radius around the wetland (e.g., 300 m), with greater weight given to land use in areas upstream or upslope of a site, that is, the "contributing watershed." Although GIS information can be a powerful tool for defining a disturbance gradient, it is not a replacement, or a good surrogate, for the IBI itself or for biological monitoring (Karr and Chu 1999). To supplement GIS mapping data, onsite visits are generally required to improve the assessment of human disturbances. Several states (i.e., Ohio, Oregon, Florida, and Maryland) have prepared data forms to assist in the assessment of onsite conditions for rapid field assessment of environmental risks or disturbances

Figure 2: Minnesota Wetland Disturbance Analysis

PRELIMINARY DRAFT RATING FORM (2/08/01)

The forms shown below are presented to show the concepts and framework under development in Minnesota for rating the degree of human disturbance to wetlands and their immediate landscape. These forms are to be completed while referencing various, current digitized spatial information, such as land use, wetland border, aerial photos, digital raster graphs, hydrologic data themes and field notes from site visits. These forms and the corresponding evaluation method are likely to be considerably revised and changed as the scores and method are further tested against additional data sets (Helgen and Gernes in press).

Site: **Study:** **Raters:** **Date:**

Factor 1. Buffer landscape disturbance

points

Extent and Intensity

	Best--as expected for reference site, no evidence of disturbance	(0)
	Mod.--predominately undisturbed, some human use disturbance	(6)
	Fair--significant human disturbance, buffer area nearly filled with human use	(12)
	Poor--nearly all or all of the buffer human use, intensive land use surrounding wetland	(18)

	BEST		MOD.
	Mature (>20 yr.) woodlot, forested		Old field, CRP or rangeland,
	Mature prairie		Restored prairie (<10 yr.)
	Other long recovered area		Young (<20 yr.) second growth woodlot
	Other wetlands		Shrubland

	FAIR		POOR
	Residential with unmowed areas		Urban development
	Active pasture		Industrial development
	Less intensive agriculture		Intensive residential/mowed
	Turf park, Golf course		Intensive agriculture
	Newly fallowed fields		Mining in or adjacent to wetland
	High road density in buffer area or impervious surfaces		Active construction activity

Remarks or comments:

Factor 2. Landscape (immediate) Disturbance

points

Extent and Intensity

	Best--landscape natural, as expected for reference site, no evidence of disturbance	(0)
	Mod.--predominately undisturbed, some human use disturbance	(6)
	Fair--significant human disturbance, landscape area nearly filled with human use	(12)
	Poor--nearly all or all of the landscape in human use, isolating the wetland	(18)

	BEST		MOD.
	Mature (>20 yr.) woodlot, forested		Old field, CRP or rangeland,
	Mature prairie		Restored prairie (<10 yr.)
	Other long recovered area		Young (<20 yr.) second growth woodlot
	Other wetlands		Shrubland

	FAIR		POOR
	Residential with unmowed areas		Urban development
	Active pasture		Industrial development
	Less intensive agriculture		Intensive residential/mowed
	Turf park, Golf course		Intensive agriculture
	Newly fallowed fields		Mining in or adjacent to wetland
	High road density or impervious surfaces in immediate landscape		Active construction activity

Factor 3. Habitat alteration—immediate landscape

(within and beyond buffer)

points

Severity and extent of alteration

	Best--as expected for reference, no evidence of disturbance	(0)
	Mod.--low-intensity alteration or past alteration that is not currently affecting wetland	(6)
	Fair--highly altered, but some recovery if previously altered	(12)
	Poor--almost no natural habitat present, highly altered habitat	(18)

Vegetation removal disturbances

	Mowed, Grazed		Shrub removal
	Tree plantations		Course woody debris removal
	Tree removal		Removal of emergent vegetation

Substrate/soil disturbances and sedimentation

	Grading/bulldozing		Vehicle use
	Filling		Sediments input (from inflow or erosional)
	Dredging		Livestock hooves
	Other		

Other

	Fish stocking or rearing		Other

Remarks or comments:

Factor 4. Hydrologic alteration

points

Severity and degree of alteration

	Best--as expected for reference, no evidence of disturbance	(0)
	Mod.--low-intensity alteration or past alteration that is not currently affecting wetland	(7)
	Fair--less intense than "poor," but current or active alteration	(14)
	Poor--currently active and major disturbance to natural hydrology	(21)

	Ditch inlet		Berm or dam
	Tile inlet		Road bed or RR bed
	Point source input		Levee
	Installed outlet, weir		Unnaturally connected to other waters
	Dredged		Dewatering in or near wetland
	Graded or fill		Source water changes
	Other		Drainage

Remarks or comments:

points	**Factor 5. Chemical pollution**

Severity and degree of pollution

	Best--chemical data as expected for reference and no evidence of chemical input	(0)
	Mod.--selected chemical data in low range, little or no evidence of chemical input	(7)
	Fair--selected chemical date in mid range, high potential for chemical input	(14)
	Poor--chemical input is recognized as high, with a high potential for biological harm	(21)

Checklist:

	High C1 conc. (water)		Known MMCD treatment
	High P conc. (water)		Evidence of altered DO regime
	High N conc. (water)		Other treatment
	High Cu conc. (sediment)		High input potential
	High Zn conc. (sediment)		Other

Remarks or comments:

Additional factors and concerns

points Used in exceptional cases as described below

Maximum of (4) additional points added to the cumulative disturbance total for reasons described below. Apply on factors 4 and 5.

