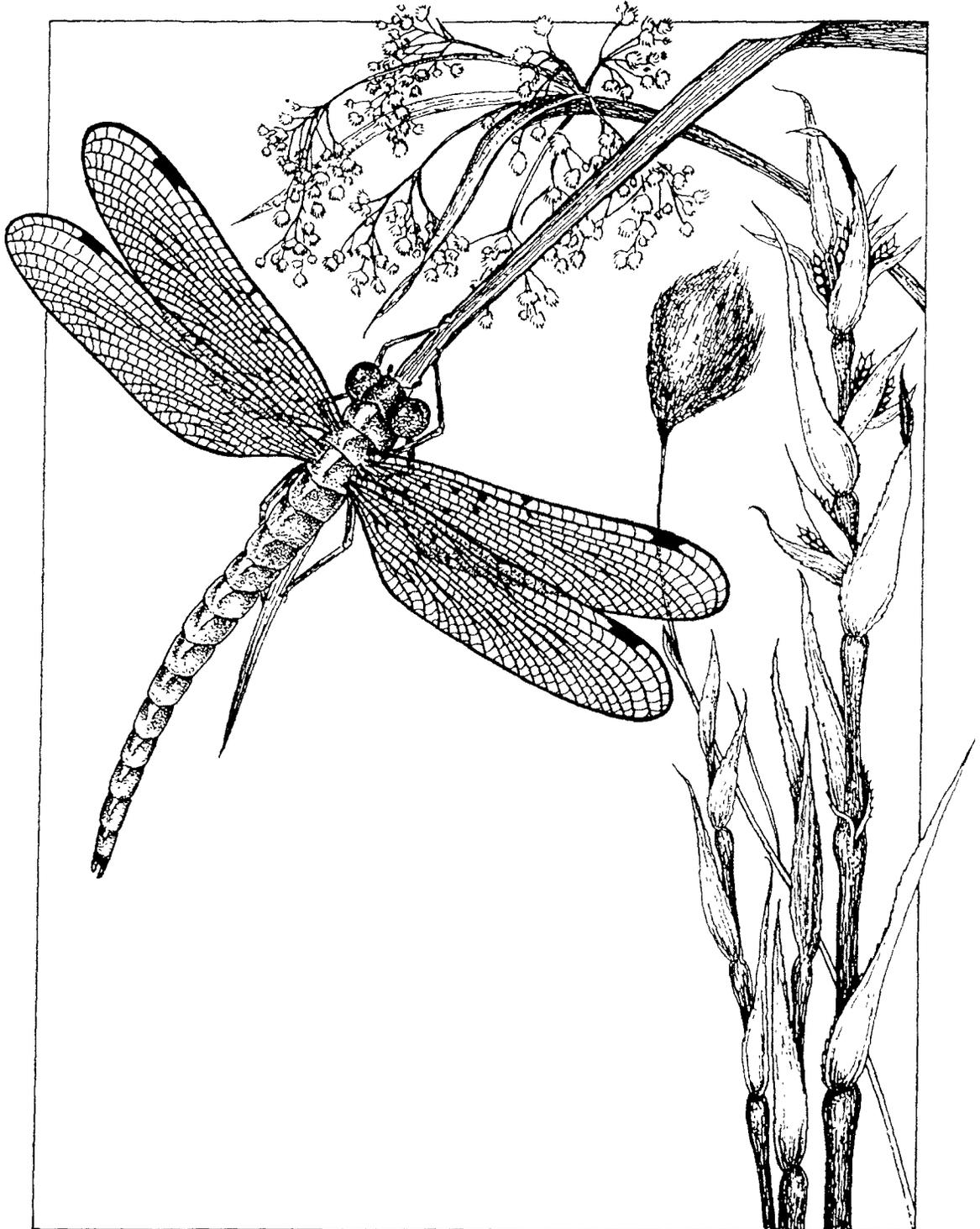




Bioindicators for Assessing Ecological Integrity of Prairie Wetlands



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of Prairie Wetlands**

by

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Notice

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Executive Summary

This document is designed to assist State agencies and other users in developing programs to monitor biological communities of prairie pothole wetlands and ultimately to develop biological criteria appropriate for protecting this valued resource. The document emphasizes one aspect of biocriteria development: the selection (targeting) of assemblages of biological indicators (bioindicators) and metrics as a basis for designing and conducting biosurveys. Before meaningful biocriteria can be developed and implemented, appropriate bioindicators must be identified and tested. Bioindicators are species, species assemblages, or communities whose presence, abundance, and condition is indicative of a particular set of environmental conditions.

As a foundation for developing biocriteria, the document is a compilation of current knowledge regarding responses of various organisms to natural and anthropogenic stresses, and a summary of the utility of various organisms as indicators of these stresses. Information is organized by chapters covering each of the major assemblages of related species: microbes, algae, vascular plants, invertebrates, amphibians, and birds. For each, the document reviews past and ongoing biological monitoring programs in the prairie region's wetlands. The document provides quantified estimates of spatial and temporal variability of various biological groups from a limited number of existing data sets. As an additional aid to future monitoring, the document broadly describes field-sampling methods potentially applicable to the region's wetlands. By documenting the ecological roles of each biological group, the document also attempts to clarify understanding of interactions among ecosystem components and justify the use of particular biological groups as indicators. Several appendices of the document are in electronic format and provide a database of information on environmental tolerances, life history, habitat preferences, and other characteristics of individual species as well as tabulate results of the analyses of indicator variability.

