METHODS FOR EVALUATING WETLAND CONDITION

#9 Developing an Invertebrate Index of Biological Integrity for Wetlands

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Prepared jointly by:

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

and

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

APPROPRIATE CITATION

U.S. EPA. 2002. *Methods for Evaluating Wetland Condition: Developing an Invertebrate Index of Biological Integrity for Wetlands.* Office of Water, U.S. Environmental Protection Agency, Washington, DC. EPA-822-R-02-019.

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

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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

*T*he invertebrate module gives guidance for developing an aquatic invertebrate Index of Biological Integrity (IBI) for assessing the condition of wetlands. In the module, details on each phase of developing the IBI are given. First, in the planning stage, invertebrate attributes are selected, the wetland study sites are chosen, and decisions are made about which stratum of the wetland to sample and what is the optimal sampling period or periods. Then, field sampling methods are chosen. The module describes field methods used in several States, and gives recommendations. Laboratory sampling procedures are reviewed and discussed, such as whether and how to subsample, and what taxonomic level to choose for identifications of the invertebrates. Specific categories of attributes, such as taxa richness, tolerance, feeding function, and individual health are discussed, with examples. Appendices to the invertebrate module give details about the advantages and disadvantages of using invertebrates, of the different attributes, of various field sampling methods, and of lab processing procedures as used by several State and Federal agencies. The module and appendices give a detailed example of one State's process for developing an invertebrate IBI, with a table of metrics with scoring ranges, and a table of scores of individual metrics for 27 wetlands. A glossary of terms is provided.

Purpose

*T*he purpose of the invertebrate module is to describe the advantages of using aquatic invertebrates for assessing the condition of wetlands, and to present approaches for developing IBIs for wetlands.

Introduction

*T*his module describes the advantages of using aquatic invertebrates for assessing the condition of wetlands and presents approaches for developing an invertebrate IBI for wetlands. Processes, methods, and examples of invertebrate IBIs for wetlands are presented, along with summaries of approaches currently used in several States. The module is based primarily on work done on ponded freshwater wetlands, but the approaches described can be modified for other types of wetlands. The module describes development of the IBI, but other indexes and approaches for assessing the condition of wetlands, particularly multivariate techniques (Davies et al. 1999, Reynoldson et al. 1997), can be used. A glossary of terms is also provided.

Why use aquatic invertebrates to assess wetlands condition?

- Because they respond to many kinds of stressors to wetlands, as shown in Figure 1.

What are the advantages and disadvantages of using aquatic invertebrates?

Several advantages and disadvantages of using aquatic invertebrates are reviewed in Appendix A. Briefly, some of the advantages of using invertebrates for biological assessments of wetlands are:

- They are commonly and widely distributed in many types of wetlands (Batzer et al. 1999).

- They respond with a range of sensitivities to many kinds of stressors; they are commonly used for toxicity testing and ecological assessments in waterbodies (e.g., see Barbour et al. 1999, Beck 1977, Cairns and Niederlehner 1995, deFur et al. 1999, Euliss and Mushet 1998, Hart and Fuller 1974,

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Heliovaara and Vaisanen 1993, Helgen and Gernes in press, Hellawell 1986, Kerans and Karr 1994, Lewis et al. 1999, Morris et al. 1989, Rainbow 1996, Saether 1979, Servos 1999, Stuijfzand et al. 2000, Warwick 1980).

■ Many aquatic invertebrates complete their life cycles in wetlands; they are exposed directly to physical, chemical, and biological stressors within the wetland (Clarke 1981, Driver 1977, Hanson and Swanson 1989, Fairchild 2000, Klemm 1982, Larson 1987, Mackie, in press; Merritt and Cummins 1996a; Walker 1953, 1958, Walker and Corbet 1975, Westfall and May 1996, Wiggins 1996, Wiggins et al. 1980, Wrubleski 1987).

■ Aquatic invertebrates are important in wetland food webs of wildlife (Batt et al. 1992, Colburn 1997, Deutschman 1984, Eldridge, J. 1990, Fredrickson and Reid 1988, King and Wrubleski 1998, Krapu and Reinecke 1992,

Swanson et al. 1977, Swanson et al. 1985, Wissinger 1999).

■ They have public appeal in citizen monitoring programs (see Module 8, Volunteers and Wetland Biomonitoring; Helgen and Gernes 1999).

Many wetland invertebrates complete the life cycle from egg to adult in a wetland, and therefore are directly exposed to wetland conditions and stressors. In infrequently flooded, seasonal, and temporary wetlands, invertebrates will have shorter life cycles of days to weeks (Schneider and Frost 1996, Wiggins et al. 1980). In more regularly flooded, more permanent wetlands, invertebrates with longer life cycles of weeks to months, such as dragonflies or crayfish, will be present. Populations of invertebrates with shorter life cycles, such as fairy shrimp and mosquitoes, will respond more quickly to human disturbances, but they may recover more quickly, either from resting eggs or from recolonization by adult insects. Invertebrates with

FIGURE 1: INVERTEBRATE IBI SCORE PLOTTED AGAINST AN ESTIMATION OF HUMAN DISTURBANCE ON LARGE DEPRESSIONAL WETLANDS IN MINNESOTA.

The IBI has 10 scored metrics; the gradient combines factors of chemical pollution and alterations in the buffer zone and near wetland landscape.

longer life cycles, such as dragonflies, some finger-nail clams, and snails, will experience longer exposure to wetlands conditions. In some cases, recovery from losses of juveniles may take longer, because of more limited seasons of egg laying by adults and longer development times from egg to adult (Corbet 1999).

The disadvantages of using aquatic invertebrates for biological assessments are detailed in Appendix A. The biggest challenge is the amount of staff time and expertise that is needed for picking the organisms from the samples and for identifications. Techniques for reducing the amount of picking time, such as using a screen under vegetation while dipnetting, using activity traps, or by subsampling, will be described below. Some State agencies or organizations may lack the laboratory facilities to do the work in-house and will need to contract out the work.

When work is contracted out, it is important for the project managers to provide the contractor with explicit protocols and procedures for identifications and analysis in relation to the needs of the program. An advantage of doing the work in-house is that the staff involved can provide active input into the development of the biological indexes and can participate in implementing the findings into the State's programs. Whether or not the identifications are contracted out, it is vital to have scientists on staff to provide active input into biocriteria development and implementation.

In sum, disadvantages of using macroinvertebrates are:

- Sample processing takes a lot of staff time or resources.

- Organizations may lack facilities for processing and identifying invertebrates.

Process for Developing an Invertebrate IBI for Wetlands

A flow chart for developing an invertebrate IBI for wetlands is shown in Figure 2. A similar flowchart showing an example of the process for developing an invertebrate IBI in Minnesota is shown in Appendix K. Overall, the process involves sampling invertebrate attributes of several wetlands of similar class and region representing a range of human disturbance or impairment, from least to most disturbed. After sampling, the information on the degree of impairment is related to the measures of various invertebrate attributes to see which of the attributes show predictable responses to impairment. These attributes will constitute the metrics for the IBI. Scores are assigned to each metric to indicate the level of response to human disturbance (see Module 6: Developing Metrics and Indexes of Biological Integrity). The metric scores are summed for the total IBI score. Decisions are made as to which range of IBI scores indicates a poor condition, i.e., not attaining designated use (if one exists), or a moderate or excellent condition. This module will focus primarily on the steps in the process that relate to developing invertebrate IBIs.

Additional detail for this process can be found in Module 4: Study Design, Module 7: Wetlands Classification, Module 6: Developing Metrics and Indexes of Biological Integrity, and Module 17: Landscape Characterization for Wetlands Assessments. Additional information on biological monitoring of wetlands is also available in Rader et al. (in press).

It needs to be stated that other approaches are successful in evaluating the condition of waterbodies with invertebrates. Multivariate methods for ana-

STEP 1:
SELECT STUDY
SITES ————————

- Select ecogeographic region
- Decide on wetland class
- Select sites within wetland classes that exhibit a range of disturbances

STEP 2:
PLAN INVERTEBRATE ————
SAMPLING

- Select invertebrate attributes of sensitivity, richness, tolerance, trophic structure, or other attributes likely to respond to human disturbance
- Gather literature about regional invertebrates for the wetland class
- Determine which strata of wetland to sample
- Select optimal seasonal sampling period for maturity of invertebrates

STEP 3:
FIELD SAMPLING ————

- Select appropriate sampling methods for objectives of the program
- Pretest sampling methods to determine number of samples to be taken per site, and to assure the desired types of invertebrates are collected
- Decide if samples will be preserved and processed in the lab, or sorted and identified in the field; write Standard Operating Procedures
- Sample sites within the index period, at the same time collect samples for chemical analysis and assess the surrounding landscape

STEP 4:
SAMPLE PROCESSING ————

- Decide to pick entire sample or subsample
- Decide on taxonomic levels for identifications establish database of taxa lists and ITIS codes
- Develop Standard Operating Procedures and set up a Quality Assurance plan for repicking and for independent verifications of identifications
- Establish a reference collection with several specimens of each taxon

STEP 5:
METRIC ANALYSIS ————

- Plot attribute data against human disturbance gradient
- Select most responsive 8-12 metrics
- Score metrics by trisecting data or other method
- Sum the metric scores for IBI, plot IBI against disturbance gradient

FIGURE 2: FLOWCHART FOR DEVELOPING AN INVERTEBRATE INDEX OF BIOLOGICAL INTEGRITY.

lyzing invertebrate data are used for stream assessments in the Biological Monitoring Program in Maine (Davies and Tsomides 1997, Davies et al. 1995, 1999). Discriminant analysis of invertebrate data predicts the degree of impairment by comparison with known biological characteristics of the state of Maine's four water quality management classes. (See also Marchant et al. 1997, Norris 1995, Wright 1995, Hawkins and Carlisle in press, U.S. EPA 1998, Lake and Reservoir Bioassessment and Biocriteria Appendix E). Reynoldson and others (1997) found estimates of accuracy and precision to be higher when multivariate techniques were used for data analysis compared with multimetric methods. If multivariate analysis is used, it is important to use a method that allows interpretation of proportional attributes rather than one that is limited to data on the presence or absence of taxa (see Reynoldson et al. 1997). A disadvantage of multivariate methods is the complexity of setting up the data analysis.

Step 1. Select study sites

To reduce natural variability, an ecoregion or ecological region and a class of wetlands are chosen for developing the IBI (see Module 7 on Classification). It is necessary to select specific wetland classes, because invertebrate assemblages may differ among classes of wetlands (in Batzer et al. 1999; see Huryn and Gibbs, Leslie et al., Marshall et al., Smock et al., and others; Carlisle et al. 1999, Weisberg et al. 1997). It is important to include several least impaired or reference wetlands along with a range of wetlands that are affected by human disturbances. Sufficient numbers of least-impaired reference wetlands and wetlands experiencing a range of impairments are needed to detect significant dose-response relationships between the invertebrate attributes (Y axis) and the measures of human disturbances (X axis). See the Glossary for definitions of disturbance and human disturbance.

The impaired wetlands can be selected to target the major types of human-caused stressors to wet-lands, those that are most likely to be causing impairment to the invertebrates within the region and wetland class. Invertebrates exhibit a wide range of sensitivities to human-induced stressors such as pesticides, metals, siltation, acidification, loss of vegetation or vegetation diversity, nutrient enrichment, and changes in the oxygen regime (Adamus 1996, Beck 1977, Eyre et al. 1993, Helawell 1986, Resh and Rosenberg 1984, Saether 1979). Macroinvertebrates respond to disturbances in wetland vegetation because invertebrates are dependent on the vegetation as part of their food source (Wissinger 1999), for attachment sites, refugia (Corbet 1999, p. 164, Orr and Resh 1989), and egg laying. Some dragonflies and damselflies lay eggs on specific types of aquatic vegetation (Sawchyn and Gillott 1974, Corbet 1999, p. 591).

Step 2. Plan the invertebrate sampling

Selection of invertebrate attributes

Attributes are measures of the invertebrate composition to be tested to see if they show a graded response, or dose-response, to human disturbances such as chemical pollution, siltation, or habitat alteration. If a response is seen, the attribute will be selected as a metric and scored as part of the overall IBI score. See Module 6: Developing Metrics and Indexes of Biological Integrity. More detailed information on the selection of invertebrate attributes is given near the end of this module in Step 5 on Metric Analysis. Appendix F lists advantages and disadvantages of different kinds of attributes, and Appendix I shows metrics used by Minnesota for large depressional wetlands. Appendix G shows the attributes used or tested by several States.

Attributes are selected from major categories of invertebrate composition (Barbour et al. 1996, Resh et al. 1995), such as measures of taxonomic richness, measures of tolerance proportions and intolerant taxa, measures related to trophic structure and functional feeding groups (see Merritt et al. 1996,

1999) and other measures related to longevity, introduced or exotic species, and invertebrate health or condition (see Diggins and Stewart 1998, Hudson and Ciborowski 1996, Warwick 1980).

Several invertebrate attributes are tested, and the data for the attributes are related to measures of human influence. From the attributes that show significant relationships with human disturbances, a set of 8 to 12 are selected as metrics. An attribute that does not show a significant response to human disturbances will likely be discarded. However, if there is some reason to think the attribute might show a response to different kinds of stressors that were not included in the study design, it might be retained for further testing.