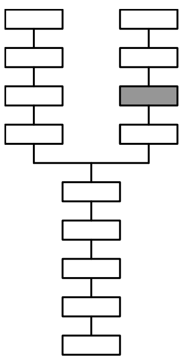
	Factor 1 - Buffer and landscape
	Factor 2 - Landscape (immediate disturbance)
	Factor 3 - Habitat alteration
	Factor 4 - Hydrologic alteration
	Factor 5 - Chemical pollution
	Additional factors

Total final disturbance score

Site: ____________________

to wetlands (e.g., the Ohio Rapid Assessment for Method for Wetlands) (Ohio EPA 2001). Independent observers should be able to repeat the assessments of disturbances and obtain the same results, that is, the assessments should be highly precise. Nonetheless, a relatively high degree of judgment usually is required to assess, a priori, the relative level of human disturbance a site is likely to be enduring. Many impairments are not clearly visible or assumable (e.g., subsurface drainage tile and chemical contamination).

Sample Within the Wetland



After wetlands have been properly classified and selected, sampling within a wetland may begin. One approach for sampling is to collect data from all habitat zones. Another approach is to find a single zonal transition area that may be indicative of the entire wetland (a wetland equivalent to the riffle zone used in streams). The goal is not to measure every attribute of a wetland, but rather to find efficient indicators of health that are expressed adequately with a minimal amount of sampling. For example, a Minnesota study collected data from the nearshore shallow emergent zone and based all comparisons on that area. That approach led to accurate assessments without the need for the more time-consuming sampling from each zone. In addition, human access to some parts of wetlands may be difficult or impossible, which would preclude sampling from every zone.

Although sampling from a single zone may, in certain instances, provide enough information to accurately assess wetland health, there are instances when it will not. For example, in streams, it is often necessary to survey several habitats (e.g., riffles, pools, runs, and backwater areas) to collect the variety of taxa needed for IBIs to be sensitive to the array of possible impairments. If the decision is made to sample several zones, the number of samples that need to be collected will increase as the area of the wetland and its heterogeneity increase. At a minimum, samples should be collected from the following zones if they are present: permanently inundated areas, seasonally inundated areas, and areas saturated but seldom inundated. For purposes of IBI development, within these three zones, samples should be collected in a manner that will yield unbiased data from the widest variety of disturbance conditions and seral stages. For quantitative metrics (such as relative abundance and percent cover) to be used in multimetric indexes, random or systematic (grid- or transect-based) location of sampling or observation points within zones may be justified, and a much larger number of samples will be necessary. The sampling strategy must be clearly described and the number of samples must be adequate to capture the variablity of the attribute being measured. For more detail on sampling, see Module 4: Study Design for Monitoring Wetlands.

In general, the number of samples or observations per zone should be roughly proportional to the area of the zone if the goal is to characterize the wetland as the assessment unit. (However, a fixed number of samples should be taken to avoid problems with increasing species richness with increasing sample numbers.) One approach to address sample adequacy is to construct species/area curves to determine the number of samples required to produce diminishing returns (an asymptotic curve) in the cumulative number of species of the target taxa (De Vos and Mosby 1969) (Figure 3). Another approach is to plot the cumulative coefficient of variation (in, e.g., the diameters of trees that were measured) against the number of samples (e.g., the number of trees measured). Other approaches for estimating sampling adequacy may be preferred, depending on assessment objectives (see Elliott 1970). Also, the boundaries of the unit to which the collected data will be extrapolated need to be clearly defined and stated. For example, will a few samples collected on the fringe of a large roadside wetland be claimed to represent the entire wetland or only the roadside fringe?

A basic premise of IBI is that the biota have been sampled in their true relative abundance without bias toward taxa or size (Karr et al. 1986). As this assumption is relaxed, the reliability of inferences based on the IBI is reduced. However, with any single method, there are inherent biases that affect the tendency to capture or notice particular organisms. Therefore, method limitations should be well understood. Standard sampling protocols should be adhered to, and the resulting metrics should be tailored to the known limitations of the methods, protocols, and sampling equipment. In addition, data collected by one sampling method should not be mixed or compared with data collected by another method.

Temporal variations may also affect the quality of the sample or limit its comparison with other data. This may be true particularly in temporary or seasonal wetlands that support organisms that are equally as periodic in nature. Therefore, it is important to know the best time of the year for the collection of certain taxa and to specifically relate that information to the protocols that govern sampling. When sampling assemblages or habitats with specific sampling periods tied to weather conditions, such as amphibians in vernal pools, it may be important to maintain flexibility in sampling periods to be able to adjust to the time of emergence. Other sections of this document discuss this in greater detail. Several methods may be appropriate for sampling the biota of wetlands, and it is important to be able to duplicate these methods from site to site and to be as consistent as possible. Sampling should always be conducted in a way that maintains both precision and accuracy (see Module 4: Study Design for Monitoring Wetlands).