The document concludes that in most prairie wetlands the possibility of ongoing or recent past exposure to excessive sedimentation is probably best indicated by species composition of algae and invertebrates, with emphasis on the epibenthic forms (organisms that live on the top surfaces of the sediment). Epibenthic and epiphytic (attached to plants) algae and invertebrates are also useful indicators of excessive enrichment, removal of vegetative cover, and turbidity that is occurring either currently or during past years as determined by analysis of decay-resistant remains. Ongoing or recent past changes of water regime and salinity as well as overgrazing in individual wetlands are perhaps best indicated by species composition of vascular plant communities. Longer-term changes in these factors can be inferred by examining seed banks and decay-resistant remains of invertebrates. Exposure to pesticides and heavy metal contaminants can sometimes be inferred from species composition of invertebrates and from various biomarkers in amphibians and birds. For bioaccumulative contaminants, tissues of individual plants and birds can be examined. Birds are also uniquely valuable for spatially integrating information on the hydrologic stresses to wetlands across entire regions.

1. Introduction

1.1 Need for This Document

Under the Clean Water Act (CWA), wetlands are legally considered "waters of the State." Thus, States are required to adopt narrative standards and criteria for protecting quality of wetlands (USEPA 1987, 1989), just as States have developed standards and criteria for other surface waters.

The US Environmental Protection Agency (USEPA) is responsible for developing regulations, policies, and guidance to help States implement a water quality standards program. USEPA policy requires that States adopt biological criteria as part of their water quality standards for wetlands. USEPA recommends that States use biological criteria to supplement the chemical and physical water quality standards they have used traditionally. USEPA has taken this approach because biological criteria measure the actual time-integrated response of the resident aquatic community to all environmental stresses, rather than inferring biological impairment from a comparison of values derived from laboratory bioassays with instantaneous field measurements of the same or similar contaminants (USEPA 1990).

To satisfy USEPA requirements for biocriteria development, State agencies need technical information. Specifically, they need to know which biological resources to monitor in wetlands, how to monitor them, how to analyze and interpret the data, and what it costs. To adequately monitor wetlands, develop sound criteria and standards, and evaluate ecological risks, States also need information on what levels of various contaminants (or other regulated stresses) will impair the integrity of various kinds of wetland communities. USEPA is mandated to provide States with such technical guidance and information, drawn from comprehensive synthesis of literature, research, and expert knowledge.

This document is intended as a contribution to the effort to establish biocriteria in one region of North America: the prairie region. The document compiles biological information on a single wetland type in this region: prairie potholes. These are wetlands that during most years are unconnected by surface water to lakes, rivers, or streams. The acreage of prairie pothole habitat has declined dramatically over the years as a result of human activities (Dahl 1990, Dahl et al. 1991), underlining the importance of monitoring and maintaining the quality of what habitat remains.

Processes for developing biocriteria may include:

- developing and testing consistent and biologically meaningful classifications of ecoregions and wetland types
- designing and conducting biosurveys, e.g., to establish and characterize reference sites and conditions
- developing and calibrating sample metrics

- evaluating data to assess environmental effects
- analyzing collected data to devise biocriteria.

This document assumes that the reader is generally familiar with these processes, and thus it does not discuss all of them in depth. Rather, the document emphasizes just one aspect of biocriteria development: the selection (targeting) of assemblages of biological indicators (bioindicators) and metrics as a basis for designing and conducting biosurveys. Before meaningful biocriteria can be developed and implemented, appropriate bioindicators must be identified and tested.

Bioindicators are species, species assemblages, or communities whose presence, abundance, and condition is indicative of a particular set of environmental conditions. Bioindicators can include assemblages of species that are important because they play pivotal roles in wetland ecosystems or assemblages that show outstanding sensitivity to, or strong correlation with, particular anthropogenic or natural factors (stressors). Bioindicators can be used both to assess wetland condition and to help measure and diagnose the actual causes of impairment. Bioindicators that are cost-effective and sensitive to particular stressors are useful for measuring attainment of other wetland management objectives as well, such as criteria for successful restoration of wetlands. The most useful bioindicators are likely to be ones that can distinguish between natural variation (e.g., phenological changes, annual wet-dry cycles) and anthropogenic stresses because the latter often mimic (and are overlaid upon) natural stresses, varying only in terms of relative magnitude.

Once bioindicator data have been collected, efforts are often made to integrate the data into metrics (e.g., density estimates, species counts) and ultimately to combine multiple metrics into indices of ecosystem condition. However, in the case of prairie wetlands, our knowledge of the relative performance of various metrics is severely limited, and no attempts have been made yet to devise and test indices of wetland integrity. For ecosystems generally, several excellent texts describe methods for developing and interpreting metrics and indices of condition (e.g., Green and Vascotto 1978, Gauch 1982, Pielou 1984, Isom 1986, Jongman et al. 1987, Ludwig and Reynolds 1988, Magurran 1988).

When developing biocriteria, it is seldom practical to address the environmental needs of all species within a particular assemblage of organisms (Landres 1992). Thus, many past efforts have focused on identifying functionally similar assemblages (or "guilds") of species and life stages. "Functionally similar" generally means similar with regard to reproductive strategy, food habits, and/or habitat preference. Examples of assemblages specific to wetland or aquatic species are discussed by Merritt and Cummins (1978) (macroinvertebrates), Dean-Ross and Mills (1989) (bacterial communities), Short (1989) (birds), Boutin and Keddy (1993), and Hills et al. (1994) (plants). A limitation of the functional assemblage approach is that much of the basic natural-history information needed to validate the appropriateness of the assemblages and classifications for prairie wetlands is currently lacking.