It is important to plan to measure the types of invertebrates that would be expected to inhabit the class of wetland in the ecological region of interest. The taxonomic composition of the invertebrates will likely differ in bogs, playa lakes, temporary ponds, prairie potholes or riparian wetlands, for example (see Batzer et al. 1999). Information can be sought from regional scientific literature and from invertebrate biologists in the area. Whatever the class of wetland might be, the major categories of attributes will be represented, such as taxa richness and tolerant and intolerant taxa, even if the taxonomic composition differs. If there is little preexisting information on species composition for the particular wetland class, it may be helpful to do preliminary sampling in reference wetlands to develop a list of species and then select the attributes to be tested. This could be done when the sampling methods are being tested.

Determine which stratum or zone(s) of the wetland to sample

One decision is whether to attempt to sample the entire wetland and all of its habitats, or to sample defined zones or strata within the wetland. Wetlands have several zones that are related to the influence of hydrology and/or plant communities. For example, freshwater marshes have shallow water, emergent macrophyte, and floating-leafed and submersed aquatic plant zones. Bottomland forest wetlands may have zones ranging from aquatic to swamp to semipermanently flooded to seasonally then temporarily flooded (Mitsch and Gosselink 1993). It may not be necessary to sample all the zones in a wetland to assess its condition. Either enough of the wetland habitat should be sampled to assess its condition, or the area most sensitive to impairment should be assessed. In a small wetland, sampling all the habitats may be possible, but in larger wetlands the work effort may necessitate choosing strata for the sampling and maintaining a consistency among wetlands of the habitats that are sampled. The choice can include the following considerations:

- Sample in the zone or stratum that is likely to have the greatest variety and production of macroinvertebrates,

- Sample in the zone or stratum that is considered to be most vulnerable or most affected by human disturbances, or

- Select a habitat type that is representative of the wetland, as opposed to unique or minor in extent; sample enough of the habitats to integrate the conditions in the wetland.

See also Module 4: Study Design for Monitoring Wetlands. Examples of the zones that were selected by different States involved in wetland monitoring are given in Appendix B.

Aquatic macroinvertebrates occur in association with benthic sediments, emergent vegetation, submergent vegetation, and open-water habitats. They colonize hard substrates such as tree roots or rocks. Some feed on the microflora that colonize the surfaces of plants and hard substrates; some are predators on the smaller herbivorous or detritivorous invertebrates. Different invertebrate assemblages are associated with these different

FIGURE 3: ILLUSTRATION OF SAMPLING SITES IN A WETLAND WITH EMERGENT VEGETATION SHOWN AS SYMBOLS.

Two standardized dipnetting samples are taken from the wetland about 20-30 meters apart (DN1 and DN2). Each sample consists of three to five sweeps repeated in two efforts (represented by two DN1s and two DN2s). Ten activity traps (BT) are placed at the edge and in representative areas of the near shore emergent vegetation zone in water < 1 m deep. Wetland is not drawn to any scale. Method is used by Minnesota (MPCA). See Appendixes B and C.

habitats, even with different kinds of wetland vegetation (Burton et al. 1999, Gathman et al. 1999, King and Brazner 1999). In preliminary studies of coastal wetlands, some invertebrate attributes differed among types of emergent vegetation zones. Attributes were selected that gave consistent, rather than contradictory, responses to human disturbance among sites across four defined plant zones (Burton et al. 1999).

The vegetated areas of wetlands have been observed to have more taxa of chironomids (Driver 1977) and aquatic beetles (Aitkin 1991, Timms and Hammer 1988) and other taxa (Dvork 1987). Emergent vegetation areas had greater richness when compared with open water areas that lacked submersed vegetation (Olson et al. 1995, Voigts 1976). If fish are present, open-water areas may have more predation by fish on invertebrates

(Hanson and Riggs 1995). Having more taxa in vegetated areas may not reflect direct herbivory on the plants, but the conversion of macrophytes into detritus is an important source of nutrition for invertebrates (Euliss et al. 1999). Also, as stated previously, macrophytes provide refugia, substrates for growth of algae and small organisms, and egg laying sites.

An example of sampling locations in a depressional wetland in Minnesota is given in Figure 3.

Select the optimal seasonal sampling period

The seasonal index period is the window of time when the sampling of the wetland is optimal to obtain the most representative, mature invertebrate community and the maximum number of identifiable taxa. The different and seasonal life cycle strat-

egies of invertebrates present a challenge as to when to sample, especially if the sampling is to be done just once in a season. If the wetland is sampled too early in the year, the invertebrates may be less mature, making the identifications more difficult. If the wetland is sampled later in the season, there may be emergences of aquatic insects from the wetland and immigration of adult insects that fly into the wetland from other waterbodies. If the wetland is sampled too late in a season, the smaller wetlands may have dried down or become choked with vegetation.

The seasonal index period will differ across different regions of the United States and it will differ for different types of wetlands. Invertebrates are known to undergo seasonal changes in populations and species that inhabit a waterbody. Ideally, the wetland should be sampled in more than one season; however, this may not be practical for State programs. In selecting an index period, the following should be considered:

- The invertebrates should have developed sufficiently to be identified by biologists.

- The index period should bracket a time when as many resident taxa as possible are present.

- The index period is not during a time that the wetland is likely to dry down or become choked with vegetation (so it is still sampleable).

- The index period could attempt to precede maximum fish predation, if any, while still encompassing the season of maximum richness and development of invertebrates.

- The index period should be shifted somewhat to account for unusually late or early seasons (the main goal is to optimize the number of invertebrate taxa present and mature).

See Appendix B for the approaches taken by different States and Appendix H for addresses of State contacts. Maine samples in June, to obtain the most

mature of some of the invertebrates (Davies et al. 1999), Minnesota (Gernes and Helgen 1999, Helgen and Gernes in press) samples during June when there is greater maturity in the Odonata and more water in the wetlands. Wetlands in the more southern location of a study set are sampled earlier in the index period. Montana (Apfelbeck 1999) samples the Plains Ecoregion in April to mid-June, the Intermountain Valley and Prairie Ecoregion from June to August, and the Rocky Mountain Ecoregion from mid-June to September. *Rapid Bioassessment Protocols for Use in Streams and Wadeable Rivers* (Barbour et al. 1999) contains a discussion of the sampling seasons for stream invertebrates.

STEP 3. FIELD SAMPLING METHODS AND DECISIONS

Before the actual sampling for monitoring wetlands takes place, several decisions need to be made. These decisions will be based in a large part on the program objectives or information needs that the invertebrate IBI is addressing:

- What strata or zones of the wetland to sample (see discussion of selecting sampling stratum above and see Module 4 on Study Design)

- Whether to sample once or more than once during the season (see discussion on selecting seasonal index period above)

- Whether to sample all habitats in the stratum or zone (multihabitat approach), or selected habitats

- Whether the approach collects the range of invertebrates needed for the attributes

- What sampling methods will be used to collect the desired invertebrates efficiently

- How many samples can be processed in the lab

Recommendations for field methods

Several methods for collecting invertebrates in wetlands are described below and listed with ad-

vantages and disadvantages in Appendix C. The following are recommended, but may not be applicable or suitable for researchers or State agencies under certain circumstances.

- Sample a stratum of the wetland that contains most of the macroinvertebrates: often the shallow areas have emergent or submerged vegetation; very small wetlands can be sampled all around the edges.

- Sample once during the season, after determining when the maximum development of the invertebrates occurs, and take more than one sample at that time; if resources permit, sample in two seasons taking more than one sample on each visit date.

- Sample all habitats within the stratum or zone, or sample selected habitats if they are known to have a wide representation of invertebrates.

- Use a dipnetting method with a standardized and repeatable protocol; combine this with activity traps to collect the motile predators (see discussion of these and other methods below).

Determining the number of samples to take: species richness and sampling effort

Once these decisions have been made, the sampling methods should be pretested in the field to ensure they collect the invertebrates that are needed for the attributes and to determine the numbers of samples that will be needed. In practice, States, Tribes, and researchers often have to balance the limitations of resources against the requirements for validation of data. It is useful to test methods in reference wetlands to determine how many samples are needed to obtain the desired representation of the invertebrate fauna.

It is well known in ecological research that the number of species or taxa collected frequently increases with the sampling effort (Magurran 1988). A quantitative sampling method (e.g., cores or Gerking samplers) will yield data on the density of taxa or species per unit area sampled. A semiquantitative sampling method (dipnetting, activity traps), as described below, will record the number of taxa per total sample count. This is called numerical taxa richness. In either case, the number of species or taxa usually increases with sample size, i.e., with the number of samples taken or the area sampled. For the purposes of comparing sites, it is essential that the number of samples and area sampled be the same at all sites.

Several samples should be taken with consistent methods at all sites to determine how many samples are necessary to collect a desired percentage of the total number of species collected. Ideally, enough samples will be collected to achieve the plateau level at which taking more samples does not increase the number of species. However, this may not be practical, because a plateau may not be reached even with many samples (see Mackey et al. 1984). Sparling et al. (1996) collected multiple samples at the same sites and recorded the increases in the percent of total invertebrate taxa with the increase in the number of samples analyzed. In 1, 2, 3, 4, 5, and 6 samples there were, cumulatively collected, 40%, 56%, 60%, 64%, 68%, and 72% of the invertebrate taxa, respectively. In addition to comparing the taxa gained by added sampling effort, the effects on the final evaluation of the wetland, which lead to the IBI or other index score, should be gauged for each level of effort. To reduce variability, a minimum of three samples is recommended, although each method should be tested for its variability if possible.

It may not be possible to do a power analysis at the beginning of IBI development. Power analysis determines how many samples need to be taken to obtain enough statistical power to detect differences among sites using the IBI scores. To do this, several samples would be taken in each of several wetlands that have a range of human disturbances. For discussions of power analysis and the number of samples to take, see Eckblad (1991), Allan

(1984), and Green (1989). Several papers may be helpful (Davies et al. 1995, 1999, Davies and Tsomides 1997, Fore et al. 1994, Rankin and Yoder 1990, Rathbun and Gerritsen in press). Variability in the biological data may increase with greater impairment, as seen in biological monitoring in Ohio streams (Rankin and Yoder 1990, Yoder 1991).

Select the appropriate sampling methods

The methods appropriate for sampling will depend on the type of wetland and the goals of the sampling. Appendix B describes sampling protocols used by several states with advantages and disadvantages. The emergent or submerged vegetation in wetlands presents a challenge for sampling. Selected methods used in wetlands studies are summarized in Appendix C with advantages and disadvantages. More detail on the methods can be obtained from references cited in the text to follow. See Batzer et al. (in Rader et al. in press) on sampling invertebrates in wetlands.

There is a need for comparisons among the most commonly used sampling methods for sampling invertebrates, particularly for differences in invertebrate composition and attributes. Brinkman and Duffy compared Gerking samplers, cores, activity traps, and artificial substrates (1996) and found Gerking samples collected significantly more taxa than cores. Hyvonen and Nummi (2000) compared activity traps with corers, finding fewer active invertebrates in core samples. Some of the articles cited below have comparative studies of methods.

Is a quantitative sample necessary?

A quantitative sampling method collects invertebrates by trapping the column of water and/or the bottom sediments from a known dimension of bottom area. This permits calculating the number of invertebrates per unit area of wetland bottom. Quantitative methods may be particularly useful in assessing the productivity of wetlands in relation to waterfowl production. They are not as necessary

for developing IBIs (Karr and Chu 1999). Examples of quantitative sampling methods for macroinvertebrates are the Gerking box sampler (Gerking 1957, Anderson and Smith 1996); the stovepipe sampler (see Wilding sampler in Welch 1948, Turner and Trexler 1997); and various methods for collecting (Gates et al. 1987) or coring the benthic sediments (Hyvonen and Nummi 2000, Swanson 1978, 1983). Some have used an Ekman grab sampler mounted on a pole.

The Gerking box sampler—a quantitative sampler

The Gerking box sampler is lowered into the water until the open bottom of the sampler (0.3 m^2) is pushed into the sediments leaving the open top projecting above the water's surface. Everything within the sampler is collected from the sediments, vegetation, and water column. A flat 1 mm mesh plate is slid across the bottom before the sampler is pulled up and drained, and the sample is rinsed into a sieve. The advantages of this device are the quantitative estimates of the invertebrates and the fact that it captures many of the organisms from the benthos, vegetation, and water column. Gerking samplers collect greater abundance and greater numbers of invertebrate taxa than activity traps or artificial substrates (Brinkman and Duffy 1996). The chief disadvantage of the Gerking box is the labor involved in processing the large samples that it collects. In addition, the device is bulky, requiring two people to carry it to the site and a crew of three to four to operate. It is not useful if woody vegetation is present. This method has been used in projects at the Patuxent National Wildlife Refuge (see Appendix B).

Core sampler—a quantitative sampler

The advantage of using core samplers is the quantitation per unit bottom area of wetland and the shorter collection time. A disadvantage is the fact that core samples contain fewer invertebrate taxa than Gerking samplers or sweep nets (Hyvonen and Nummi 2000, Cheal et al. 1993) and the organisms have to be processed from the mud. Cores

sample a smaller bottom area than the Gerking box and they do not capture the motile taxa. The frequently anaerobic benthic sediments of some wetlands would not be expected to have the range of taxa found in other habitats. Cores are appropriate if the goal is to analyze the benthic invertebrates, such as oligochaete worms, benthic molluscs, and chironomids, or if the type of wetland has low water or saturated conditions (see Hershey et al. 1998). Only 2 taxa of chironomids were collected from cores pooled from benthic sediments in depressional wetlands, whereas a mean of 12 taxa were collected by a dipnet method (Helgen et al. 1993).