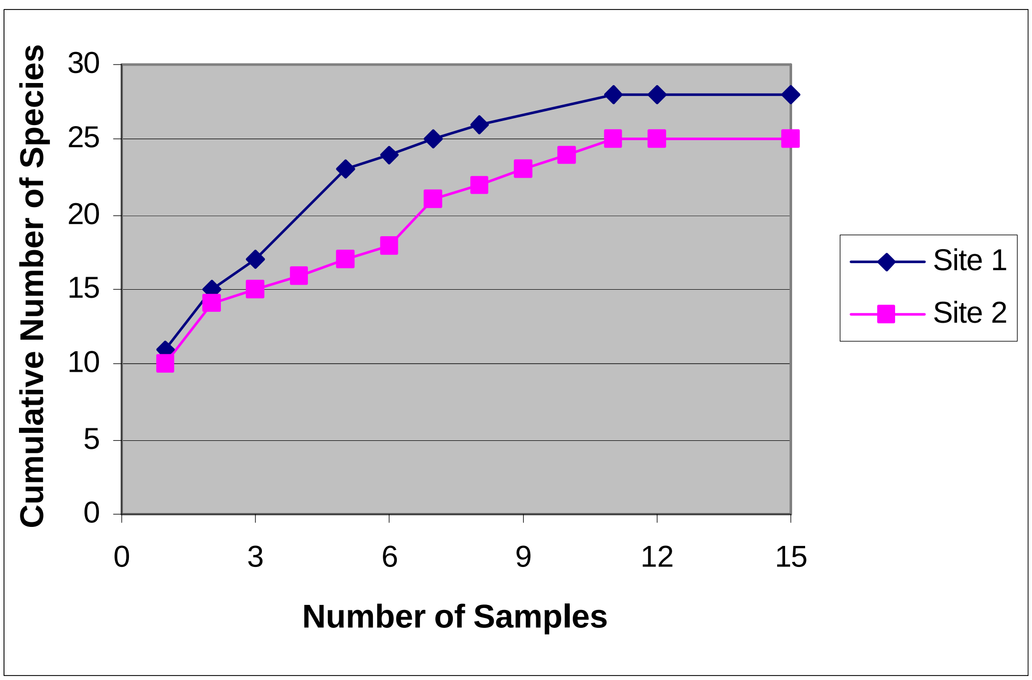


Figure 3: Species/area curves for wetland invertebrate samples.

Demonstrates the asymptotic relationship (leveling of the curve) that should be considered for the determining the level of sampling effort (preliminary data from Sparling et al.).

In addition to sampling the biota, most projects involving the development of bioassessment methods also sample some physical and chemical attributes of the study sites (Sparling et al. 1996; Fennessy et al. 1998; Gernes and Helgen 1999). This information is particularly useful for verifying that wetlands are classified properly and for investigating how a wetland is damaged. Data or methods from existing functional assessments may be useful for helping to characterize the structural characteristics of wetlands. For example, the hydrogeomorphic approach (Brinson 1993) or state rapid assessments can provide valuable information to support the development of IBIs for wetlands. Ohio EPA (2001), for example, uses information from its Ohio Rapid Assessment Survey to gather information on structural characteristics of wetlands. Ohio EPA also collects additional information from water quality tests. Collecting physical and chemical information for wetlands that can be useful for developing IBIs should include hydrological indicators and soil characteristics.

Hydrological indicators include the following:

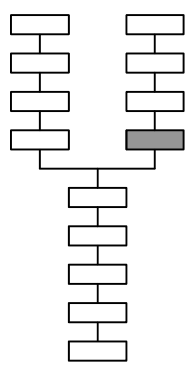
- Presence of conducted stormwater or agricultural drainage
- Water marks, stained leaves, sediment deposits, or soil saturation in the top 30 cm (see U.S. Army Corps of Engineers 1987)
- Water chemistry parameters (If the system is seasonally flooded and water chemistry data are required, this information must be taken into account when developing the sampling design. It may be necessary to sample water chemistry at different times than vegetation sampling. Parameters to analyze might include pH, dissolved oxygen, conductivity, total phosphorus, nitrogen, total suspended solids, chloride, metals (in some instances), and total organic carbon)
- Percent salinity and conductivity
- HGM class, for data on site hydrology
- Temporal and spatial patterns of water area and depth

Soils can be easily characterized using a standard soil probe. The following soil data should be recorded in each vegetation community type:

- Thickness of the organic layer
- Soil texture
- Color as determined by a Munsell Color (1975) soil chart
- Presence of mottles and their size and color as well as the presence of oxidized root channels

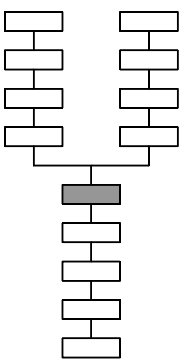
Depending on the study goals, it may be necessary to collect soil samples for analysis. Standard analysis includes pH, percent organic matter, nutrients, and perhaps metals. Total phosphorus can be a good indicator of disturbance and the deposition of heavy sediment loads. Also, calcium levels in the soil may be a good index of stress severity.

Summarize Sample Data by Biological Attributes



A spreadsheet or database is useful at this point to summarize sample data. Many spreadsheet applications (e.g., Lotus 1-2-3 and Microsoft Excel) are easy to learn, but they can be cumbersome and difficult to use for exploratory analysis. Database applications are more difficult to learn, but they offer more features and flexibility for conducting exploratory analysis and constructing lists based on certain parameters. Some states use a combination of spreadsheets, databases, and statistical software. Regardless of the type of computer software used, the data from field data sheets must be entered into a computer and summarized on the basis of the list of attributes. Field data sheets often include a list of species or taxa with a number next to those found at a site. The number depends on the assemblages monitored (e.g., the number of individuals or the percent cover of a species). After the data are entered into the computer, they must be summarized based on the attributes.

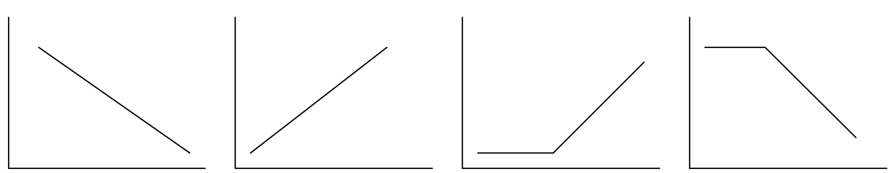
Evaluate Attribute Performance Across a Gradient of Human Disturbance



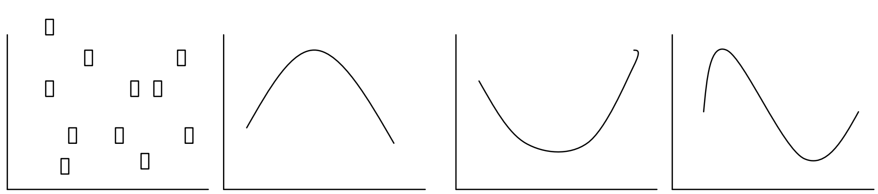
The need to test and validate biological responses of attributes and metrics across degrees of human disturbance is a core assumption of the IBI (Karr and Chu 1999). Ideally, metrics that are incorporated into a multimetric index should be relatively easy to measure and interpret. They should change in a predictable fashion. The metric should be sensitive to a range of biological disturbances and not narrowly focused on one particular aspect of the community (e.g., species richness). Most importantly, metrics must be able to discriminate between human disturbances and the background "noise" of natural variability. Human impact should be the focus of biological monitoring (Karr and Chu 1999).