1.2 Document Organization

Biological monitoring (biomonitoring) generally focuses on one or more broad assemblages of related organisms. For this reason, this document uses common taxonomic nomenclature for designating assemblages of organisms (algae and microbes, vascular plants, invertebrates, amphibians, birds) as main section headings. Fish and mammals were not discussed in this document because of their relatively low diversity in prairie wetlands and the paucity of information. No attempt was made to give equal coverage to all topics in this document because availability of data varies greatly. The document provides information on each of the major assemblages of organisms in separate subsections:

x.1 Ecological Significance and Suitability as an Indicator: This describes why the particular assemblage is important to a wetland's functioning. That is, the subsection provides a rationale for using the assemblage as an assessment endpoint. The subsection also summarizes advantages and disadvantages of using the assemblage as an indicator of the ecological integrity of wetlands, and it identifies assemblages that are conventionally defined as subsets of the larger taxonomic assemblage.

x.2 Potential Indicator Metrics: This subsection lists the metrics (measurable aspects that summarize biological structure or function, e.g., species richness) that show promise as indicators of the ecological integrity of wetlands when applied to the taxonomic assemblage. Metrics were included if they had been used previously in prairie wetlands and/or were judged by the author to show promise (due to sensitivity, cost, and other factors) for reflecting wetland integrity. These lists of metrics are by no means definitive. Readers should not assume because a metric is listed, that it has been "proven" by research, and they should be aware that the listing is not all-inclusive. Many indicators deserve considerably more investigation and fine-tuning before they are used routinely. Whenever possible, users should obtain assistance from local wetland scientists when using the indicators to interpret wetland condition.

x.3 Previous and Ongoing Monitoring in the Region: From a review of over 400 publications, this subsection summarizes studies in the region that have addressed the taxonomic assemblage and the most common themes among these studies. Some ongoing research (circa 1994) is also noted, but listings are not necessarily comprehensive. This subsection is provided to help readers understand the relative extent of knowledge about different taxa and stresses. Understanding the extent of the knowledge base allows users to exercise proper caution in interpreting statements in this document. Such an understanding also can help focus future research on important topics that previously have been understudied.

x.4 Response to Stressors: This is the largest of the subsections, and for each major taxonomic assemblage (primary headings), it compiles and organizes all available prairie wetland literature according to various stressors (secondary headings) and metrics (tertiary headings). Information on both anthropogenic and natural stressors is presented together because few prairie studies have reliably distinguished any differences in the responses of biological communities to the effects of these. Stressors are not necessarily "bad" for maintaining wetland resources and functions of interest to humans. Indeed, some degree of disturbance or stress, whether natural or anthropogenic, is vital to the evolution and sustainability of prairie wetlands, whereas excessive levels (too much or too little) of a stressor can spell the eventual loss of

wetland function. Anthropogenic stressors are of special interest because they not only affect the structure and function of biological communities but also influence an ecosystem's ability to rebound from natural stresses. They are also, by definition, more amenable to human control.

Information in the stressor subsections describes how each metric (e.g., biomass, richness) responds to each stress, with the caveat that much of this information is derived only from single, perhaps unrepresentative, studies. When information is sufficient, this subsection gives physiological thresholds for impacts occurring at broad taxonomic levels, e.g., the level of salinity at which most wetland plants are incapable of reproducing.

Stressors discussed in this subsection are factors that are most likely to impair the ecological integrity of prairie wetlands when present at levels (or times) that differ greatly from their usual natural occurrence, and belong to the following categories. Six categories of stressors have been recognized:

x.4.1 Hydrologic Stressors: Changes in levels of surface water or water tables, i.e., drought or flood conditions, whether natural or aided by anthropogenic factors, e.g., drainage, groundwater withdrawal, global climate change.

x.4.2 Vegetative Cover Conditions: Changes in areal cover and density of vascular plants, whether natural, e.g., from muskrat consumption, or aided by anthropogenic factors, e.g., grazing, burning, mowing, herbicide application.

x.4.3 Salinity: Changes in total dissolved solids in the water column, soils, or sediments, whether natural or aided by anthropogenic factors.

x.4.4 Sedimentation and Turbidity: Physical changes in a wetland's benthic (bottom) substrate and/or changes in light penetration caused by introduction or resuspension of living or (especially) non-living matter as aided by either natural or anthropogenic factors, e.g., tillage, erosion.

x.4.5 Excessive Nutrient Loads and Anoxia: Occurrence of available phosphorus and nitrogen at greater-than-natural-background levels, usually because of the introduction of animal fecal material or application of fertilizers, and the resultant spread of anoxic conditions (i.e., lack of dissolved oxygen) throughout sediments and the water column.

x.4.6 Pesticide and Heavy Metal Contamination: Occurrence of insecticides, herbicides, fungicides, heavy metals (e.g., mercury), and selenium at greater-than-natural-background levels usually because of intentional application to crops or leaching from drained, irrigated, or mined soils. The relative ecological risks of these stressors to all wetland functions (not just biota) were assessed in an earlier comprehensive review of the prairie pothole literature (Adamus 1992). Also, it is important to recognize that few stressors act alone; cumulative interactions among stressors are usually important, and these interactions are summarized in Section 1.3.

x.5 Monitoring Techniques: This subsection describes techniques, equipment, and general considerations for sampling the particular assemblage of organisms. This is intended to be a

general description rather than a prescriptive manual or standard operating procedure. Information is intended to be sufficient to allow users to make choices among various types of equipment and protocols.

x.6 Variability and Reference Points: This subsection summarizes what is known about the spatial and temporal variability of each metric, e.g., the degree to which species richness changes within a wetland, among wetlands, within a year, and among years. Also, this subsection summarizes published maximum numeric values or ranges of numeric values for several metrics, e.g., densities of macroinvertebrates, in order to provide crude reference points that are useful for planning a monitoring program, calibrating wetland models, conducting realistic simulations, interpreting monitoring data, evaluating the success of restoration projects, and (perhaps) defining "optimum" conditions. However, these referenced numeric values are not necessarily representative of the prairie wetland population generally because the studies from which they are drawn were not located according to a probability-based sampling scheme. These numeric values reflect only the wetland that was studied, the season and year it was studied, and the equipment and techniques used to study it. Typically there is insufficient detail in descriptions of study areas and methods to permit meaningful comparisons among values or to distinguish natural variation from anthropogenic effects. Moreover, species richness values are notoriously difficult to compare because of additional biases introduced by variation in sample sizes, sampling frequency, and the levels of resolution in identification.