In samples that include benthic sediments, such as cores or stationary samplers, the organisms can be floated from the sediments in dense (30%) sucrose or salt solutions (Anderson 1959, Ritchie and Addison 1991). As much sediment as possible is washed out of the sample with running water on a No. 30 (600 mm) mesh sieve. After water is drained from the residual debris and sediment, the 30% sucrose solution is added to float out the organisms from the residual. This technique is mostly limited to muddy benthic sediment samples that are very difficult to pick.
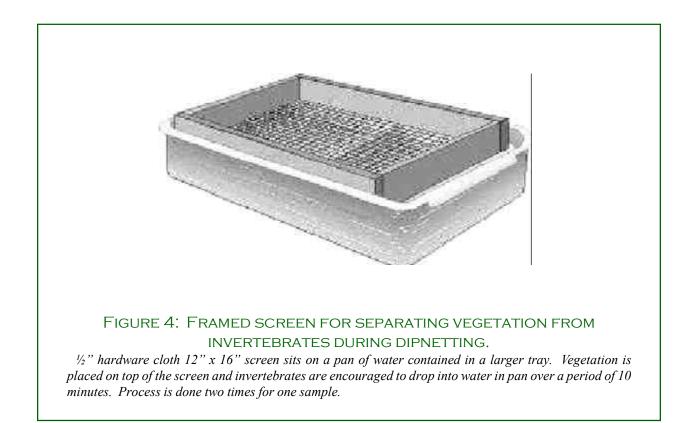
Semiquantitative sampling methods
Dipnet or sweep net samples. Dipnetting, also referred to as sweep netting, is probably the most common method for sampling invertebrates in shallow vegetated wetlands (see Appendix C). With consistent, standardized protocols, dipnetting yields semiquantitative data on invertebrate abundances and taxa richness. Without a consistent effort, dipnetting yields only qualitative results. See Appendix B for methods used by different States to produce semiquantitative data with dipnetting. Repeatability is dependent on the standardization of protocols and the training and skill of the field crews.

Ways to assure repeatability in dipnetting protocols include:

- Defining the number of sweeps (Minnesota, Florida)

- Defining the amount of time for sweeps (Montana, Ohio, Merritt et al. 1996, 1999)

- Defining the distance of sweeps and the number (Florida, Maine)

- Doing consistently repeated efforts at each site (Minnesota)

Dipnetting samples a large area and the range of wetland habitats. Dipnets have been considered a useful method because they capture a high richness of species, comparable to that obtained with the Gerking box sampler (Cheal et al. 1993, Kaminski 1981). Dipnets collect more taxa than are collected by cores or artificial substrates (Mackey et al. 1984), and collected more taxa of chironomids than corers, artificial substrates, or activity traps in Minnesota (Helgen et al. 1993). An advantage of dipnetting is that experienced crews can collect samples quickly over a wide range of habitats.

See Appendix C for more details of dipnet procedures. In Florida, 20 0.5-m sweep net efforts are distributed in proportion to the representation of the habitat type, with emphasis on the "productive habitats" (Florida DEP 1994, 1996, and FL SOP). In Minnesota, 3 to 5 sweeps are done twice for each sample, i.e., a total of 6 to 10 sweeps per sample, and 2 samples are collected per wetland (Gernes and Helgen 1999, Helgen and Gernes in press). In Ohio, a 30-minute multihabitat dipnetting is done and invertebrates are handpicked from substrates that could not be sampled by dipnet (Fennessey et al. 1998). Montana samples all habitats in the wetland for 1 minute, 3 to 4 times, depending on the wetland size and complexity, or until at least 300 organisms are collected into one composited sample (Apfelbeck 1999). Merritt, Cummins, and others have used 30-second sweeps with D-frame nets to assess the status of vegetated riparian systems (Merritt et al. 1996b, 1999,

FIGURE 4: FRAMED SCREEN FOR SEPARATING VEGETATION FROM INVERTEBRATES DURING DIPNETTING.

½" hardware cloth 12" x 16" screen sits on a pan of water contained in a larger tray. Vegetation is placed on top of the screen and invertebrates are encouraged to drop into water in pan over a period of 10 minutes. Process is done two times for one sample.

Cummins and Merritt in press; Cummins et al. 1999). A time-constrained dipnetting protocol may be difficult when wetlands are particularly mucky or habitats are farther apart.

A disadvantage of dipnetting is the amount of vegetation and other debris that gets trapped in the net. This adds greatly to the picking time needed in the lab. Unless debris is removed in the field, it increases the amount of sample that must be preserved. Minnesota reduces the vegetation in samples by laying the net contents on a framed ½" 12" x 16" hardware cloth screen that sits on a pan of water contained in a floatable tray (see Figure 4 and Appendix C). The vegetation is gently teased apart periodically over a 10-minute period and the invertebrates are encouraged to drop into the water in the pan beneath the screen. This process is done twice for one dipnet sample, then the pan of water is poured through 4" cylindrical 200 micron mesh sieves. Florida reduces the amount of vegetation in its multihabitat dipnet samples by washing

it in the net and removing the larger pieces of vegetation, as does Ohio.

Some habitats are not amenable to dipnetting, e.g., areas with a lot of woody debris or roots or very dense vegetation, or water that is too shallow. Coring methods may be necessary in very shallow, saturated wetlands or during drought cycles (Hershey et al. 1998). Also, dipnetting tends to miss some of the very motile invertebrates, e.g., the large predatory beetles and bugs. This problem is overcome by combining the use of dipnets and activity traps.

Multihabitat dipnetting methods. The multihabitat dipnetting method takes samples from most of the habitats within the wetland. The sampling can be distributed among the habitats in proportion to the habitat type or by other consistent protocols such as time constraints. Training field crews to assess the habitats and to sample consistently using

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standardized protocols is important. See Appendix C for the variations on the multihabitat method used by Florida, Montana, and Ohio.

Advantages of the multihabitat method are that the sample represents the complexity of the wetland and collects most of the invertebrate taxa, except the very motile taxa. Disadvantages of the method are the time needed for processing the large composite sample, or, alternatively, the need to subsample or use a minimum count (e.g., 200 or 300) or organisms (see Appendix C). Multihabitat samples tend to have a lot of vegetation and debris, unless it is removed in the field. Also, wetlands may differ in habitat types (Burton et al. 1999).

While it would be preferable to preserve separately the samples from each habitat, for efficiency, the sample is usually a composite of collections from all the habitat types. Separate preservation would require added effort in the field. An advantage of keeping the collections from the habitat types separate would be in the analysis of the data to determine which habitats show the most response to human disturbance.

Activity traps—a semiquantitative method. An activity trap is a passive sampler usually containing a funnel-shaped opening and an enclosed container (jar or cylinder) that receives and traps organisms that swim into the trap. With a funnel opening around 2.5cm diameter, macro-invertebrates can pass into the trap. When placed horizontally in the wetland, activity traps (AT) give semiquantitative data on the motile wetland invertebrates, effectively trapping the motile predators (e.g., the leeches, aquatic beetles, and bugs) better than dipnets (Hilsenhoff 1987b, 1991, Turner and Trexler 1997). They are less suitable for collecting the nonmotile types of invertebrates (Hyvonen and Nummi 2000).

Activity traps are variously styled as funnel traps (Swanson 1978, Fennessey et al. 1998, Hanson et al. 2000, Gernes and Helgen 1999, Helgen and Gernes in press, Murkin et al. 1983, Swanson 1978). They are left out at least one night, so the night-active invertebrates swim or crawl into the funnel openings. The style of trap that is used by Minnesota is pictured in Figure 5. Traps used by Minnesota and Ohio EPA are described in Appendix C.

Figure 5: Activity traps used by Minnesota.
Ten activity traps are placed horizontally in near shore area of wetlands. a. Trap is held to ½" 4 ft dowel by a sliding bracket of 3" thin wall PVC. A wingnut unites this and the larger bracket that holds the bottle; b. funnel is cut from the top end of a 2 liter beverage bottle. Four grooves 1/8 x 2" are cut into rim of funnel to snap into bottle, c. bottle is held by a 4" PVC open bracket. (see Wik D. in References)

Activity traps should be placed in shallow water (<1 m to a few cm deep), because the active invertebrates, such as predaceous beetles and bugs, feed there. If they are placed too deep, fewer invertebrates are collected. Typically, the traps are placed horizontally, although vertical placement has been used, primarily for zooplankton with smaller opening size in the funnel (Whiteside and Lindegarrd 1980). The traps are usually retrieved after 24 to 48 hours, depending on the water temperature, with a longer period in colder water.

An advantage of activity traps is that they provide semiquantitative data. Also, less training and time are required to set out and collect several traps in a repeatable way. Activity traps collect a sample that is clean of most vegetation and requires less processing time. Minnesota averaged 2.3 hours of processing time for 10 activity traps per wetland.

A disadvantage of activity traps is the need to revisit the site to collect the traps after one or two overnights. Activity traps do not collect the range of macroinvertebrates needed for the IBI. They must be used in conjunction with another sampling method, such as dipnetting, if the goal is to have a broad representation of the invertebrates for the IBI attributes. There is also a concern that predation within the trap might alter the invertebrate composition. One study (Elmberg et al. 1992) suggests that fish, but not invertebrate predators, may affect richness, but not abundance, of the invertebrates.

Another disadvantage of activity traps is the possible collection of large numbers of tadpoles so dense they seem to exclude macroinvertebrates. Decomposition was advanced even after 24 hours in the water (Peter Lowe, Patuxent Wildlife Research, personal communication; Sparling et al. 1995). Dead organisms in the traps might attract some predators to the traps, although this has not been evaluated.

More study is needed on the effectiveness of the different designs for activity traps, particularly the size of the funnel opening and how it might affect the size of organisms, including vertebrate predators, that can enter the traps. The effect of trap volume and whether the trap is enclosed (plastic or glass) versus open (screen) should be reviewed for predation impact that might be reduced by declining oxygen levels in the enclosed traps. Minnesota excludes air bubbles in activity traps to reduce activity of predators inside the traps (Ralph Gunderson, St. Cloud State U., MN, personal communication). Finally, more study is needed on the relation between water temperature and efficiency of funnel traps for active macroinvertebrates. Murkin et al. (1983) suggest that water temperature was not significantly correlated with the abundance of invertebrates in activity traps, but temperature does affect invertebrate activity (Henrikson and Oscarson 1978).

Artificial substrates. Artificial substrates are passive samplers that are made of hard substrates (plates, tiles, or objects that mimic the natural substrates) that are placed in the water for a few weeks to allow colonization on the substrates by certain aquatic invertebrates. The substrates are colonized by epiphytic fauna and have been used to collect information on a number of attributes (King and Richardson, in press; King and Richardson, submitted). They were useful in studies on the impact of mosquito control agents on chironomids (Liber et al. 1998, Ferrington 1994). More taxa of chironomids were found on artificial substrates on plates that were placed up in the aquatic macrophytes than on plates placed on the bottom (Liber et al. 1998, Ferrington 1994). A disadvantage of artificial substrates is the lack of collection of actively swimming invertebrates and consequent lower taxa than obtained with dipnets (Turner and Trexler 1997).

Advantages of using artificial substrates are:

- They yield a clean sample relatively free of debris.

- The data are semiquantitative (based on area, size and number of plates).

- They are easy to deploy in the field.

Disadvantages of artificial substrates are:

- They collect chironomids, oligochaetes, snails, and other epiphytic taxa, but not Odonata or other active invertebrates collected by other methods.

- The need to put the samplers out and collect them several weeks later, with the possibility of loss or disturbance of samplers over time.

Step 4. Sample processing procedures

Several decisions need to be made concerning sample processing:

- Whether to preserve samples in alcohol in the field or chill and bring back live

- Whether to pick samples in the lab or do much of the picking in the field

- How to conduct the sample picking in the lab

- Whether to pick the entire sample or a subsample

Appendix D lists some options for the above choices, along with advantages and disadvantages. If samples are preserved in alcohol or another preservative, there should be adequate ventilation for sample processing staff even after the samples have been rinsed and placed in water. The following are recommended, but other options may be needed depending on the circumstances of the researchers or State agencies. Whatever the protocols are, they must be used consistently.

- Preservation of the sample in the field is preferable, so the processing of the sample takes place in controlled conditions in a laboratory.

- Picking the samples should be done in the laboratory, not under varying field conditions of climate and lighting.

- Staff who pick the samples should have some training in aquatic invertebrates, but they need not be the taxonomists who do the identifications.

- Picking the sample in the lab should be done either under a microscope or in a glass tray over a light box with a magnifying lamp.

- The sample should be picked into partly sorted categories to expedite identifications.

- If the entire sample is picked, it is more efficient to collect a sample that has reduced vegetation and debris.

- Preservative should be rinsed off samples picked in water; adequate ventilation should be provided for staff doing the picking and the identifications.

Subsampling or picking the entire sample?

It is recommended the entire sample be picked if the sampling method and resources permit this. To make this more efficient, it is helpful to collect samples that are not overloaded with vegetation and debris. When wetlands are sampled with a repeatable, consistent sampling effort, picking the entire sample improves proportion metrics of total sample count, taxa richness, and variability in metrics (Doberstein et al. 2000, Ohio EPA 1988, Courtemanch 1996) and allows better comparability among sites. However, with stationary samples such as the Gerking box, stovepipe, or Wilding samplers, or with certain multihabitat dipnetting methods, there may be a lot of vegetation and debris. In such cases, a subsampling process may be necessary. See Appendix C for some methods to reduce debris in these samples.

A sample can be subsampled by using a gridded screen or a grid underlying a glass tray. Random squares or rows are selected to ensure that a defined proportion (at least 20%) of the sample grid is picked. There are sample splitters and other methods, but these need to be tested and their use may be difficult with debris-laden samples from wetlands.