The process for selecting metrics for use in a multimetric index involves testing a large set of biological attributes (candidate metrics) and then selecting the ones that are most sensitive to various aspects of human disturbance. Although large numbers of attributes may need to be explored while developing a multimetric index, it is inevitable that there will be spurious relationships when considering large numbers of disturbance-attribute pairings. A good metric will show a strong signal (response) to increased disturbance and does not give mixed signals. Attributes that show large variation (extremely wide ranges in response when data from a variety of sites are plotted) will have less utility. For example, the following simplified graphs show some acceptable relationship for metrics:

The simplified graphs below show relationships that have limited acceptability. Exceptions are noted with the two middle two panels, which indicate peak attribute responses with intermediate levels of disturbance. Attributes with such responses may make appropriate metrics with proper adjustments in metric scoring.

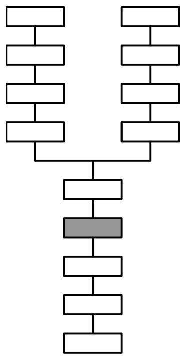


X-Axis = Increasing Human Disturbance
Y-Axis = Attribute Value



X-Axis = Increasing Human Disturbance
Y-Axis = Attribute Value

Select Metrics from Best Performing Attributes



For most taxa, at least 5 metrics (but preferably 8–12) should be defined and selected to construct a multimetric index. Each metric should reflect the quality of a different aspect of biota that responds in a different manner to disturbances to wetlands (Margalev 1963, Fausch et al. 1990, Hughes and Noss 1992). Therefore, whenever possible, care should be taken to select metrics from several different metric categories (e.g., species composition and richness, tolerance and intolerance, trophic composition, and population characteristics) to ensure that response is derived from a range of influences and that redundancies are minimized. In general, the wider the range of environmental influences represented by the metrics the better (Table 2). For example, if long-lived taxa requiring years to mature are present, one can infer that the spatial and temporal components they require are also present. Excessive production of herbivorous species may be indicative of excessive nutrients. High levels of toxic substances may be inferred from the frequent occurrence of individuals with disease or other anomalies (Karr and Chu 1999).

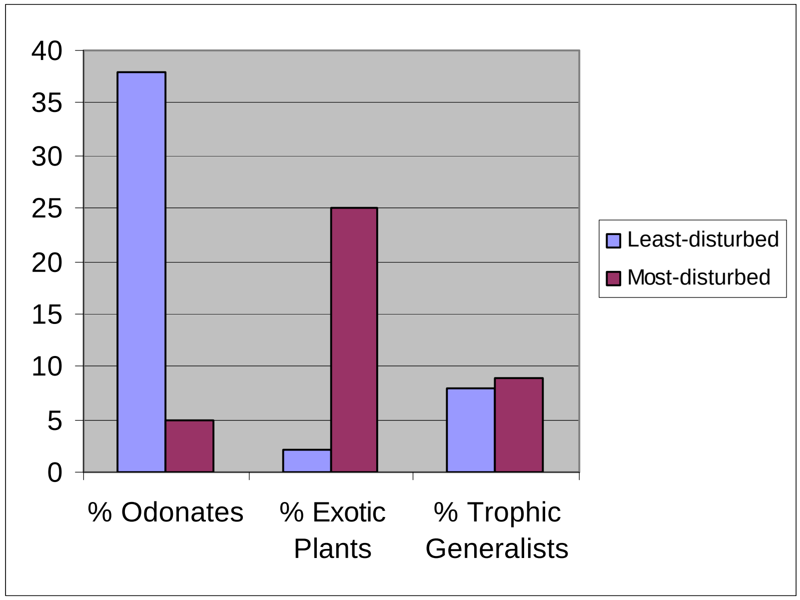
The performance of each attribute should be evaluated by assessing how well it does the following: (1) increases or decreases along a gradient of human disturbance, (2) separates the least from the most impaired sites, (3) provides similar values for similarly impaired sites, and (4) provides a unique (nonredundant) discriminatory response (Karr et al. 1997). Several graphical approaches and statistical tests may be used to evaluate attribute performance. Each may be used either individually or in concert with another to screen out attributes that do not perform acceptably and retain those that do. One frequently used approach is to create bar graphs or box plots showing means or medians and variances of a particular attribute at sites believed to be least and most impaired (Mundahl and Simon 1999). Several commercial software programs can do this. The degree of separation between the least and most impaired sites can then form the basis for retaining or discarding the attribute for subsequent analyses (Figure 4). The statistical significance of the separation can be confirmed using standard statistical tests such as *t* tests.

Another frequently used approach is to compare attribute data not just from the extreme sites, but from all sites across the spectrum of human disturbance. For that comparison, the disturbance gradient can be based on a single variable representing one or more human disturbances or several variables or an index representing multiple human disturbances (Figures 5 and 6). The relationship can be expressed either graphically (scatter plot) or by a comparison of correlation coefficients, such as Pearson's correlation coefficient (Figure 5). Based on the degree of correlation, certain attributes may be retained and others may be eliminated (Figure 7).