x.7 Collection of Ancillary Data: This subsection describes key variables that affect each assemblage because biomonitoring data are easiest to interpret when collected simultaneously with data on other (mostly abiotic) variables.

x.8 Sampling Design and Required Level of Sampling Effort: The level of effort and costs of sampling depend directly on the number and layout of samples within or among wetlands as well as the sampling frequency and duration (i.e., the sampling design). The sampling design that is most appropriate for a particular objective depends on the desired precision and accuracy. This subsection summarizes some of the sampling designs used previously to monitor the taxonomic assemblage in prairie wetlands.

x.9 Summary: The highlights of the section are presented and key findings from the research are reported in a readily usable format. Another useful feature of this document is the series of appendices at the end, which list vascular plants (Appendices A and I), invertebrates (Appendix B), birds (Appendix C), and algae (Appendix H) that occur in prairie wetlands. These lists are not comprehensive; they primarily include species that were identified in the literature as being numerically or functionally dominant in at least one prairie wetland during at least one sampling period. The appendices, A - O, were prepared in similar format so they can be linked and cross-referenced using commercially available database software if users so desire. They have several uses. First, they can be a source of ideas for candidate species for laboratory toxicity testing; thus, bioassays that are used to help establish water quality criteria would be realistic because they would be run on species indigenous to the wetland type and region. Second, vegetation information in the appendices could be used as an aid in classifying wetlands during the development of State water quality criteria. Third, the information can be used to help develop site-specific criteria, e.g., as an information source for the "recalcitration" or "resident species" procedures described in USEPA's water quality program guidance (USEPA 1991).

Fourth, the lists can serve as an aid in linking species composition with wetland condition. For example, users can compare the species they find in a particular wetland (e.g., a reference wetland or other wetland for which a State is developing a "wetland profile") with species listed in the appendices. Then, by noting in the appendices the conditions usually associated with those species, users can make inferences about the ecological integrity of the wetland and the possible identity of its stressors. As a result, the appendices help fulfill the recommendation of Smith (1991) for developing "natural community databases. . .for evaluation of changes in plant community structure to determine the biotic integrity of specific habitat types," and serve as a foundation for a "habitat requirements approach" to biocriteria development as demonstrated in the Chesapeake Bay by Dennison et al. (1993).

1.3 Cumulative Effects of Stressors

When symptoms of change are noted in prairie wetlands, it is not always possible to attribute the symptoms to a single stressor (i.e., an agent of stress) because many stressors in prairie wetlands act in concert or manifest themselves similarly (Larson 1994). Thus, if species composition, richness, density, biomass, or other metrics are to be interpreted unambiguously and used as a basis for biocriteria, it is important to understand which stressors are most likely to influence each other or exert a similar influence on a particular metric. The following examples are intended to further the understanding of the most frequently encountered interactions:

- **Hydrologic stressors** can aggravate or mitigate the effects of several other stressors. Specifically, drought (or water level drawdown) aggravates the effects of salinity, turbidity, excessive nutrient enrichment, and contamination (chemicals within a wetland become concentrated and bottom sediments are more likely to be disturbed by wind mixing). However, floods (or water level increases) also can decrease salinity in wetlands (Neill 1993). Floods can increase turbidity and nutrient enrichment in wetlands if they deliver chemicals and sediments to the wetland via runoff and groundwater input.
- **Changes in vegetative cover** are almost always the result of changes in hydrology, salinity, sedimentation/turbidity, nutrient enrichment, and/or contaminants. Droughts can decrease cover by allowing greater access to the center of usually flooded wetlands by vehicles, livestock, and fire, or can increase cover in the long term by increasing the dominance of "drawdown" species (plants whose germination depends on periodic absence of water or shallow conditions). Floods usually decrease cover by drowning rooted wetland plants.
- **Turbidity** can increase the toxicity of some herbicides (Hartman and Martin 1984, 1985) but can reduce the availability of others. Excessive sedimentation can increase the frequency of drought experienced by a wetland by decreasing wetland depth and isolating the wetland substrate from the water table. Flooding can increase, however, in downslope wetlands as water is displaced to these wetlands.

Thus, users who desire to fully know, for example, the biological effects of hydrologic alteration (or natural hydrologic cycles) will remember to look not only in the hydrologic stressor subsection of this document, but also in subsections on salinity, excessive nutrient enrichment, sedimentation and turbidity, and pesticide and heavy metal contamination.

Figures 1–4 also illustrate some of these relationships, and a more complete analysis can be derived from the qualitative models of prairie wetlands detailed by Adamus (1992).