Alternatively, picking the sample until a minimum number of macroinvertebrates, typically 100, 200 or 300, is found will reduce the picking effort. There are disagreements on the validity of taking this kind of subsample. For issues of subsampling see Sovell and Vondracek (1999), Barbour and Gerritsen (1996), Courtemanch (1996), Vinson and Hawkins (1996), Somers et al. (1998), and Doberstein et al. (2000).

Some investigators facilitate the sample picking by staining the invertebrates with Rose Bengal stain (100 mg Rose Bengal in 1,000 mL preservative, see Lackey and May 1971).

Taxonomic resolution and identifications of invertebrates

The level of taxonomic identifications, to order, family, genus, or species, will depend on the attributes or metrics that are used, the degree of certainty needed in the wetland assessments, and the ease or difficulty of identifying the particular groups of invertebrates. More information about the condition of a waterbody is obtained when organisms are identified to the genus or species level. Among genera within a family there are often differences in sensitivities to factors causing impairment, likewise among the species within a particular genus. A very rapid assessment approach or a citizen monitoring program might identify to the family level.

- It is recommended to identify the invertebrates to the lowest possible taxonomic level, at least to genus and to species where possible, because of the different sensitivities within some taxonomic groups.

- It is recommended, where possible, to have the identifications done by biologists who are on staff and trained in taxonomy; these staff can also participate in the development of biological monitoring tools and help guide the biological monitoring program.

- It is recommended that reference collections be maintained with several specimens of each organism that was identified, and to have these identifications verified by outside taxonomists if the taxonomist on staff lacks expertise in particular groups (e.g., chironomids).

- It is recommended that a database management staff person be dedicated to the biological database and participate with the biological staff in its development of the program.

- It is recommended that the taxonomic names be coded in the database with the national Integrated Taxonomic Information System (ITIS) codes (www.itis.usda.gov); unique codes will be needed for the taxa that are not yet coded by ITIS.

- It is recommended that the known functional feeding groups for each taxon be built into the database so that functional feeding group attributes can be tested.

Identifications at least to the genus level are recommended for biological assessments to be used for resource agency decisions. A number of excellent keys and sources are available (for example, Merritt and Cummins 1996a; Hilsenhoff 1995, Thorpe and Covich 1991, Walker 1953, 1958, 1975, Needham and Westfall 1954, Klemm 1982, Westfall et al. 1996, Clarke 1973, 1981, Mackie in press, to name a few). Some taxa will be identified to the lowest practical level, depending on available taxonomic sources, staff expertise, and work effort for identifications. For some groups or life stages, reliable taxonomic keys may not be avail-

able. For other groups, such as the fingernail clams and leeches, identifications are difficult because of the time needed to process the shells to view the hinge teeth, or to relax certain leeches and dissect the reproductive structures. Some snails can be identified to species, others only to genus level.

Once the choices are made, they should be consistently applied in the assessment protocol by all staff working on the identification of the samples. For the total taxa metric, a clear definition of which taxa to be included must be determined for the standard identification procedures in the lab. The staff biologists need to keep track of changes in taxonomy and maintain literature sources used for the identifications. The ITIS codes should be regularly updated (see Appendix E).

Some agencies contract out the identifications of certain special groups, such as chironomid larvae to genus level. If chironomids are identified only to the four subfamily/tribe levels, information about condition is lost compared to identifications to the genus level, where more than 12 to 16 genera may be found in one wetland (Diggins and Stewart 1998, Helgen and Gernes 1999, Gernes and Helgen in press, Beck 1977).

For some of the functional feeding group metrics, taxonomy is carried to the level needed to describe the function (Merritt and Cummins 1996a). In this case, all Odonata would immediately be classed as predators, or all members of a family might be classed in one functional feeding group. In other cases, identifications to lower taxonomic levels are needed to define the functional feeding groups (see Mihuc 1997). Therefore it is prudent to identify the macroinvertebrates to the lowest possible taxonomic level, genus or species, where possible.

Immature specimens and morphospecies
Some specimens will be too immature to identify to genus and will have to be left at the higher level

of identification, e.g., family. Others may be damaged and not identifiable. In some cases the experienced taxonomist may be able to extrapolate, with caution, from other specimens in the collection. Some specimens will be identified to genus but will be distinctly different from others in the same genus in the sample. These specimens must be recorded so that they are recognized as an additional species in the sample, i.e., as a "morphospecies" (Oliver and Beattie 1996). If species are carefully identified as distinct morphologies, this level of identification can produce accurate measures of species richness, but only when the experienced biologist is certain the differences are not simply due to differing stages of development.

It is important to define standard protocols for counting the number of different taxa when the identifications of different taxa are at different levels of taxonomic resolution. For the counting rules used by Maine, see Davies and Tsomides (1997).

Contracting out the identifications of the invertebrates
Contracting the services of taxonomic experts is an option when staffing levels or limitations in expertise and facilities prevent analyzing the invertebrates "in house," especially for particular invertebrates that require specialists to identify. Contracting some invertebrate taxonomy can free the staff to develop the indexes and analyze and report data. This can be a cost-efficient way to obtain reliable data, however it entails staff time in forming and tracking contracts.

It is important to give the contractors clear guidance on the groups of invertebrates to identify, the taxonomic levels, the protocols, and the attributes desired, and not to let the contractor make these decisions. It is important to have in-house staff with expertise in macroinvertebrates give guidance for the work of any outside consultants. It is recommended to have all of the macroinvertebrate groups

identified to allow calculation of new or improved metrics that may not have been previously used.

A. SELECTION OF ATTRIBUTES

Selecting invertebrate attributes

As stated in Step 2, attributes are candidate metrics that are measures of the invertebrate community. They are tested to see if they show a dose-response to increasing levels of impairment, such as chemical pollution, unnatural hydrologic or habitat alterations, or siltation. If a response to impairment is seen, the metric will be scored and its score will contribute to the overall IBI score for a wetland.

An IBI is more robust if it is composed of 8 to 12 metrics selected from different categories of attributes that represent patterns of responses to changes in the physical, chemical, and biological integrity of the wetland and its surrounding landscape. The major categories of attributes and their expected responses to increases in impairment (in parentheses) are:

- Measures of taxa richness (decrease)

- Measures of tolerance (increase) and intolerance (decrease)

- Measures of trophic structure and functional feeding groups (varies)

- Measures of life cycles, such as longevity and reproduction (decrease)

- Measures of poor condition or poor health of individuals (increase)

Advantages and disadvantages and expected responses to human disturbance of these and other categories of attributes are reviewed in Appendix F. Attributes in use by several States are given in Appendix G. Some of the categories of attributes are discussed below, with a rationale for the expected responses.
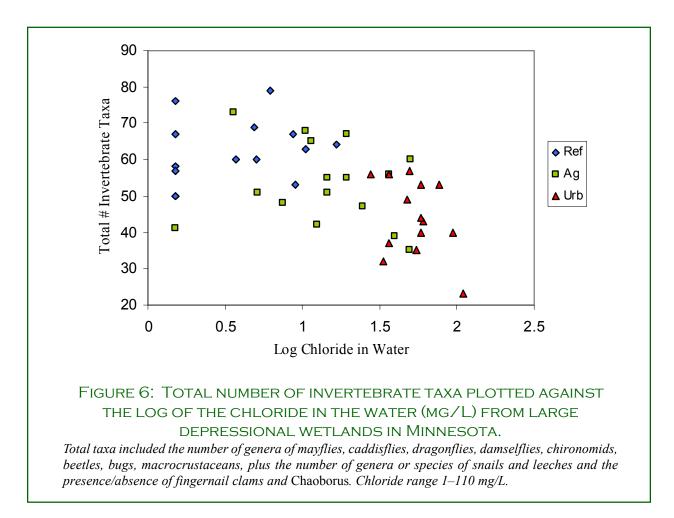
Taxa richness

Taxa richness is the count of the number of types of invertebrates that inhabit an ecosystem. The taxa richness, primarily genera or species, of a wetland is enumerated by counting up all the types of invertebrates collected from the sampling effort. It is important to use a standard protocol for counting invertebrate taxa when the taxonomic level for the identifications differs among taxa. The number of taxa commonly declines as human disturbance increases (Barbour et al. 1995, Kerans and Karr 1994, Fore et al. 1996). This attribute shows a high statistical power for detecting differences among sites (Sandin and Johnson 2000). However, there are exceptions, e.g., when low-nutrient wetlands receive more nutrients (Rader and Richardson 1994), or forested canopy areas are opened up over wetlands that were shaded and less productive before the forest clearing (King et al. 2000). Plotting the taxa richness against the measure of disturbance will show the response curve. If there is a unimodal response, with a peak of taxa richness at the intermediate level of human disturbance, the metric may not be useful.

In Figure 6, the number of invertebrate taxa decreases as the concentration of chloride increases in the water in the wetland. The increased chloride may alter the active pumping systems of invertebrates for maintaining osmotic balance in body fluids. Urban wetlands (Urb) receiving stormwater runoff are especially high in chloride.

Tolerance

Tolerant taxa inhabit a wide range of habitats and tolerate a wide range of conditions. The number of tolerant taxa may not change with impairment, but the relative abundance of tolerant organisms tends to increase as the amount of impairment to the site increases. This might be measured by the proportion of known taxa, or by the proportion repre-

FIGURE 6: TOTAL NUMBER OF INVERTEBRATE TAXA PLOTTED AGAINST THE LOG OF THE CHLORIDE IN THE WATER (MG/L) FROM LARGE DEPRESSIONAL WETLANDS IN MINNESOTA.

Total taxa included the number of genera of mayflies, caddisflies, dragonflies, damselflies, chironomids, beetles, bugs, macrocrustaceans, plus the number of genera or species of snails and leeches and the presence/absence of fingernail clams and Chaoborus. *Chloride range 1–110 mg/L.*

sented by the dominant two or three taxa to the sample count.

Tolerance values assigned to stream invertebrates are based largely on organic enrichment from data sets of stream invertebrates (Hilsenhoff 1987a). The Hilsenhoff Biotic Index for streams (HBI, see Hilsenhoff 1987a, 1995) is calculated from tolerance values assigned to species of various stream invertebrates in relation to their sensitivities to organic pollution. New England has a list of family-level tolerance values derived primarily from EPA's listing (EA Mid-Atlantic Regional Operations 1990) with some regional modifications. Hilsenhoff also has developed a family-level HBI, the FBI, for a rapid field assessment or organic pollution (Hilsenhoff 1988).

Tolerance values assigned to stream invertebrates may not be applicable to wetlands invertebrates because many wetlands invertebrates are tolerant of, or adapted to, the fluctuating oxygen conditions in wetlands. In addition, there are many invertebrates in wetlands that are "wetland specialists": water boatmen; backswimmers; diving beetles and marsh beetles; fairy shrimp, clam shrimp, and tadpole shrimp; mosquitoes; marsh flies; biting midges; horse and deer flies; the snails in the families Physidae, Lymnaeidae, and Planorbidae; and fingernail clams (see Wissinger 1999). For these and other species that are predominantly wetland specialists, there is little or no existing information on their tolerances to human caused impairments.
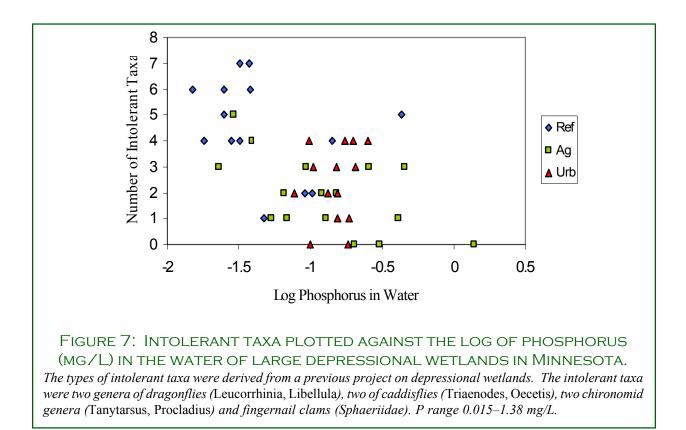
Attributes that relate to tolerances of macroinvertebrates in wetlands need to be derived

for the invertebrates of the wetland class and region. Decisions about which taxa are "tolerant" need to be based on examination of data sets from a range of high-quality and impaired wetlands. Tolerant taxa might be present in the range of wetlands, but they would show a proportionate increase in wetlands with greater human disturbance. The designations of tolerance then need to be tested on another data set for validation. Karr and Chu (1999) suggest that about 10% of the taxa, or 5% to 15%, be defined as either tolerant or intolerant. One caution on designations of tolerance values, if done at the genus level, is the possibility that the species within a genus may differ in their tolerances (Hilsenhoff 1998). It is preferable, but not always possible, to designate species rather than genera as tolerant or intolerant.

Intolerant taxa, by definition, are more likely to disappear under impaired conditions. Their presence indicates good conditions. Preliminary determinations of intolerant species would require the examination of the data set to see which taxa tend to disappear from the more impaired wetlands but are found in reference sites. Again, designations of intolerant taxa need to be tested with other data sets. An example of intolerant taxa, originally derived from data on depressional wetlands and applied to a new data set on large depressions is shown in Figure 7.