Attributes that contain many of the same taxa may be considered redundant, and although some redundancy is acceptable, seek to reduce double counting or using the same taxa over and over. Redundancy can be tested statistically, for example, through factor analyses (Hatcher 1994) or by simply examining similarities in the taxa groupings that form each attribute. Simple tables can be constructed to compare metric performance over the various tests.

FIGURE 4: EVALUATION OF METRIC PERFORMANCE.

Examples of bar graphs illustrating the degree to which attributes separate least- from most-disturbed sites (expressed in mean percentages). Based on degree of separation two of the attributes may be retained for further evaluation (% Odonates and % Exotic Plants) while the other may be eliminated (% Trophic Generalists) (hypothetical).



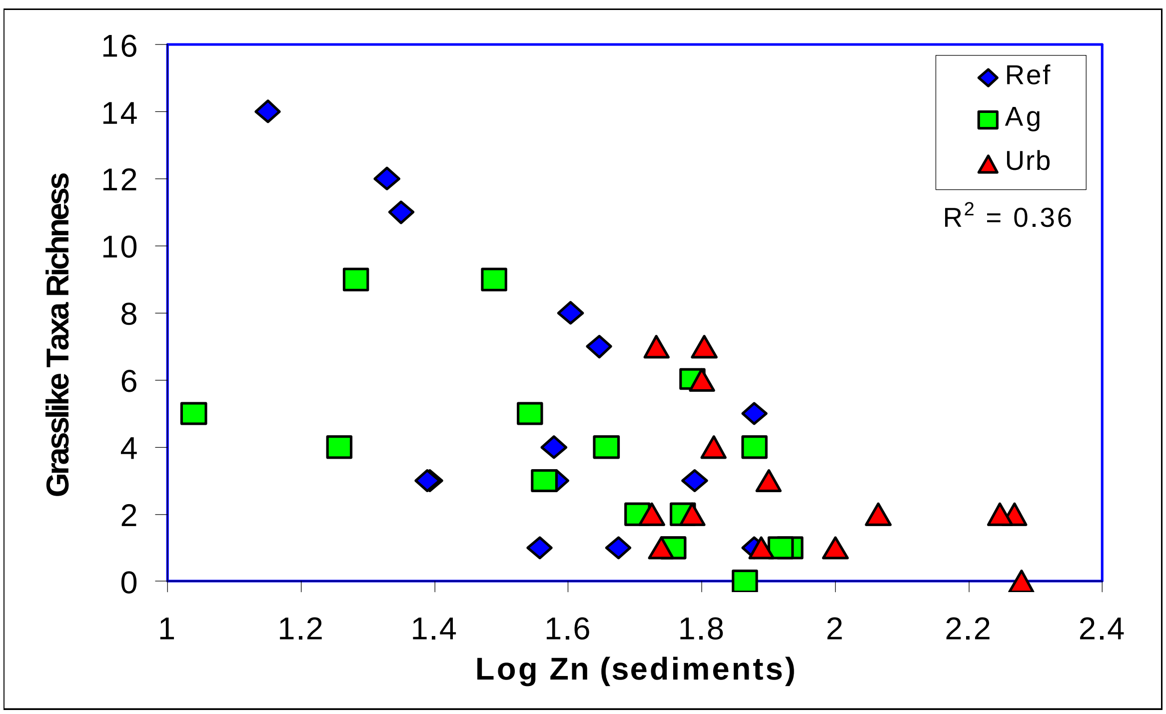
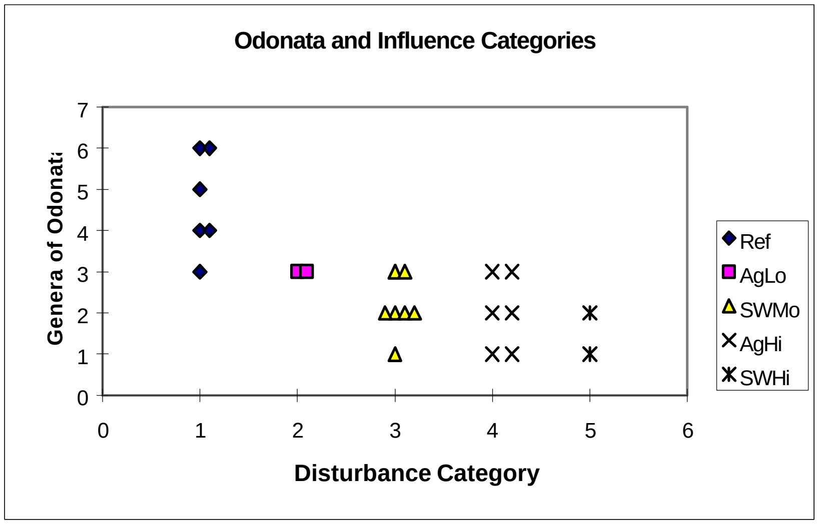


FIGURE 5: SCATTER PLOT ILLUSTRATING METRIC (GRASSLIKE TAXA RICHNESS) RESPONSE TO A SINGLE VARIABLE (ZINC CONCENTRATIONS) REPRESENTING THE GRADIENT OF HUMAN DISTURBANCE.

R^2 = Pearson's correlation coefficient; Ref = reference wetlands; Ag = wetlands with agricultural impairments; Urb = wetlands with urban impairments (Helgen and Gernes in press).

Figure 6: Evaluation of attribute performance over a combined set of disturbances.