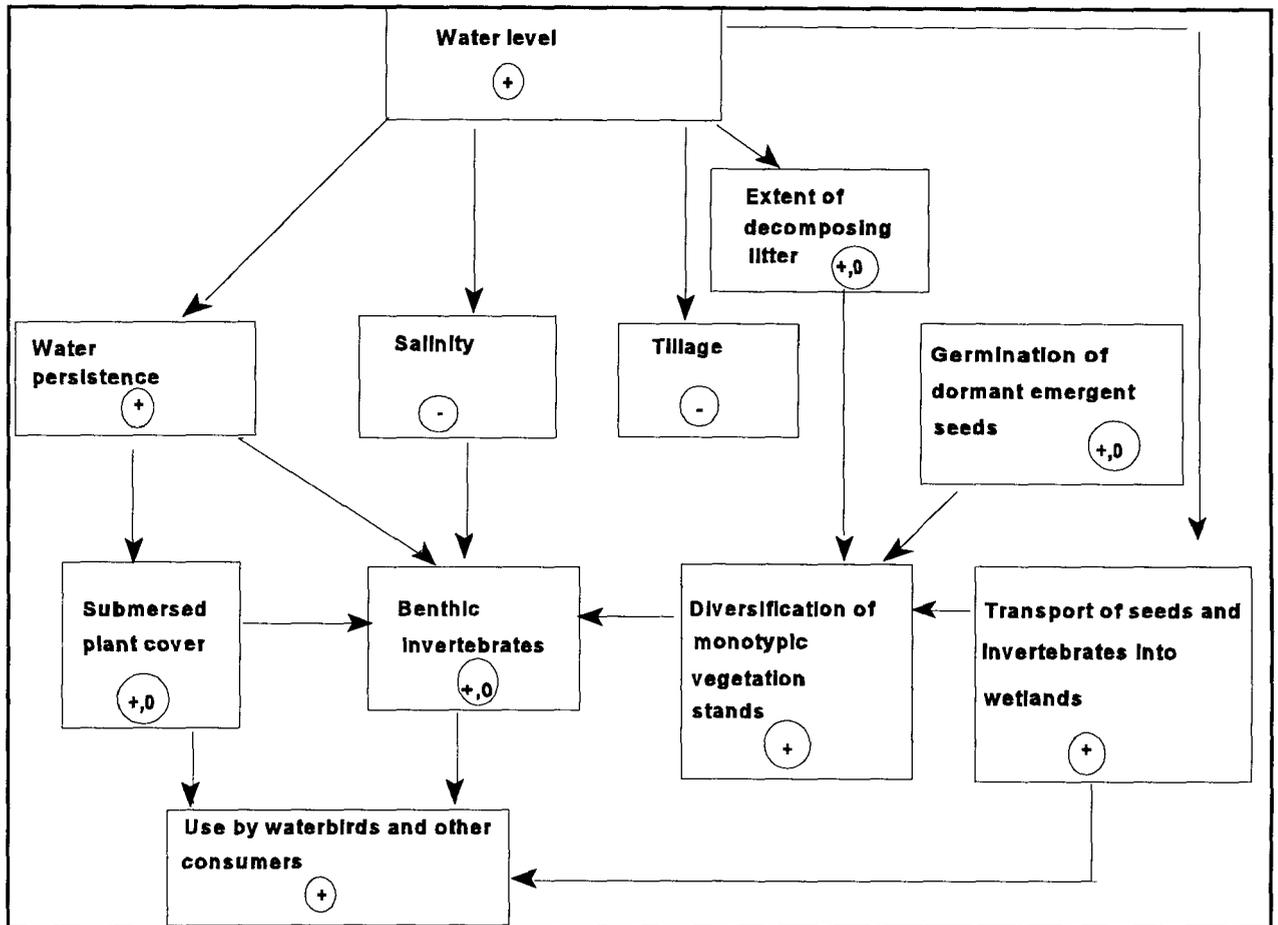


Figure 1. Paths of effects that can result from increases in water levels above the long-term annual norm in a prairie wetland.

+ = increase in the variable, - = decrease in the variable, 0 = no change in the variable

This diagram simplifies the processes involved. The extent and actual probability of these effects occurring may depend partly on the wetland type (e.g., semipermanent vs. temporary), initial condition (e.g., the point in a long-term wet-dry cycle the wetland is currently in), seasonal timing, presence/absence of fish, and characteristics of the specific water level process that trigger the effects (e.g., the type, frequency, duration, intensity, and timing of water level changes). See text for citations of supporting literature.