Trophic function and functional feeding group attributes

Trophic function attributes relate to the type of food eaten by the invertebrates: herbivores consume algae and plant material, predators consume animals, omnivores eat both plant and animal material, and detrivores consume decomposed particulate material. The proportion of predators is expected to decline as impairment increases (Kerans and Karr 1994). Many wetlands are lacking in fish predators and are dominated by invertebrate predators such as leeches, dragonfly and damselfly larvae, and juvenile and adult aquatic beetles and bugs

FIGURE 7: INTOLERANT TAXA PLOTTED AGAINST THE LOG OF PHOSPHORUS (MG/L) IN THE WATER OF LARGE DEPRESSIONAL WETLANDS IN MINNESOTA.
*The types of intolerant taxa were derived from a previous project on depressional wetlands. The intolerant taxa were two genera of dragonflies (*Leucorrhinia, Libellula*), two of caddisflies (*Triaenodes, Oecetis*), two chironomid genera (*Tanytarsus, Procladius*) and fingernail clams (*Sphaeriidae*). P range 0.015–1.38 mg/L.*

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(Fairchild et al. 1999, Wissinger 1999). In the fish IBI, the proportion of individuals that are top carnivores (Simon and Lyons 1995) is expected to show a decrease in response to human disturbance. More work is needed in testing of attributes of trophic function against gradients of human disturbance in wetlands.

Attributes of functional feeding groups (FFGs) merit further exploration. Such groups are based on the mode of food acquisition rather than the type of food eaten (Merritt and Cummins 1996b, Merritt et al. 1996, 1999). Merritt's work in defining functional feeding groups of many macroinvertebrates has laid a foundation for the analysis of FFG attributes. The FFGs of each taxon, if defined, should be included in databases to facilitate the testing of FFG metrics.

In streams, the analysis of functional feeding groups has been useful for understanding the changes in stream ecology from losses of riparian vegetation or conversion from a shaded, leafy stream to an open system in which algae, rather than leaf litter, become the primary food source for the invertebrates (Merritt et al. 1999, see also Rawer-Jost et al. 2000, Hannaford and Resh 1995, Resh 1995). Cummins and Merritt (2001) and Cummins et al. (1999) have analyzed FFGs in wetland riparian areas.

Functional feeding group attributes that decreased with increasing human disturbance in streams were the proportion of grazers and the proportion of predators (Kerans and Karr 1994). An attribute that tended to increase as human disturbance increased was the relative proportion of filterers (Kerans and Karr 1994). Much more testing is needed of functional feeding group attributes in relation to human disturbance in wetlands. See Merritt et al (1996) for criteria levels for FFG ratios for evaluating ecosystem parameters in streams.

Some functional feeding group attributes have shown greater variability than taxa richness measures, partly because some of the FFG attributes have been expressed as ratios (e.g., scrapers/gatherers) rather than proportions (Resh 1988, Resh 1994, Stan Szczytko, University of Wisconsin, Stevens Point, WI, personal communication). Ratios are susceptible to variability if both of the variables are changing. Other FFG attributes are expressed as proportions and these may be more robust when tested in wetland systems. Taxonomic levels needed for FFGs vary widely. Some groups, e.g., Odonata, can be entirely classed as predators, whereas others (see Merritt and Cummins 1996a) will require identifications to genus or species level. It is recommended that identifications be done routinely to the lowest taxonomic level.

Other invertebrate attributes to consider

Other attributes can be considered; some are in Appendix F. Some can be found in the literature (Barbour et al. 1996, Resh et al. 1995, Kerans and Karr 1994). A few that might apply to wetlands in the future are discussed below.

Condition or health of individual invertebrates

In the fish IBI, there is a metric that records the number of deformities, erosion, lesions, and tumors in individual fish in the sample (Sanders et al. 1999). This metric is most responsive in highly contaminated areas, as was found in Ohio. To date, malformations have been recorded in some aquatic invertebrates, i.e., chironomids and dragonflies. In chironomids, malformations have been shown to be more numerous in response to contaminants (Palmer 1997, Hudson and Ciborowski 1996a,b; Warwick 1980 1990). Minnesota has data on malformations in chironomids from 43 wetlands (done by Leonard C. Ferrington), but has not yet developed a metric. Malformations were found in the cast exoskeletons (exuviae) of dragonflies in Minnesota, first in a report from rivers and bog/fen areas (Steffens and Smith 1999) and then from some large

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depressional wetlands (Smith 2000). There is little monitoring data on malformations in most invertebrates. Therefore, it is unknown whether malformations are increasing in invertebrates the way they appear to have increased in frogs in the 1990s in northern North America (Helgen et al. 2000, Hoppe 2000, Ouellet et al. 1997, Northeast Natural Resource Center 2000).

A possible attribute to indicate the health of invertebrates is the proportion of coverage of aquatic insects by bacteria as an indicator of nutrient enrichment in wetlands, based on recent work by Lemly and King (2000).

Introduced or exotic species

There are numerous exotic or introduced species in freshwaters of the United States (see Cox 1999, Mack et al. 2000). Some of these invade wetlands. As an attribute, the number of exotic taxa or the proportion would be expected to increase as human disturbances increase. Introduced fish will alter the invertebrate composition (Hanson and Riggs 1995). In Minnesota, the huge oriental mystery snail, *Cipangopaludina chinensis,* has been found in urban wetlands, probably after being discarded from aquaria. It consumes submersed aquatic plants and creates open areas in the vegetation. The rusty crayfish, *Orconectes rusticus*, has been expanding its range and introduced into lakes and wetlands (Helgen 1990), where it consumes the macrophytes.

Generalists and specialists

Attributes that address the proportion or richness of generalists and specialists could be explored (see Wissinger 1999, Mihuc 1997). A community with a high proportion of wetland specialists is thought to reflect more competition and evolution of specialists, whereas one with a high proportion of generalists may indicate less competition and more use of the same resources. The number of specialist taxa, or the proportion of specialist individuals, might be expected to decrease as the human disturbance

increases in wetlands. In the Floristic Quality Assessment method (Fennessy 1998) for using plant communities to assess wetlands, plant taxa that have a higher fidelity for particular types of habitats, i.e., a higher coefficient of conservatism, will give the Floristic Quality Assessment Index a higher score (see Module 10, Using Vegetation to Assess Environmental Conditions in Wetlands).

B. Forming the IBI

To form the invertebrate IBI, the attributes are plotted as a scatter plot against stressors or human disturbance gradients (e.g., Figure 1, see Fore et al. 1996, Karr and Chu 1999). Eight to 12 attributes that show a response are selected and scored. Another method of visualizing metrics is to plot the attribute data using box-and-whisker plots to show the spread of values of an attribute in reference sites compared with the range in the impaired sites (Barbour et al. 1995, Barbour et al. 1992). Attributes that show very little overlap between reference sites and impaired sites are chosen as metrics and scored.

There are various ways to score the metrics. The examples given below are for assigning scores with a 1, 3, 5 system for metric data that are showing a linear or regular response to disturbance. Other scoring ranges can be used. For metrics whose data indicate a nonlinear response to the gradient of human disturbance, other methods, such as assigning scores on either side of inflection points, need to be used (Karr and Chu 1999). It is important that the study sites used to assign scores to metrics include the full range of impairment and the fullest range of biological response values for the metrics.

■ The range of the data values for the metric can divided evenly into three parts from the lowest data value to the highest data value. The low sector is assigned a score of one, the middle is given a three, the top third is given a five. This

method assumes the data ranges include a range of values from the most impaired to the least impaired wetlands, and the data values respond evenly to the human disturbances (Karr and Chu 1999, Helgen and Gernes in press, Gernes and Helgen 1999).

■ The data for a metric are plotted in rank order for the sites. Then the sites are divided into thirds and the associated data values are assigned scores of 1, 3, or 5 for the low, middle, and top third of ranked sites.

■ The metric values for the reference sites are arranged in quartiles. The values for the top three quartiles (top 75th percentile) are assigned a score of 5. The range of values from the upper range of the lowest quartile to the lowest data value is divided in two and assigned a 3 or 1 (Barbour et al. 1996). This method assumes there is an adequate number of reference sites including some with the least amount of human disturbance or no disturbance. See Module 6: Developing Metrics and Indexes of Biological Integrity. Once the metrics are scored, a matrix of scores is made in a table for all the sites such as the table in Appendix J. The scores are added to the total IBI score and the IBI scores are divided to indicate three or more categories of condition. Appendix I shows the scoring criteria for the 10 metrics used by Minnesota for large depressions. It indicates how many wetlands that were designated as reference, agricultural or stormwater-influenced received the scores of 5, 3, or 1.

Conclusions and Recommendations

1 Biological assessment of wetlands by invertebrate metrics should be increased because the invertebrate metrics are sensitive to a broad range of impairments to the physical, chemical, and biological integrity of wetlands. This will require:

■ More work on the development of invertebrate IBIs for different classes of wetlands

■ More information on the tolerances and sensitivities of invertebrates in wetlands

■ Consistent funding dedicated to staff to work on invertebrate biological assessments

2 Biological assessment of wetlands using macroinvertebrates will provide scientifically sound data for States, Tribes, and organizations to gauge the degree of impairment to wetlands from a mix of stressors. This will enable them to:

■ Develop invertebrate biocriteria for determining aquatic life use support

■ Report on condition of wetlands in 305(b) reports and in 303(d) (TMDL) lists and facilitate prioritization

■ Understand the condition of watersheds using carefully designed studies

■ Compare the results from biological condition assessments with assessments from other "rapid" physical assessment methods to validate the latter

■ Provide a solid foundation for citizen biomonitoring programs using protocols derived from those used by States, Tribes, and organizations

■ Provide a sound method to determine the effectiveness and suitability of permitting decisions

■ Provide a sound method to assess the results of restorations and mitigations in wetland replacements

Appendix J shows an example of the metric scores for a set of depressional wetlands in Minnesota. The sites are sorted by the IBI scores, and tentative lines were drawn to indicate which of the sites might be considered to be in excellent, moderate, or poor condition. This was done by trisecting the range of IBI scores (10 to 50 total points for 10 metrics). Although most of the sites that were considered to be reference, or least impaired, wetlands had IBI scores in the excellent range, a few candidate reference sites scored as moderate. It is expected that some sites chosen to be reference sites may not be in the highest condition, but will themselves have a range of conditions. Other approaches may be used for determining the criteria lines.

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APPENDIX A. ADVANTAGES AND DISADVANTAGES OF USING INVERTEBRATES FOR BIOLOGICAL ANALYSIS OF WETLANDS	
Advantages	Disadvantages/Comments
Invertebrates can be expected to respond to a wide array of stresses to wetlands, such as pollutants in water and bottom sediments, nutrient enrichment, increased turbidity, loss or simplification of vegetation, siltation, rearing of bait or game fish, input of stormwater or wastewater runoff, introductions of exotic species, or alterations of the landscape around the wetland.	Because it is likely that multiple stressors are present, it may not be possible to pinpoint the precise cause of a negative change in the composition of invertebrates. However, data from major sources of human disturbance, e.g., water and sediment chemistry, the nearby wetland landscape features, sources of hydrologic alteration, and other disturbance factors cam be assessed in relation to the invertebrate data to see which factors have the greatest effects.
Life cycles of weeks to months allow integrated responses to both chronic and episodic pollution, whereas algae recover rapidly from acute sources, and vertebrates and macrophytes may take longer to respond to chronic pollution	Information on short-term, pulse impairments (using algae, zooplankton) or more long-term impairments (using macrophytes, vertebrates) or more landscape-level (using birds, amphibians) impairment may be desired.
Toxicological/laboratory based information is extensive. Invertebrates are used for a large variety of experimental approaches.	Toxicological response data may not be available for all invertebrates; data for some wetlands species are less extensive than for stream species.
There is an extensive history of analysis of aquatic invertebrates in biological monitoring approaches for streams.	Using invertebrates to assess the condition of wetlands is now under development in several States and organizations.
Invertebrates are used for testing bioaccumulation of contaminants to analyze effects of pollutants in food webs.	Tissue contaminant analyses are always costly. This is true for tissue analysis of any group of organisms: vertebrate, invertebrate, or plant.
Invertebrates are important in food webs of fish, salamanders, birds, waterfowl, and predatory invertebrates.	Aquatic invertebrates tend not to be valued by the public as much as fish, amphibians, turtles, or birds. However, citizens do respond to invertebrates.
Many invertebrates are ubiquitous in standing water habitats.	Invertebrate composition will differ in different wetland classes, as will other groups of organisms (plants, birds) that might be used to assess wetlands.
Many invertebrates are tightly linked to wetland conditions, completing their life cycles within the wetlands. They are exposed to site-specific conditions.	Some invertebrates migrate in from other waterbodies, these taxa are not as tightly linked to the conditions in the specific wetland.
Many invertebrates depend on diverse wetland vegetation, some depend on particular types of vegetation for reproduction.	Loss of invertebrates may be a secondary effect from the loss of wetland vegetation, e.g., from herbicide treatments. Vegetation loss is an impairment.
Invertebrates have short and long life cycles and they integrate stresses to wetlands often within a 1-year time frame.	Many complete their life cycle within a year, they are not as "long-lived" as birds, amphibians or perennial vegetation.
Invertebrates can be easily sampled with standardized methods.	Picking invertebrate samples is labor-intensive.
Invertebrates can be sampled once during the year, if the best index period is selected for optimal development of invertebrates.	Invertebrate composition of wetlands often varies within the seasons of the yearly cycle. Invertebrates mature at different times. This necessitates selecting an "index period" for sampling once, or alternatively, sampling more than once in the season.
Invertebrates can be identified using available taxonomic keys within labs of the entities doing the monitoring. Staff help develop biomonitoring programs.	Expertise is required to perform identifications of invertebrates. Some may choose to contract out some or all the identifications. There is a cost involved.
High numbers of taxa and individual counts permits the use of statistical ordination techniques that might be more difficult with just a few species, e.g. with amphibians.	Large numbers of taxa and individual counts make the sample processing more labor intensive than other groups. Adequate training and staff time are required. More lab time is needed than for some other groups of organisms.
Citizens can be trained to identify wetlands invertebrates and become interested and involved in wetlands assessment. Citizens are excited to see the richness of wetland invertebrates.	Citizen monitoring requires training to learn many invertebrates in a short time, a structured program, and a commitment by volunteers and local governments; citizens may tend to underrate high quality wetlands.