Ref = reference wetlands; AgLo = wetlands with low levels of agricultural impairment; SwMo = wetlands with moderate levels of stormwater impairment; AgHi = wetlands with high levels of agricultural impairment; and SwHi = wetlands with high levels of stormwater impairment. Based on the relationship, one attribute (number of Odonate genera) may be retained, while the other (number of leech genera) may be eliminated (Gernes and Helgen 1999).



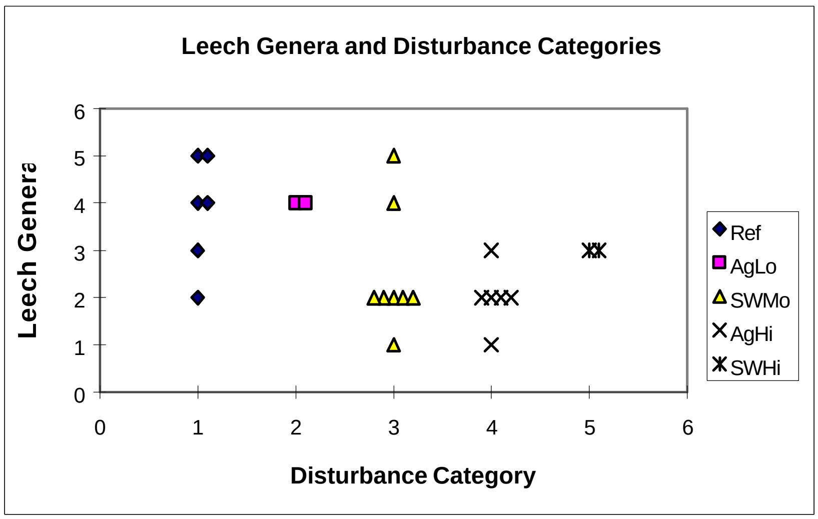
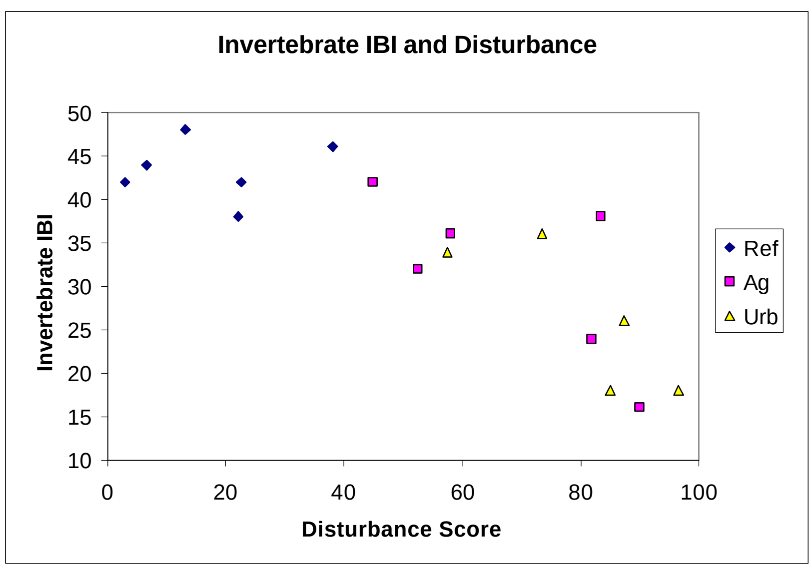
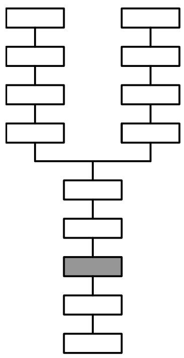


Figure 7: Scatter plot demonstrating close correlation between a wetland macroinvertebrate IBI and a disturbance gradient derived from a combination of human disturbances.

Ref = reference wetlands; Ag = wetlands with agricultural impairments; Urb = wetlands with urban impairments (Helgen and Gernes in press).



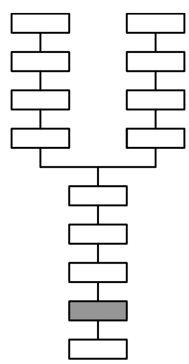
Score Each Individual Metric



The metric evaluation process should cull attributes, even those that may show some relationship to the gradient of human disturbance, to select those few metrics that are highly sensitive to impairments yet not redundant, to form the IBI. The selected metrics then can be scored by assigning values, such as 5, 3, or 1, depending on whether the data they represent are comparable to, deviate somewhat from, or deviate greatly from values exhibited by the least impaired wetlands (Karr et al. 1986). Some metrics will decrease with added human disturbance (positive metrics) and others will increase (negative metrics); each should be scored accordingly. Several techniques have been used to score metrics, each with the objective of assigning relative values to reflect the degree to which each metric departs from expected conditions. Scoring may be accomplished by determining the range in values (minimum and maximum) for each metric and then dividing those data into equal thirds. Metric values falling in the higher third of the range are assigned a score of 5, those in the middle third scored a 3, and those in the lower third scored a 1; if the data are negatively correlated, the scoring is reversed (Table 3).

Another scoring alternative has been used in streams, in which a variable, such as size of stream drainage, is known to influence a metric's response. In such cases, a trisection technique is used to divide and score scatter plot data (size of stream drainage vs. metric value) to account for the influence of the variable (Lyons 1992). Although yet to be documented for wetlands, it is possible that similar influences exist and should be taken into consideration when individual metrics are scored. Maximum species richness lines or lines that represent the upper limit of scoring for other metrics can be calculated though regression analysis; species/area curve plotting; or by best-fit lines, using professional judgment. Care should be taken to avoid outliers in data that may skew the scoring. This process should be as objective as possible; rules should be designed for the scoring procedure. Note that scoring should apply only to wetlands in the same class. For example, take care to avoid having the best bog receive a score of 25 out of 50 and the best marsh a 49 out of 50, which would incorrectly imply that bogs are not as healthy as marshes. A pristine bog should receive a 50 compared with other bogs, and a pristine marsh should receive a 50 compared with other marshes.