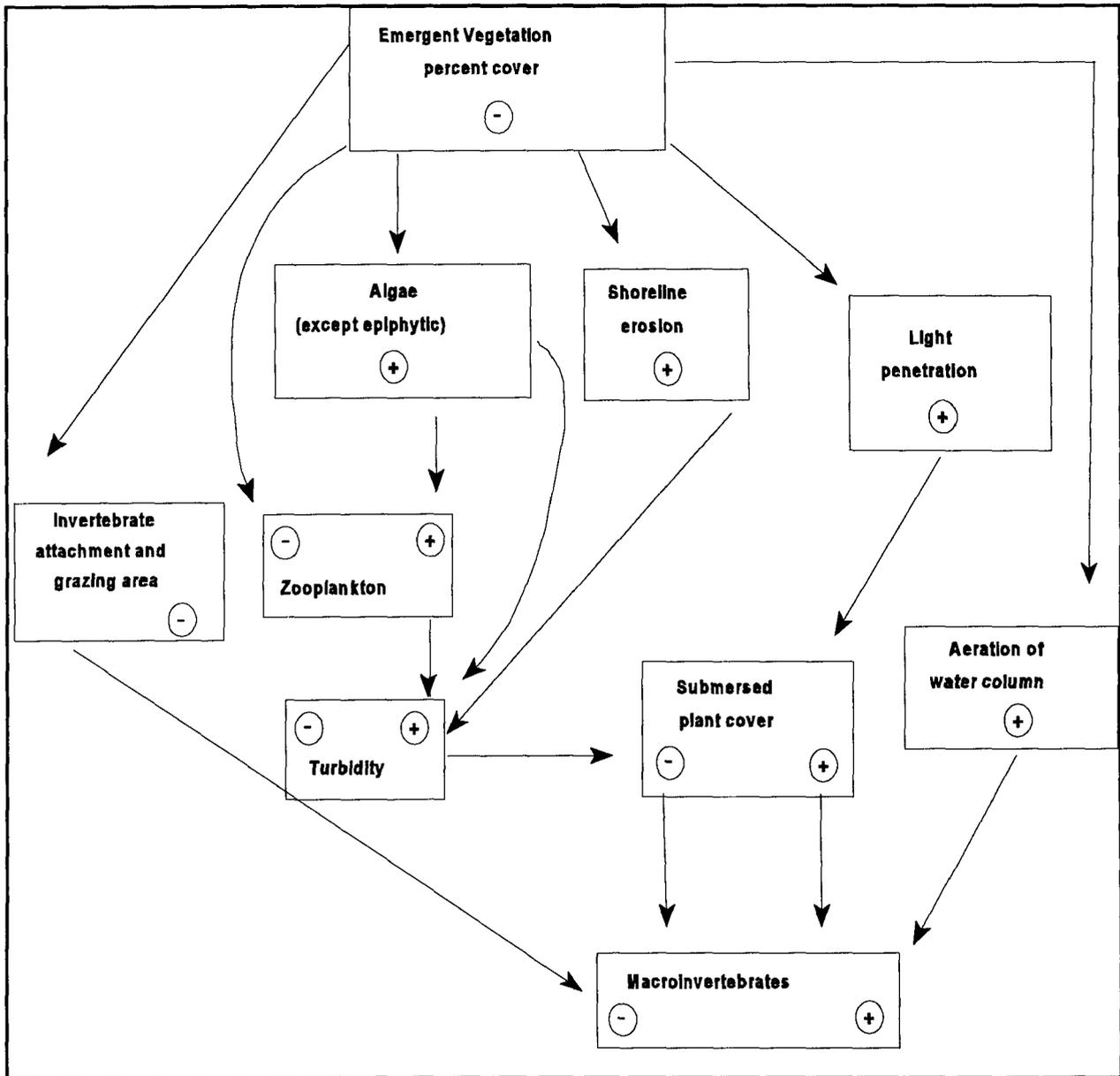


Figure 2. Paths of effects that can result from decreases in the density and percent cover of emergent vegetation in a prairie wetland.

+ = increase in the variable, - = decrease in the variable

This diagram simplifies the processes involved. The extent and actual probability of these effects occurring may depend partly on the wetland type (e.g., semipermanent vs. temporary), the initial condition (e.g., the point in a long-term wet-dry cycle the wetland is currently in), seasonal timing, presence/absence of fish, and characteristics of the specific vegetation removal process that trigger the effects (e.g., the type, frequency, duration, intensity, and timing of herbicide application, grazing, fire, water level increase, etc.). See text for citations of supporting literature.

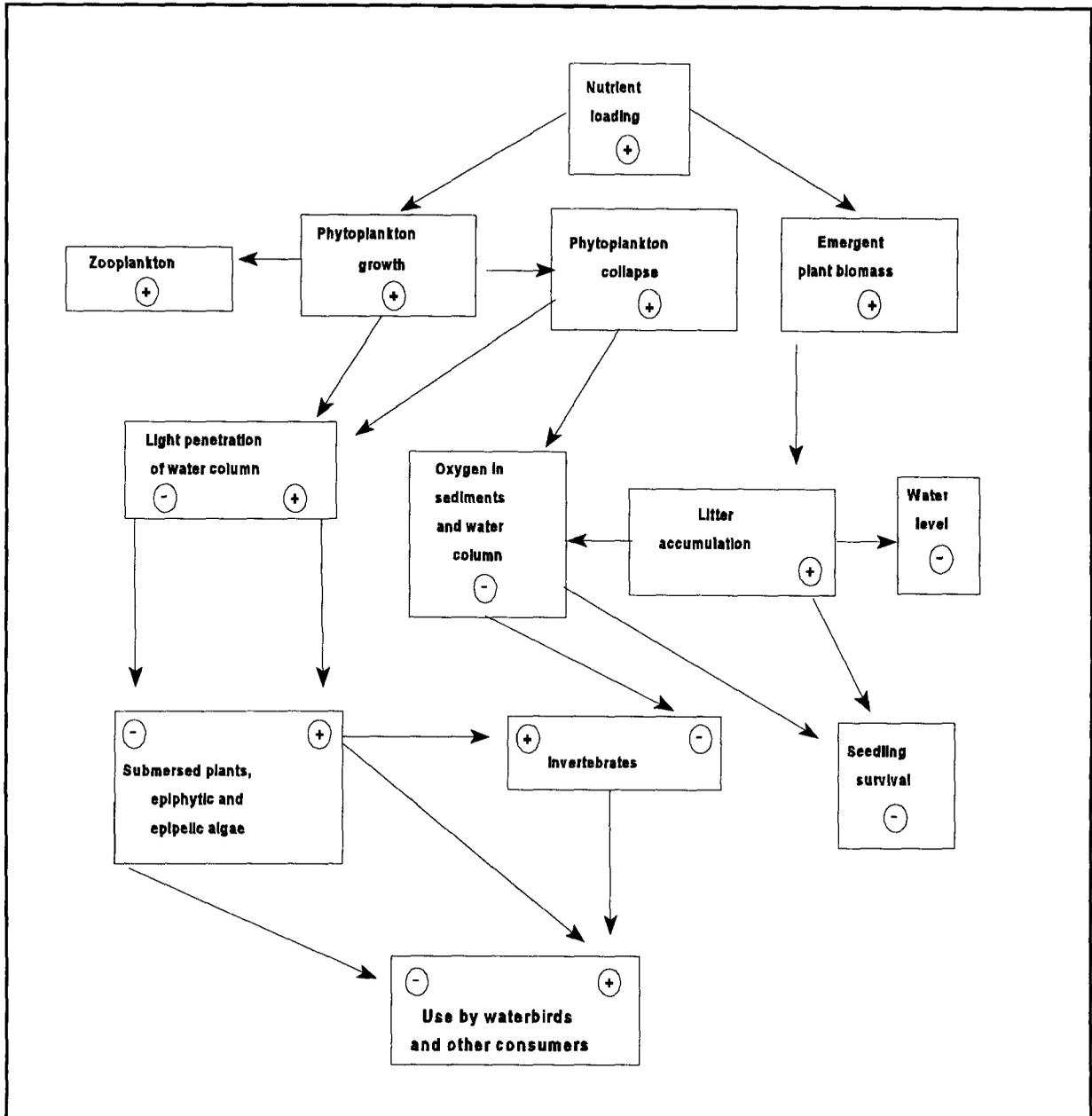


Figure 3. Paths of effects that can result from increases in sediment deposition in a prairie wetland.