STATE OR AGENCY	FLORIDA	OHIO	MAINE	MINNESOTA	MONTANA	PATUXENT WILDLIFE REFUGE
Wetland class	Depressional, emergent, herbaceous and forested wetlands	Depressional wetlands, forested and emergent; shrub-scrub wetlands	Depressional, riverine and lacustrine fringe	Depressional emergent marsh wetlands	All wetland types that had surface water (focus on depressional)	Depressional, restored/created emergent marshes
Region studied or ecoregion	Florida peninsula and panhandle	Eastern Corn Belt Plains and Erie Ontario Drift and Lake Plains	Casco Bay watershed, expanding to other areas of southern Maine	North Central Hardwood Forest ecoregion through central MN	Entire state of Montana, focus on NW Montanavalleys; 3 ecoregions	Mid-Atlantic coastal plain
Strata sampled	Near shore emergent zone < 1 m deep, all habitats	Edge area from 6" to 12" for AT; DN all habitats	Near shore emergent zone, < 0.5m for bucket, <1m for dipnet	Near shore emergent zone < 1 m deep	Near shore emergent zone < 1 m deep	Random transects, deepest spot to near shore
Methods; # of samples taken/date/site	Dipnets, 20 sweeps composited to one sample, multihabitat method	Dipnet, hand picking from substrates, and activity traps; one composite DN, 10 ATs	5 gallon open bottom bucket, 10 handnet sweeps/bucket 3/site; dipnets, testing 3 1m sweeps and multihabitat composite sampling	2 dipnet samples per site, each having two efforts, screen used for vegetation removal, 10 activity traps per site	4-5 dipnet efforts using multihabitat method for one minute each; subsampled and composited in field; one sample per site	Quantitative Gerking box sampler; 6 samples/site
Index period(s), # of dates	April through October, sample 1x per year	March-April; May; and June-July, 3 dates	June, one sample date	June, one sampling date	April-Sept., once, date depends on ecoregion	June - September; sample 4-5 dates
Pick in field or lab	Pick in lab	Pick in lab	Pick in lab, except for multihabitat samples	Pick in lab	Pick in lab	Pick in lab
ID in field or lab	ID in lab	ID in lab	ID in lab (of contractor)	ID in lab	ID in lab (of contractor)	ID in lab
Contract out IDs?	No	No	Contract out all IDs	No, except chironomids	Contract out all IDs	No
Pick entire sample or subsample?	Subsample and pick 100 to 150 organisms	Pick entire subsample, except subsample extremely abundant taxa	Pick entire sample	Pick entire sample except Neopleia (counted in tray on light box)	Subsample in field, and pick 200 to 300 organisms in lab	Split sample to ¼ or ½ to reduce picking time
Taxonomic level of identifications	Genus or species, depending on the keys	Mostly to genus, some to species including some chironomids	Lowest practical level (genus if possible); taxa aggregated to genus level for richness metric	Mostly genus, some to species, fingernail clams to family	Mostly to genus, some to species	Mostly genus, chironomids to tribe and subfamily
Disturbance gradient?	Landuse Development Index (Mark Brown)	Ohio Rapid Assessment Method (ORAM) v 5.0	Landscape disturbance score, with hydrologic alterations, and physical chemical data	Score landscape plus hydrologic alteration plus chemistry data into one score of human influence	Assessed landscape disturbance plus hydrologic alteration plus chemistry	Under development
Indexes used to assess wetlands	IBI under development	Indexes under development	Metrics and index under development	IBI composed of ten metrics	IBI	Metrics and IBI under development
Scoring method if using metrics	Under development	Under development	Exploring multivariate analysis methods	Trisect data for each metric	Scores were weighted based on metric response to stressors	Under development

Method	Description	Advantages	Disadvantages/comment	Sample processing time in lab
Gerking box sampler (Patuxent National Wildlife Refuge projects)	39 cm x 76 cm base, 61 cm tall box with sliding 1 mm mesh screen on bottom, box is pushed down on bottom through water column and vegetation; tray is slid through top cm of benthic mud.	Is quantitative, samples 0.29 m² area. Captures benthic, water column and vegetation-associated invertebrates	Sample is very large (1 gallon), vegetation needs rinsing and picking, laborious to pick large sample, use in field requires 3-4 staff, usually subsampled	80 - 125 minutes to pick, sort and identify when samples have been subsampled (to ¼ or ½ of sample)
Stovepipe style sampler (bucket sampler)(Maine)	5 gallon plastic pail with bottom removed pushed into sediment; remove vegetation and rinse in sieve bucket, take 10 sweeps with small 500 micron net from within bucket, 3/site are composited to sample	Semiquantitative, samples water column, vegetation and sediments; easy in field; one trip to site	Large amounts of detritus, much labor to pick samples; current sampler is restricted to water 40 - 50 cm deep.	Up to 8 hours per sample, varies with sample
Dipnetting with removal of vegetation on screen (Minnesota)	3-5 sweeps done with D-frame net (600 micron mesh) through water, contents of net are placed for 10' on 12" x 16" ½" hardware cloth screen over pan of water, vegetation then removed, done twice per sample. Sample filtered through 200 micron sieve.	Large number of invertebrate taxa; most vegetation is left at the site, less effort picking out the invertebrates, can pick entire sample	Semiquantitative, but done as a consistent, repeated effort	Pick average 3.6 hrs each DN sample, ID time for two DN plus 10 AT samples, 4.2 hrs
Dipnetting with multihabitat sampling (Montana)	Time-constrained, one minute of collecting from all habitat types in the wetland done 3-4 times per site and composited, 1 mm mesh dipnet used, everything is preserved	Large number of taxa from all habitats in wetland; wetland complexity is represented in sample	A lot debris and vegetation is kept, extensive time in sorting the samples, needed to pick a 200-organism subsample	Picking took up to 4-18 hours per sample (done by contractor)
Dipnetting with multihabitat sampling (Florida)	20 discrete sweeps done in "productive habitats," if one is present 10 plus 10 in minor habitat, if two present, 7 in each and 6 in minor, if 3 present, 5 in each and 5 in minor, etc. Organisms are dislodged from debris to reduce sample volume.	Better spatial coverage of site, less sampling error; better characterization of community structure; FL uses multihabitat method in streams	Training staff is important both in assessing the habitat and in the dipnetting; takes 1-2 hours to sample a site	Sample processing is 15 - 30 hours for a site
Dipnetting with multihabitat sampling (Ohio)	30 minutes sampling with triangular 30 mesh (595 micron) net all habitats; pick through vegetation in net or tray of water to remove invertebrates; manual picking of substrates with forceps until crew feels there are no new taxa being found; invertebrates are picked out of debris in the field.	Quick to collect, samples a variety of habitats	Not quantitative, collections from all habitats are pooled, so can't associate taxa with specific habitat, don't get motile or nocturnal taxa, may be hard to standardize crews	Process in field 0.5 - 1.5 hours, ID one sample in 2-3 hours with chironomids; if diverse site, up to 8 hrs to process in lab
Activity trap: 2 liter enclosed plastic funnel trap (Minnesota)	2 liter clear plastic beverage bottle cut so top is inverted funnel, placed horizontally in shallow water, 10 traps/site, left out overnight two nights in pairs in near shore emergent zone in water < 1 m deep. Funnel opening 7/8" (ca 2.3 cm).	Captures actively swimming predators, leeches, beetles and bugs that aren't caught as well in the dipnets; predators tend to die in trap as DO declines	Need to be left out two nights, requires revisit to the site, doesn't capture full richness, need to use with DN method; death of vertebrates in trap	2.3 hours to pick 5 pooled samples from 10 traps; ID time for 2 DN + 10 AT samples, 4.2 hrs
Activity trap: minnowrap style made of alumunum window screen (Ohio)	18" cylinders of aluminum window screen 8" diameter, funnels at both ends made of fiberglass window screen with 1.75" (ca 4.5 cm) openings, 10 traps/site spaced around perimeter of wetland (in small wetlands), place in 6" - 12" water	Quick to collect, easy to standardize, captures broad range of large active and nocturnal taxa, free of debris, get fish and amphibians for relative abundances, use in variety of habitats	Don't get density data/m², don't collect sedentary taxa effectively, activity of some taxa may relate to weather and temperature, requires a second visit to site	0.4 - 0.8 hours each AT to process and ID

APPENDIX D. LABORATORY PROCESSING PROCEDURES, ADVANTAGES AND DISADVANTAGES

Procedures	Options	Advantages	Disadvantages/Comment
Preservation	Preserve with 95% alcohol in field to final conc of 70-80%, represerve in lab	Maintains the samples for later work; keeps samples for QA procedures; no chance of predation in sample; sample can be put in water for picking and identifications	Must represerve heavy samples, not suitable if vertebrates present (use 8% formalin), cost of alcohol, need flame proof storage, lose color and behavior characteristics of live organisms
	Collect and chill sample in field, store refrigerated in lab	Specimens retain color, saves cost of alcohol, can dispose of bulk of debris before preserving in alcohol	Must process samples in short time, possibility of predation in samples, requires refrigeration space
Picking samples	Pick out invertebrates in lab	Sample will be processed under consistent conditions, with light box, magnifying lens or microscope; subsampling can be controlled with grid, lighting is adequate	Takes time, depending on how much debris is in the sample.
	Pick invertebrates in field	Reduces time for sample picking in lab, less material needs to be preserved and brought back to lab	Conditions of weather and light may make for inconsistency in quality of picking, lack of magnification, slows down the field work, may reduce taxonomic resolution
Sample jars	Plastic	Light weight, unbreakable, good for use in the field	May not be good for long term storage, may need to transfer to glass jars unless using nalgene-type jars with alcohol-tight lids
	Glass	Good for long-term storage, with solvent proof seals, can see sample	Too heavy for field work, breakable
Aids to sample picking	Glass tray (8"x12" x 1.5") and light box	Good for viewing sample over light box, make from window glass, seal or tape the top edges, use with magnifying lamp, light from below and above makes sample clearer	Difficult if sample has a lot of debris, this can be overcome by doing smaller portions of sample diluted with water so organisms are visible and spread out in tray until done
	Dissecting microscope	Good for picking small organisms such as chironomids	Smaller amount of sample is processed at one time
	White pan or tray	Inexpensive, use with magnifying lamp, useful for citizen monitoring	Not as good as glass tray with light box, no light from below the sample
	Stains, Rose bengal, Congo red	Makes some preserved invertebrates (e.g. chironomids) stained and more visible	Stain must be in the alcohol used to preserve sample in field (Lackey and May 1971, Brinkman and Duffy 1996)
	Density gradient float	Adding 30% sucrose to sieved sediment causes invertebrates to float up, works better with live sample	Is messy, needs to be done until no further organisms float up, may not work as well with preserved samples (Anderson 1959, Ritchie and Addison 1991)
Amount of sample to pick	Pick entire sample	Counting entire sample gives maximum information on species in sample and allows better estimation of relative abundances in sample (see text)	Requires greater work effort, especially if the sample has a lot of debris
	Subsample	Reduces effort in picking sample especially when a lot of debris is present	May lose information on the composition of the invertebrates at the site
	Pick to a fixed count	Reduces effort in picking sample, is done to a minimum of 100, 200 or 300 organisms	Compared to total sample count, fixed count has lower taxa richness and more variability in data for metrics (see text)

Any biological monitoring program should have its own written Standard Operating Procedures (SOP's) for each assemblage that is used, and for all field and laboratory protocols and data analysis methods. Detailed guidance for developing a Quality Assurance Project Plan is available from the U.S. Environmental Protection Agency, Guidance for the Data Quality Objectives Process (EPA QA/G-4, EPA/600/R-96/055, ORD) and EPA's Content Requirements for Quality Assurance Project Plans for Water Division Programs (EPA Region V, Environmental Sciences Division, August 1994). For examples of SOPs see Florida DEP SOP Draft 2000, North Carolina DWQ 1997 SOP.

The following text describes some considerations specific to biological assessment using macroinvertebrates.

1. At least 10% of the samples should be checked and verified for the accuracy of taxonomic identifications by a second taxonomist and/or by comparison to the reference collection. Changes in taxonomy must be tracked and continuously updated, using current taxonomic references and regional experts. At least 10% of the picked residuals of samples should be checked by the experienced taxonomist to assure all the macroinvertebrates were picked out.

2. Samples should be stored carefully for a defined period of time, if not indefinitely, for later verifications or quality assurance, especially if the assessments are part of a regulatory decision. They should not be disposed of right after the identifications are completed. Taxonomists are revising certain groups of invertebrates as new information is gathered. Archived samples can be reanalyzed for the group in question if necessary.

3. The lab should have defined rules for counting of taxa of each invertebrate group to be identified at different levels of taxonomic resolution, for identifying immature specimens, and for when it is acceptable to identify "morphospecies."

4. *Taxonomic coding.* In addition to having a lab list of taxa identified with unique codes, the wetland invertebrates are coded in the database using the national Integrated Taxonomic Information System (ITIS) codes (www.itis.usda.gov/). This is a partnership of Federal agencies in the United States and Canada to improve and standardize biological nomenclature nationwide. The ITIS database includes taxonomic information with authority, synonyms, common names, a unique taxonomic serial number (the code), publications, experts, and data quality indicators. There should be a routine procedure for checking the ITIS database to update and change any codes in the invertebrate database.