Calculate Total IBI Score for All Sites



An IBI is composed of the summed response signatures of the individual metrics that collectively provide a relative measure of biological condition and individually point to likely causes of degradation at different sites (Karr et al. 1986, Yoder and Rankin 1995). An IBI score can be calculated for each site by applying the scoring criteria to the data from each site (Table 4). If the metrics selected are closely correlated to the gradient of human disturbance, then the IBI that is derived from the composite of those metrics will be also (Figure 7). Once metric scores have been totaled, various interpretations can be made. For example, the same application can be used for other wetlands in the region and before and after studies can be performed to assess the relative effect of various conservation practices on wetlands. Biological criteria can be established to help support water quality objectives. Or, analyses can be performed to determine which

Table 4: Individual metric scores for invertebrate metrics

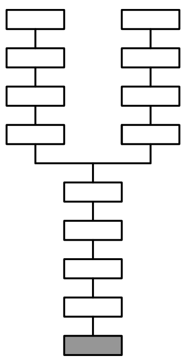
Site	Chiro	Odon	Corix%	Leech	ETSD	Erp%	TaxTot	Intol	3Dom%	SnailTx	Type	IBI	Condition
1	5	5	5	5	5	5	5	5	5	5	Ref	50	Exc
2	5	5	5	5	5	5	3	5	5	5	Ref	48	Exc
3	3	3	5	5	5	5	5	5	3	5	Ref	44	Exc
4	3	5	3	5	5	5	5	3	5	5	Ref	44	Exc
5	5	5	5	3	5	5	3	5	5	1	Ref	42	Exc
6	1	5	5	3	5	5	3	5	3	3	Ref	38	Exc
7	3	3	5	5	3	3	1	1	5	5	Ag	34	Mod
8	1	3	5	5	3	5	5	1	3	1	Ag	32	Mod
9	3	3	5	3	1	3	3	1	3	3	Ag	28	Mod
10	3	1	3	3	3	1	5	1	3	3	Ag	26	Mod
11	3	1	1	3	3	3	3	1	3	5	SW	26	Mod
12	3	3	3	1	3	5	3	1	3	1	SW	26	Mod
13	3	1	1	3	3	5	3	1	3	3	SW	26	Mod
14	1	3	5	5	1	5	3	1	1	1	SW	26	Mod
15	3	1	3	3	3	5	1	1	3	3	SW	26	Mod
16	3	1	1	5	1	5	3	1	1	3	SW	24	Mod
17	3	1	3	3	1	5	3	1	1	3	Ag	24	Mod
18	3	1	3	3	1	3	5	1	1	1	SW	22	Poor
19	1	1	3	3	3	5	3	1	1	1	SW	22	Poor
20	1	3	3	3	3	3	1	1	1	3	SW	22	Poor
21	3	1	1	3	1	5	1	1	1	3	Ag	20	Poor
22	1	3	1	1	1	1	3	1	3	3	Ag	18	Poor
23	1	1	1	3	1	1	3	1	1	3	SW	16	Poor
24	1	1	1	3	1	1	1	1	3	3	SW	16	Poor
25	1	1	1	3	1	1	1	1	3	1	Ag	14	Poor

Chiro, Chironomid genera; Odon, Odonata genera; Corix%, Corixidae proportion; Leech, leech genera; ETSD, ; Erp%, Erpobdella proportion; TaxTot, total taxa; Intol, # of intolerant taxa; 3Dom%, proportion of dominant 3 taxa; Snail Tx, snail taxa. Type Ref, reference wetland; Ag, agricultural-influenced wetland; SW, stormwater-influenced wetland. Exc, excellent; Mod, moderate

Note: Condition classification = 37-50 as excellent, 24-36 as moderate, and 10-23 as poor (Gernes and Helgen 1999).

metrics are contributing most heavily to sites with low scores, thus helping to diagnose causes of impairments for particular sites (Figure 8).

Interpret IBI and Report Data



Perhaps the greatest benefit of an IBI is that it summarizes and presents complex biological information in a format that is easily communicated to managers and the public. Most people can understand plant and animal IBIs more easily than they can understand complex statistical calculations or abstract chemical and physical wetland functions. To help with the interpretations, integrity classes can be developed to classify sites, along with narrative descriptions of relative biological condition (Table 5). Although an IBI score is helpful for quickly communicating the overall condition of a wetland, most of the valuable information lies in the individual metrics. When bioassessment results are reported, the IBI score should be accompanied by (1) a narrative description of overall biotic condition in comparison to reference wetlands of the same region and wetland type, (2) numeric values of each metric, and (3) narrative descriptions of each metric in comparison to reference conditions of the same region and wetland type. For more information about how IBIs can be used to improve wetland management, refer to Module 5: Administrative Framework for Implementation of Wetland Bioassessment Program.