+ = increase in the variable; - = decrease in the variable

This diagram simplifies the processes involved. The extent and actual probability of these effects occurring may depend partly on the wetland type (e.g., semipermanent vs. temporary), the initial condition (e.g., the point in a long-term wet-dry cycle the wetland is currently is), seasonal timing, and characteristics of the specific sediment deposition processes that trigger the effects (e.g., the type, frequency, duration, intensity, and timing of deposition). See text for citations of supporting literature.

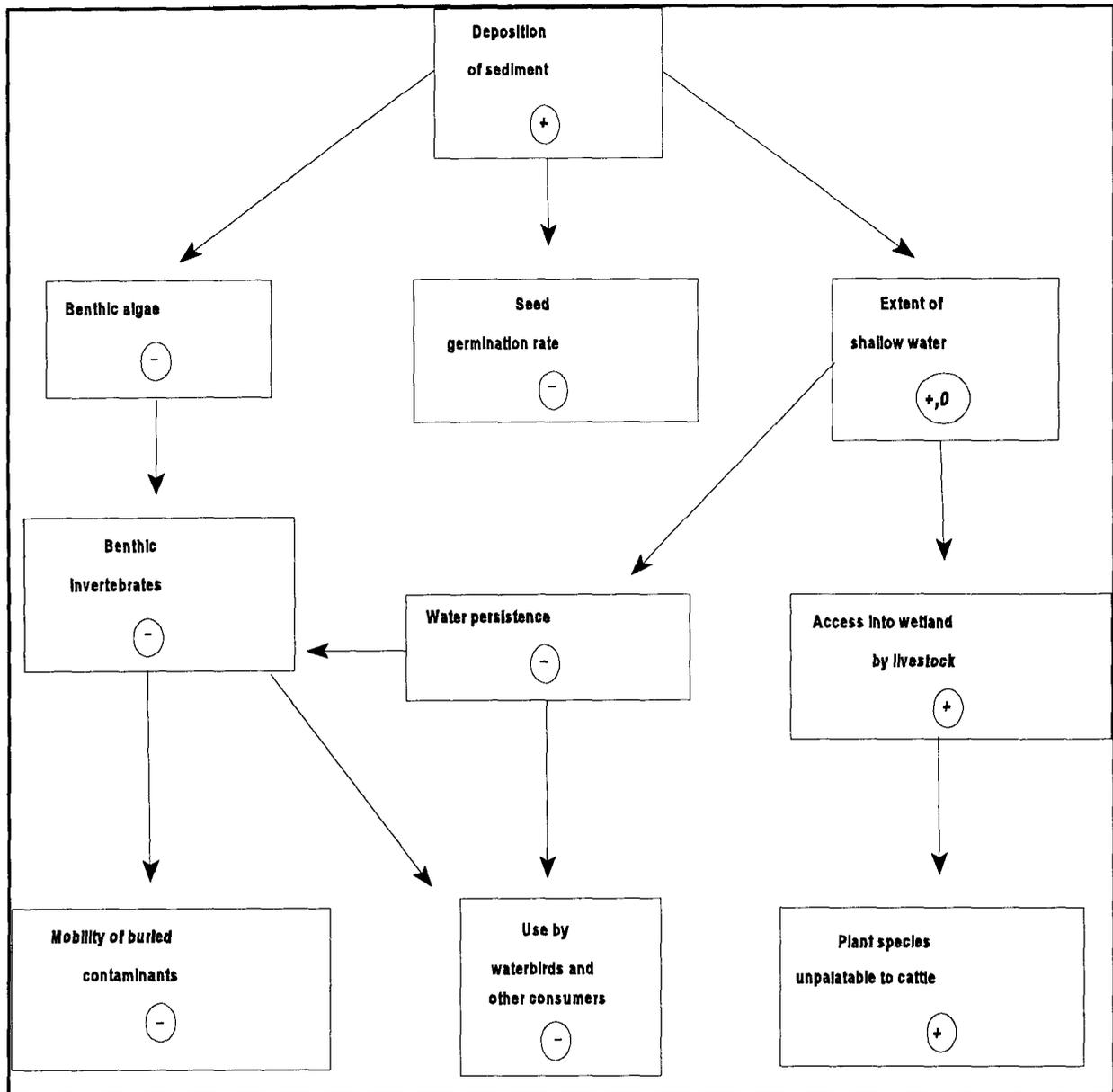


Figure 4. Paths of effects that can result from increases in nutrient loading to a prairie wetland.

+ = increase in the variable; - = decrease in the variable; 0 = no change in the variable

This diagram simplifies the processes involved. The extent and actual probability of these effects occurring may depend partly on the wetland type (e.g., semipermanent vs. temporary), overall water chemistry, initial condition (e.g., the point in a long-term wet-dry cycle the wetland is currently in), seasonal timing, and characteristics of the specific nutrient loading process that trigger the effects, such as the frequency, duration, intensity, and timing of increased inputs (e.g., more fertilizer or greater seasonal runoff) or increased mobilization of nutrients previously immobilized in sediments. See text for citations of supporting literature.