One problem is that not all wetland invertebrates have received the codes or serial numbers in the ITIS system. So the agency must, temporarily, create its own codes for uncoded taxa. Minnesota is coding taxa not found in the ITIS database with unique negative numbers. These can be easily flagged and have no possibility of interfering with ITIS codes. ITIS is requesting input from biologists, so states can notify them of uncoded taxa. The ITIS database should be searched periodically

to see if uncoded taxa have received codes so the in-house, temporary codes can be replaced with ITIS codes.

5. ***Database and records management.*** It is important to have a database management staff person assigned to ongoing work with the biological data and the associated physical and chemical wetlands data. This is especially true during the time of development of the biological database and the selection of metrics and indexes. But the work is never static; changes occur and new directions arise as the biological assessments are made and applied to reports and decisions. Taxonomy is updated, and new needs arise in linking the biological data to other data sets and in making assessments accessible to the public through agency web pages. Data should be backed up routinely.

6. ***Field data forms.*** Standardized field forms should be developed to ensure that methods and site conditions are thoroughly recorded and all procedures are consistently documented. Backup photocopies should be stored separately from original data forms and field notebooks in case data are lost or destroyed. It is important to reconcile issues of data comparability between old and new methods. In addition to the standard locational, wetland habitat, and physical sampling information, the invertebrate sampling method, numbers of samples collected, and the depth and locations of sampling should be included on a field sketch. GPS (Global Positioning System) locations of latitude and longitude of sampling locations should be recorded.

7. ***Sample labeling and tracking.*** All sample containers should be clearly labeled with pencil or India ink on labels of 100% cotton or alcohol-proof paper placed inside the jar and include the following information: sampling method, sample number, station ID, date, collector, site name and location (township, county), and sample jar number (e.g., jar 1 of 2, etc.) if more than one jar is used for a single sample. Standard procedures for sample tracking and chain of custody should be established as part the monitoring program's quality assurance plan.

8. ***Laboratory Procedures.***
a. Coding and recordkeeping. In the laboratory, a unique identification code should be assigned to each sample. This code should be included in all subsequent records associated with the sample (vial or jar labels, tally sheets, databases, etc.). All laboratory data forms should be standardized and should include the information described above (see Sample Labeling and Tracking) in addition to the sample identification code and laboratory staff name(s). As with field data forms, backup photocopies should be made of all laboratory records.

b. Sample sorting. Macroinvertebrates should be sorted from detritus (picked) by trained personnel working under the supervision of a professional biologist. Picking procedures should be included in Standard Operating Procedures. It is recommended that a 10% random subset of all samples be repicked by different experienced laboratory personnel working under the supervision of a professional biologist to determine sorting completeness. In all cases, initial sample sorting and quality control repicking should be completed by different individuals.

c. The *Standard Operating Procedures* should clearly define which groups of macroinvertebrates are to be picked, what if any methods can be used to subsample, and what level of taxonomic resolution should be used for the identifications for each group of invertebrates.

APPENDIX F. INVERTEBRATE ATTRIBUTES FOR BIOLOGICAL INDEXES, ADVANTAGES AND DISADVANTAGES.

Attribute category	Advantages	Disadvantages/Comments
Richness measures: total taxonomic richness	Information-rich, the total number of taxa most often declines significantly as impairment increases; richness measures often have the lowest variability or lowest coefficients of variation	May actually increase in moderate disturbance (e.g., canopy opening), requires taxonomic expertise; not all groups can be identified to species
Proportion of tolerant invertebrates	The proportion of tolerant invertebrates to the total sample count tends to increase as impairment increases because they are more generalist and more able to feed broadly	Which taxa are tolerant needs to be derived from datasets by detecting which taxa become disproportionately represented under greater impairment; need to retest on other datasets
Number of intolerant taxa	Intolerant taxa are sensitive to impairments; they are most likely to decline under human disturbances to wetlands; they will occur in least impaired reference sites	Information as to which taxa are intolerant or tolerant needs to be derived from datasets for the wetland class and retested on other datasets
Trophic structure	A high proportion of predators may reflect complex community structure and abundance of prey types eaten by the predator	More testing is needed to relate attributes of trophic structure to gradients of human influence in wetlands to see how well the attributes respond
Functional feeding groups (FFG)	Based on food gathering and feeding morphology, the feeding groups may shift as conditions of heterotrophy or autotrophy change; an increase in the proportion of filterers to all others may indicate a shift to planktonic algae from attached algae	More testing is needed to relate attributes of functional feeding groups to show responses to gradient of human disturbances in wetlands
Longevity of taxa	Longer-lived wetland taxa (e.g. Odonata) experience longer exposure to wetland conditions than very short-lived taxa (e.g. mosquitoes and fairy shrimp); expect long-lived taxa to be reduced by chronic impairment to sites	Wetland taxa are shorter lived than some vertebrates; more permanent wetlands will have longer lived invertebrates, more seasonal wetlands will have fewer; need to classify wetlands for IBI
Invasive exotic species	Invasive exotic species may negatively alter the invertebrate community structure; expect an increase in invasive species with greater human influence	Exotic invertebrates may not be found in many wetlands
Soil resting egg bank	Provides information on resident species that deposit eggs in soil to survive dry cycles; useful in more temporarily ponded wetlands	Not useful in more permanent wetlands, requires processing soils, keys to resting eggs are inadequate, need to rear eggs for identifications
Abundance or biomass measures	Of interest for understanding overall secondary production of the wetland, human disturbance can reduce abundances of many but not all taxa	Most variable of the attribute categories, more subject to seasonal changes and sampling error; tolerant individuals may increase with disturbance
Ratios vs. proportions	Can indicate when one group is changing in relation to the entire community (proportion) or in relation to another invertebrate group (ratio); proportions are recommended	Proportions based on partial sample count (e.g., 200 organisms) need to be tested for variability compared with counting the entire sample; ratios are vulnerable to error when both groups are varying
Invertebrate health	Malformations indicate contaminants, have been shown in chironomids in contaminated sediments, have occurred in dragonfly exuviae	Requires knowledge of baseline level of malformations; requires expertise, however limiting, to clear malformations (missing or extra teeth in chironomids) requires less expertise

Metrics	Maine	Minnesota	Montana	Ohio	Patuxent
APPENDIX G. EXAMPLES OF INVERTEBRATE ATTRIBUTES USED AS METRICS IN DIFFERENT STATES FOR BIOLOGICAL ANALYSIS OF WETLANDS. ATTRIBUTES BEING TESTED ARE STARRED.					
Richness	Total genera richness*	Total taxa	Total taxa	Total taxa*	Total taxa*
	EOT genera*	Chironomid genera	Chironomidae taxa	Chironomid taxa*	# Snail genera*
	Odonata genera*	Leech taxa	Leech, sponge and clam taxa	Mollusca taxa*	# Odonata genera*
	Ephemeroptera genera*	Odonata genera	POET taxa	# Intolerant taxa*	# Ephemeroptera and Trichoptera gen.+ presence of Sphaeriidae and dragonflies*
	Trichoptera genera*	Snail taxa	Mollusca+Crustacea taxa	—	—
	Chironomid genera*	—	Odonata + Trichoptera taxa*	—	—
Composition	—	—	%Chironomidae*	—	—
	—	—	%Mollusca+Crustacean taxa*	—	—
	—	—	%Orthocladiinae to total Chironomidae*	—	—
	—	—	%Diptera*	—	—
	—	—	%Tanytarsini*	—	—
	—	—	%Trichoptera*	—	—
	—	—	%Odonata*	—	—
	—	—	Ratio POET to POET and Chironomidae*	—	—
	—	—	%Pelycypoda*	—	—
Tolerance/ intolerance	% of total count for :	ETSD metric	%1, 2, 5 dominant taxa	% dominant 3 taxa of total count	—
	EOT*	# of intolerant taxa	—	Physella snail abundance	—
	Odonata*	Tolerants % of total count	—	% Tanypodinae and Tanytarsini of Chironomidae	—
	Ephemeroptera*	Erpobdella % of total count	—	—	—
	Trichoptera*	Dominant 3 % of total count	—	—	—
	Gastropoda*	—	—	—	—
	Isopoda*	—	—	—	—
	Oligochaeta*	—	—	—	—
	Amphipoda*	—	—	—	—
Trophic structure		Corixidae % beetles + bugs	% shredders		Corixidae % beetles + bugs*
	—	—	—	—	% Total indiv as scrapers*
	—	—	—	—	% total indiv as shredders*
	—	—	—	—	—
Individual health	—	Chironomid malformations*	—	—	—
Other metrics	—	—	—	—	% total abundance of 3 most abundant taxa*
	—	—	—	—	Ratio Ephemeroptera + Odonata+Trichoptera abundance to chironomids*
	—	—	—	—	Shannon-Weaver Index*

APPENDIX H. CONTACT INFORMATION FOR STATES/AGENCIES IN APPENDIX B AND IN TEXT

	Maine	Minnesota	Montana	Florida	Ohio	Patuxent
Name	Jeanne DiFranco	Judy Helgen, Joel Chirhart	Randy Apfelbeck	Russ Frydenborg	Mike Gray	Donald Sparling or Peter Lowe
Agency	Maine Department of Environmental Protection	Minnesota Pollution Control Agency	Montana Dept. of Environmental Quality	Florida DEP	Ohio EPA	USGS Patuxent Wildlife Research Center
Program	Biological Monitoring Unit	Biological Monitoring Unit	Water Quality Monitoring Section	Environmental Assessment Section	DSW	
Address	312 Canco Rd., Portland, ME 04103	520 Lafayette Rd.,St. Paul, MN 55155	2209 Phoenix Ave., Helena, MT 50620	2600 Blair Stone Rd.Tallahassee FL 32399	4675 Homer Ohio Lane Groveport OH 43125	11510 American Holly Dr.Laurel, MD 20708-4017
E-mail	Jeanne.L.difranco@state.me.us	judy.helgen@pca.state.mn.us joel.chirhart@pca.state.mn.us	rapfelbeck@state.mt.us	russel.frydenborg@dep.state.fl.us	mike.gray@epa.state.oh.us	Donald_Sparling@USGS.GOVPeter_Lowe@USGS.GOV
Web address	http://janus.state.me.us/dep	http://www.pca.state.mn.us	http://www.deq.state.mt.us/	http://www.myflorida.com/environment/learn/science/laboratories/reports/index.html	http://www.epa.state.oh.us/dsw/wetlands/wetland_bioassess.html	www.pwrc.usgs.gov

Ten metrics with scoring criteria and data ranges for large depressions. The numbers of reference sites (ref) and agriculture- (ag) and urban stormwater-influenced (urb) sites scoring in each range are given. n is # of sites scoring in the ranges. Codes are given for each metric. These codes are used in Appendix J.

Metric	Metric data range	Ranges	Score	Ref	Ag	Urb	n
1. Total invertebrate taxa Code: TaxaTotal	23-29 taxa	>59-79	5	9	5	0	14
		>41-59	3	5	8	8	21
		<23-41	1	0	3	6	9
2. Odonata Code: Odonata	1-7 general	5-6	5	8	6	3	17
		3-4	3	6	7	4	17
		0-2	1	0	3	7	10
3. Chironomid taxa Code: ChirTaxa	0-21 general	14->20	5	11	4	3	18
		7-13	3	3	9	5	17
		0-6	1	0	3	6	9
4. Leech taxa Code: LeechTaxa	0-9 taxa	6-9	5	6	2	0	8
		3-5	3	3	8	6	17
		0-2	1	5	6	8	19
5. Snail taxa Code: SnailTaxa	1-9 taxa	7-9	5	6	3	2	11
		4-6	3	8	8	5	21
		0-3	1	0	5	7	12
6. ETSD* Code: ETSD	1-10	7-10	5	8	2	1	11
		4-6	3	3	9	9	21
		0-3	1	3	5	4	12
7. Number of intolerant taxa Code: IntolTaxa	0-7 taxa	5-7	5	7	1	0	8
		3-4	3	4	5	7	16
		0-2	1	3	1	7	20

8. Tolerant taxa proportion of
    sample count
    Code: Toler%
	13-92%	13-39%	5	6	2	2	10

		>39-65%	3	5	5	6	16
		>65%	1	7	5	6	18
9. Leech *erpobdella* Code: Erpo%	0-14%	0-<1%	5	13	7	9	29
		1-5%	3	1	5	3	3
		>5%	1	0	4	2	6

10. Corixidae proportion of beetles
    and bugs in activity traps
    Code: Corix%
	14-87%	<33%	5	9	8	8	25

		33-67%	3	4	4	3	11
		>67%	1	1	4	3	8

*ETSD metric is total of number of genera of mayflies (*Ephemeroptera*), caddisflies (*Trichoptera*) plus the presence of fingernail clams (*Sphaeriidae*) and dragonflies.

Codes in Appendix I.