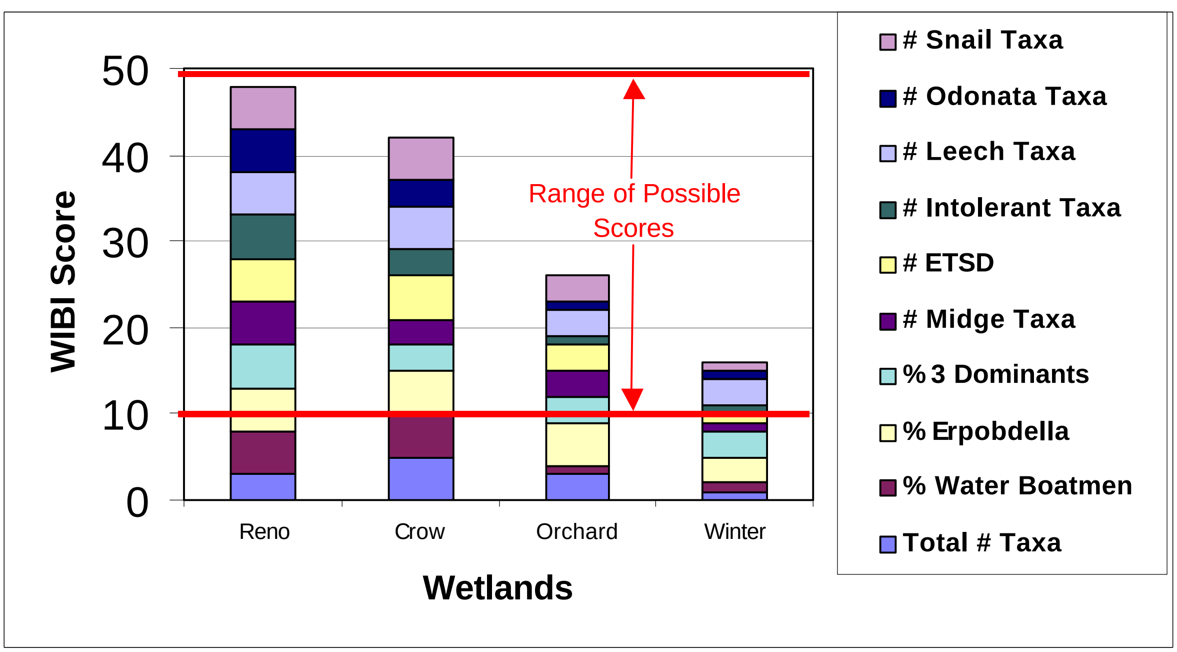


Figure 8: Example of Wetland Index of Biological Integrity scores.

From a Minnesota study for wetlands showing the range in scores and contribution of each metric (Gernes and Helgen 1999). Reno and Crow are reference sites, Orchard receives urban stormwater, Winter has heavy influences from agriculture.

Table 5: Total IBI scores, integrity classes, and their attributes for streams in a regional reference

Total IBI score (sum for 12 Integritymetric ratings)	Class	Attributes
50-60	Excellent	Comparable to the best situations in the regional subclass without human disturbance; contains all species expected for the region, including the most intolerant forms; exhibits balanced trophic structure and reproductive success.
40-49	Good	Species richness somewhat below expectation, especially due to the loss of themost intolerant forms; some species are present with less than optimal abundances; trophic structure and reproduction shows some sign of stress. Presence of some invasive or non-native species.
30-39	Fair	Signs of additional deterioration include loss of intolerant forms, fewer species, highly skewed trophic structure (e.g., increasing frequency of omnivores or tolerant species); older age classes or top predators may be rare.
20-29	Poor	Dominated by omnivores, tolerant forms, and habitat generalists; few top carnivores; reproductive and condition factors commonly depressed; hybrids or diseased individuals often present. Invasive or non-native species abundant.
10-20	Very Poor	Dominated by highly tolerant forms or invasive species; hybrids may be common; disease, lesions, parasites, and other anomalies may be regular. Complete absence of less tolerant forms.

Source: Modified from Karr et al. 1986.
Note: Narrative description of integrity classes may differ substantially for wetlands.

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Glossary

Assemblage An association of interacting populations of organisms in a wetland or other habitat. Examples of assemblages used for biological assessments include algae, amphibians, birds, fish, macroinvertebrates (e.g., insects, crayfish, clams, snails), and vascular plants.

Attribute A measurable component of a biological assemblage. In the context of biological assessments, 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 bodies of water.

Biological assessment 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 magnification The increase in concentration of some material in organisms compared with its concentration in the environment.

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

Biota The plants and animals living in a habitat.

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.

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.

Continuum A gradient of change.

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 influence" 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 abundance of one or more species in relation to the abundance of other species in samples from a habitat.

Ecosystem The community plus its habitat; the connotation is of an interacting system.

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

Functional feeding groups (FFGs) Groupings of different invertebrates based on the mode of food acquisition rather than the category of food eaten. The groupings relate to the morphological structures, behaviors, and life history attributes that determine the mode of feeding by invertebrates. Examples of invertebrate FFGs are shredders, which chew live plant tissue or plant litter, and scrapers, which scrape periphyton and associated matter from substrates (see Merritt and Cummins 1996a,b).

Functional groups A means of dividing organisms into groups, often based on their method of feeding (e.g., shredder, scraper, filterer, predator), type of food (e.g., fruit, seeds, nectar, insects), or habits (e.g., burrower, climber, clinger).

Gradient of human influence The relative ranking of sample sites within a regional wetland class based on the estimation of degree of human disturbance (e.g., pollution and physical alteration of habitats).

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 body of water caused by external forces.

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

Index of Biologic Integrity (IBI) An integrative expression of the biological condition that is composed of multiple metrics. It is 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.

Least 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.

Macroinvertebrates Animals without backbones 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.

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. Such sites may be referred to as impaired or disturbed sites.

Munsell color The color of soil based on its chroma and hue as determined by a chart in the book *Munsell Soil Color Charts* (Munsell Color 1975).

Mottles Spots or blotches of different color or shades of color interspersed within the dominant color in a soil layer, usually resulting from the presence of periodic reducing soil conditions.

Omnivores Organisms that consume both plant and animal material.

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

Predator An animal that feeds on other animals.

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.

Taxa A grouping of organisms given a formal taxonomic name, such as species, genus, and family. 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.

Tolerance (range of) The set of conditions within which an organism, taxa, or population can survive.

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

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)].