Site Name	SiteType	TaxaTotal	Odonata	ChirTaxa	LeechTaxa	SnailTaxa	ETSDMetr	IntolTaxa	Toler%	Erpo%	Corix%	IBI Score	Condition
Field	Ref	5	5	5	5	5	5	5	5	5	5	50	Exc
Glacial	Ref	5	5	5	5	5	5	5	3	5	5	48	Exc
Lasher	Ref	5	5	5	3	5	5	5	5	5	3	46	Exc
Minnow N	Ref	5	3	5	5	5	5	5	5	5	3	44	Exc
Bloom	Ref	3	5	5	3	5	5	5	5	5	1	42	Exc
Overby N.	Ref	5	5	5	1	3	5	5	5	5	3	42	Exc
Sheets Big	Ag	5	5	5	3	3	5	5	5	5	1	42	Exc
Kasma	Ref	5	5	3	5	3	3	5	1	3	5	38	Exc
Leman's	Ref	5	3	5	5	3	5	1	1	5	5	38	Exc
Malardi	Ag	5	3	5	3	5	1	3	5	3	5	38	Exc
Mud	Urb	3	3	5	3	3	3	3	5	5	5	38	Exc
Prairie	Ref	3	3	5	1	3	5	5	3	5	5	38	Exc
Sheets Small 1	Ag	5	5	5	3	3	3	3	1	3	5	36	Mod
Turtle	Urb	3	1	5	3	3	3	3	5	5	5	36	Mod
Zager	Ref	3	5	5	1	3	1	3	5	5	5	36	Mod
Battle	Urb	3	5	1	1	5	5	3	5	5	1	34	Mod
Donley Lg. N	Ref	5	3	3	3	5	3	1	3	5	3	34	Mod
Lake 21	Ref	3	5	5	1	3	1	3	3	5	5	34	Mod
Legion	Urb	3	5	3	1	3	3	1	5	5	5	34	Mod
New London	Ref	3	3	5	1	3	3	3	5	5	3	34	Mod
Cataract	Ref	5	3	3	5	3	1	1	1	5	5	32	Mod
Lake Park	Ag	5	3	5	1	5	1	1	3	3	5	32	Mod
Savage	Urb	3	3	3	1	3	3	3	3	5	3	32	Mod
Seter	Ag	5	5	3	1	3	3	1	3	5	3	32	Mod
Sethre	Ag	3	5	3	3	5	3	1	3	3	3	32	Mod
Skarpness E	Ag	3	3	3	3	3	3	3	1	5	5	32	Mod
Tyrone Bean	Ag	3	3	3	5	1	3	3	3	3	5	32	Mod
Bunker	Ag	1	5	5	1	1	3	3	3	5	3	30	Mod
Round	Urb	3	3	3	1	3	3	3	1	5	5	30	Mod
Sunset	Urb	1	5	1	1	5	1	1	3	5	5	28	Mod
Jones	Urb	1	1	1	3	1	3	3	5	3	5	26	Mod
New Prairie	Ag	3	3	3	3	3	5	1	1	1	5	26	Mod
Wood Lake	Urb	3	1	1	3	1	3	3	5	1	5	26	Mod
Breen	Ag	3	3	1	1	3	1	1	5	1	5	24	Poor
Davis	Ag	1	1	1	3	3	1	1	5	5	3	24	Poor
Grass	Urb	3	3	1	3	1	3	3	3	1	3	24	Poor
Sigler	Ag	3	5	3	3	3	3	1	1	1	3	24	Poor
Trappers	Ag	3	1	3	3	3	3	1	1	5	1	24	Poor
Ney	Ag	3	3	3	1	1	3	1	5	1	1	22	Poor
Casey	Urb	1	1	3	1	1	1	1	3	3	3	18	Poor
Lost1	Urb	1	1	3	3	1	1	1	3	3	1	18	Poor
Wakefield	Urb	1	1	3	1	1	1	1	1	5	3	18	Poor
Rose Golf	Urb	1	1	1	1	1	3	1	1	5	1	16	Poor
Sucker N	Ag	1	1	3	1	1	1	1	1	5	1	16	Poor

Case study flow chart for developing invertebrate IBI in Minnesota for large depressional wetlands (see text, Helgen and Gernes in press, Gernes and Helgen 1999).

STEP 1:
SELECT STUDY SITES ——

- Ecoregion: North Central Hardwood Forest (has abundant wetlands, is central to the state, includes Twin Cities metro area)
- Wetland class: large depressions
- Range of disturbances: 15 least impaired , 15 agriculture-affected, 16 urban-affected wetlands

STEP 2:
PLAN INVERTEBRATE SAMPLING ——

- Attribute selection: 10 attributes tested based on previous work, additional attributes were tested (see Appendix J)
- Sampling strata: sampling to take place in near shore emergent vegetation zone from edge to less than 1 m deep (vegetated zone contains greatest richness of invertebrates; nearshore area may be more exposed to disturbances from the near wetland landscape)
- Sampling methods: two standardized dip netting procedures plus 10 activity traps; all samples taken within approx. 40-50 m distance along shoreline
- Seasonal index period in June for optimal invertebrate development

STEP 3:
FIELD SAMPLING ——

- Sampling methods had been pretested in previous projects
- Samples were preserved in field and processed in lab; vegetation from dip netting procedure was left at the site
- Water and sediment chemistry samples were collected (for total N, total P, conductivity, chloride, chlorophyll a, metals)

STEP 4:
LABORATORY PROCESSING ——

- Entire sample was picked for macroinvertebrates (not for Ostracoda or zooplankton); *Neopleia* was counted on a grid over lightbox and not picked; chironomid identifications were contracted out, all others identified at MPCA lab
- Most taxa were identified to genus with exceptions: snails to species where possible; fingernail clams at family level
- SOP is written and reference collections made for verifcation
- Data were entered into ACCESS database using ITIS coding and MPCA coding where necessary.

STEP 5:
METRIC ANALYSIS ——

- Sites were analyzed for a human disturbance gradient composed of estimates of disturbance in 50 m buffer and in near wetland landscape, estimates of hydrologic alteration and rankings of water and sediment chemistry data as compared to reference condition; each disturbance was scored and summed to one score for human disturbances
- Attribute data was plotted against scores for human disturbance gradient for all the sites, and against water and sediment chemistry data (see Figure 6 and 7) for all sites
- Attribute data that showed a response to the human disturbance gradient or to the chemistry data was trisected for a 1, 3 5 scoring as metrics (see Appendix I)
- 10 metric scores were summed to IBI score and IBI scores were plotted against the human disturbance gradient and chemistry data (see Figure 1)
- IBI scoring range (10 – 50) was trisected for a hypothetical ranking of best, moderate and poor condition (see Appendix J)

Glossary

Abundance   The number or count of all individuals of one taxon or all taxa in a sample. When expressed per unit area or unit volume, it is called density.

Activity trap (AT)   A passive sampler, usually containing a funnel-shaped opening and a container that is enclosed, either as a bottle or jar, or with a mesh screen. The organisms swim into the funnel and are trapped in the container. The size of the funnel opening determines the size of organisms that can swim into the trap.

Aquatic life use support   The ability of a waterbody to support the native aquatic life that it is capable of supporting when there is little or no human disturbance, or the ability of the waterbody to support aquatic life as designated for that type of water in Water Quality Rules.

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 (insects, crayfish, clams, snails, etc.), and vascular plants

Attribute   A measurable component of a biological system. 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 influence.

Benthic invertebrates   Invertebrates that inhabit the bottom or benthic area of a waterbody.

Benthos   All the organisms that inhabit the bottom (benthic) area of a waterbody.

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

Biota   All the plants and animals inhabiting an area.

Bottletraps   A kind of activity trap that is constructed from a bottle with a funnel opening.

Box-and-whisker plots   Plots of data values made for individual attributes with percentile "boxes" around the median value, e.g., 25% and 75% boxes. The "whiskers" are lines extending beyond the percentile boxes for data value ranges that extend outside the percentile. These are used to compare the amount of overlap in the data range for an attribute between reference and impaired sites.

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

Detritivores   Organisms that consume decomposed organic particulate matter.

Dipnet   A sturdy, long-handled aquatic net for sampling aquatic habitats, also called sweep net. Mesh sizes range from 500 to 1000 microns.

Disturbance   "Any discrete event in time that disrupts ecosystems, communities, or population structure and changes resources, substrate avail-

ability or the physical environment" (Picket and White 1985). Examples of natural disturbances are fire, drought, and floods. Human-caused disturbances can be referred to as "human influence" and tend to be more persistent over time, e.g., plowing, clearcutting of forests, conducting urban stormwater into wetlands.

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

Dose-response In toxicology, a graded response by test organisms to increasing concentrations of a toxicant. In biological assessment, dose response indicates a graded response (up or down) of an attribute to a gradient of human disturbance.

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.

Epiphytic A layer of periphyton located on or attached to the surfaces of stems of macrophytes. See *periphyton*.

Family A taxonomic category comprising one or more genera or tribes of common evolutionary origin, and often clearly separated from other families. Family is between the categories of order and tribe (or genus).

Functional feeding groups (FFGs) Groupings of different invertebrates based upon 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).

Funnel trap See *Activity trap*.

Genus (plural genera) A taxonomic category of organisms composed of one or more species that are related morphologically and evolutionarily, the principal category between family and species.

Gradient A regularly increasing or decreasing change in a factor (e.g., a single chemical) or combination of factors (as in the *Human disturbance gradient*).

Gradient of human influence The relative ranking of sample sites within a regional wetland class based on the estimation of degree of human influence (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.

Herbivore An organism that consumes plant or algal material.

Human disturbance gradient A gradient or range of perturbations or impairments (or human-caused disturbances) that alter the physical, chemical, or biological integrity of ecosystems or habitats. The effects may persist over periods of time. Examples pertaining to wetlands are alterations in the natural hydrology, in the near wetland landscape, or in the wetland buffer; or chemical pollution, siltation, removal of aquatic vegetation, exotic species introductions, cattle, fish-rearing, conducting of urban stormwater, or agricultural runoff. Compare human disturbances with discrete events of natural disturbances.

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. Similar to economic indexes used for expressing the condition of the economy.

Index period A defined interval of the season that serves as the sampling period for biological assessments. For invertebrates the index period would be the interval of time when there would be optimal development of invertebrates and optimal presence of resident taxa that developed in the waterbody.

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.

ITIS The national Integrated Taxonomic Information System (ITIS) coding system for organisms in the United States and Canada. The database includes information on each organism with authority, synonyms, common names, and a unique taxonomic serial number (the ITIS code) for all taxonomic levels. It lists publications, experts, and data quality indicators. ITIS accepts input from investigators regarding uncoded taxa. The database is update periodically. Web site: www.itis.usda.gov/

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.

Macrophytes The visible aquatic plants that are emergent, floating, or submersed under water. They may be attached to the bottom or not. Distinguished from algae, most of which are microscopic.

Metric An attribute with empirical change in the value along a gradient of human influence. See *Attribute*. Metrics are scored individually, and each score composes the total score for the IBI.

Morphology The structure and form of an organism, both external and internal. Taxonomic identifications are mostly derived from the examination of external morphologies, with exceptions.

Morphospecies A taxon that has distinct morphologies from other similar taxa and is identified as a distinct taxon based solely on the morphological differences (see Oliver and Beattie 1996). The biologist must determine that the differences are not from developmental stages or change in features within the same taxon before the taxon can be counted in the overall taxa richness.

Multihabitat method A sampling method in which the sampling effort attempts to sample most of the habitats with the zone of study. The effort is variously distributed among the habitats; in some methods it is distributed in proportion to the representation of the habitat in the zone of study, in other methods it is distributed by giving greater effort to habitats more likely to have the desired organisms.

Multivariate analysis Mathematical analysis that examines numerous variables simultaneously. Data from communities of organisms are multivariate because there are several species with differing abundances responding to numerous environmental factors. Various statistical methods are used, e.g., ordination or discriminant analysis, to analyze several variables at once.

Omnivores Are organisms that consume both plant and animal material.

Periphyton The layer of algae that coats substrates in aquatic systems such as plant stems, rocks, logs, and benthic muds. This layer of algae coating is often also colonized by bacteria, protozoans, and rotifers and other microorganisms.

Population A group of individual organisms of the same species living in the same area.

Predators   Organisms that consume animals.

Proportion   The mathematical relation of one part to the whole, expressed as magnitude or degree, e.g., percent. An example of proportion attributes are the percent of tolerant individuals of the whole sample count or the proportion of the abundance of the top two dominant taxa to the sample abundance.

QA or QAPP   The written plan for quality assurance, or the quality assurance program plan, that provides written detail for quality assurance plans for quality assurance checks for all aspects of field and laboratory procedures.

Ratio   The numerical quotient of two variables or quantities. An example of an attribute that is a ratio is the ratio of scrapers/collector-filterers (see *proportion*).

Reference site A minimally impaired site that is representative of the expected ecological conditions and integrity of other sites of the same type and region.

Relative abundance   Is the abundance of one group or taxon of organisms in relation to the total abundance of the sample.

Replicate   A term usually reserved for the repetition of an experiment to obtain information for estimating experimental error. It is sometimes used informally to describe repeated consistent samples taken at a site with the same method, in the same strata and habitat, and on the same date.

Sample   A representative part of a larger unit used to study the properties of the whole.

SOP   Acronym for Standard Operating Procedure. This is the detailed, written description of all the methods to be used for field and laboratory procedures.

Species   A taxonomic category below genus, the fundamental biological unit, a population of organisms that share a gene pool and are able to reproduce successfully and produce young that are able to reproduce.

Stress (or stressor)   Any environmental factor that impedes normal growth, reproduction, or survivorship of organisms, or causes adverse changes in populations of organisms or in ecosystems.

Sweep net   Another term for *Dipnet*.

Taxon (singular, taxa plural)   A distinct taxonomic group of any level (e.g., family, genus or species); includes all subordinate groups. Taxon is any group of organisms that is distinct enough from other groups to be treated as a separate unit.

Taxonomic system   The hierarchy of classification of organisms.

Taxonomist   A biologist who specializes in the identification of organisms.

Taxonomy   The practice of describing, naming, and classifying organisms.

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

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.

Trisecting   The division into three parts of a range of data for scoring a metric.

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)].