1.4 Glossary, Abbreviations, and Place Names

For the sake of maintaining brevity, this document uses broadly certain terms that conventionally have a more narrow definition:

basin. In the prairie landscape, a topographic depression that normally lacks a permanent natural connection to larger rivers or lakes and contains a wetland, at least during the first weeks of the growing season.

biological criteria (biocriteria). Standards that use the condition of an organism or assemblage of organisms to describe the ecological integrity of unimpacted, least-impacted, or representative ("reference") areas. Biocriteria may be expressed in numeric or narrative terms.

biomarker. A measurement, generally of biological tissue or physiological byproducts, that indicates previous or ongoing organism response and/or exposure to general or specific environmental stresses (Huggett et al. 1992).

cover ratio. The percent open water in a wetland, where "open water" is any part of the wetland that lacks a canopy of emergent vegetation and contains water during at least part of the growing season.

density. The number of individuals per unit area or volume.

ecological (or biological) integrity. The condition or "health" of an area as defined by comparison of community structure and functions to those of unimpacted, least-impacted, or representative ("reference") areas.

macrophytes. Plants generally visible to the unaided eye, including vascular plants and some of the larger algae.

permanent basins. Prairie pothole depressions that retain surface water throughout the year, as classified by Stewart and Kantrud (1971), and that contain wetland vegetation and soils. Used synonymously with "permanent (or permanently flooded) wetland."

seasonal basins. Prairie pothole depressions that retain surface water for much of the growing season (e.g., sometimes into July), as classified by Stewart and Kantrud (1971), and that contain wetland vegetation and soils. Used synonymously with "seasonal (or seasonally flooded) wetland."

semipermanent basins. Prairie pothole depressions that retain surface water throughout most of the growing season, as classified by Stewart and Kantrud (1971), and that contain wetland vegetation and soils. Used synonymously with "semipermanent (or semipermanently flooded) wetland."

temporary basins. Prairie pothole depressions that retain surface water only during the first weeks of the growing season, as classified by Stewart and Kantrud (1971), and that

contain wetland vegetation and soils. Used synonymously with "temporary (or temporarily flooded) wetland."

species composition. The identity and relative abundance of species in a biological community. Used synonymously with "community composition."

species richness. The number of species (or any other taxonomic denomination) per sample, per wetland, per number of individuals. Used synonymously with "taxa richness" and "family richness" because many data sets combine a variety of levels of taxonomic resolution.

Throughout this document several place names and abbreviations are used without elaboration to maintain brevity. These are defined as follows:

Cottonwood Lakes. The Cottonwood Lakes Long-Term Environmental Monitoring site, a large and varied complex of prairie pothole wetlands located in Stutsman County, North Dakota, in which data on waterfowl, climate, and vegetation dynamics have been collected for decades.

Delta Marsh. A large lacustrine marsh in south-central Manitoba, Canada, a portion of which has been used by the Marsh Ecology Research Program (MERP) of Ducks Unlimited to conduct over 80 multi-year experiments using ten, 5-ha marsh cells, each with independent water-level control.

EMAP. USEPA's Environmental Monitoring and Assessment Program, a long-term program intended to regularly monitor the ecological condition of ecosystems (including wetlands) throughout the Nation using a probability-based sample design, and generate estimates of status and trends in ecosystem condition by region and ecosystem (e.g., wetland) type.

NPSC. The Northern Prairie Science Center, the Federal research facility in Jamestown, North Dakota, that has investigated wetland ecology of the prairies for decades, run by the National Biological Service (formerly by the US Department of the Interior, Fish and Wildlife Service, FWS).

1.5 Statistical Analyses: Objectives and Methods

One objective of this project was to estimate the probable number of samples needed to satisfy various purposes. To achieve these estimates, eight existing data sets were obtained from investigators in the region and were analyzed statistically. These data sets were selected based on their availability. Two of the data sets pertained to wetland plants, four to macroinvertebrates, and two to birds. Detailed descriptions of the data sets are found in Appendix L. Data were analyzed to address two questions of practical relevance to sampling prairie wetlands:

1. How many samples need to be collected to find 50%, 75%, 90%, 95%, and 99% of the taxa found in the full suite of samples collected by a particular study? (asymptotic richness)
2. Given a particular number of samples containing information on biomass, number of individuals, or number of taxa, what size difference between two means (e.g., from different wetlands or different dates) can be detected at usual levels of statistical significance? (power of detection or "precision")

Information of this type is essential to estimating costs and levels of effort required for monitoring programs. The results are presented in Sections 3.8, 4.8, and 6.8.

Information on asymptotic richness is needed to help determine if a population has been oversampled or undersampled with regard to detecting most taxa that are present. To address this objective, we used a bootstrap subsampling technique to quantify species accumulation rates (Szaró and King 1990). This reflects a basic principle of diminishing returns: as one samples a population, the number of taxa in samples at first rises rapidly, but then levels off as additional samples add only a few new taxa.

A computer program was written in SAS to estimate species accumulation rates. The program first tallied the number of taxa in the entire data set. Samples that had been collected were then selected randomly without replacement until the number of taxa they cumulatively contained reached one of the specified points (50%, 90%, 95%, 99% of species total from all samples). However, the number of samples needed to reach a particular point depended on the order in which the samples were combined. Thus, the random selection process was repeated 100 times, and the median, mean, and standard deviation of sample sizes estimated from the 100 runs were used to represent requisite sample size. Two assumptions were made when implementing the statistical analysis: 1) 100 runs were sufficient to stabilize the estimates of requisite sample size, and 2) the number of samples originally collected was sufficient to capture nearly all taxa in the target population.

Addressing the power of detection at first seemed straightforward, inasmuch as many papers in the published literature (and recently, several software programs) have defined power of detection through use of elementary equations and simplifying assumptions (Downing 1979, Schwenneker and Hellenthal 1984, Canton and Chadwick 1988, Riddle 1989, Downing 1989, Niemi et al. 1993). Although coefficients of variation calculated for all data sets (Appendix N) might have been used in such equations, the use of simplified approaches limits the generality of the results. Thus, a more involved approach (Components of Variance) was used. Variance component estimates for random factors were calculated using the SAS MIXED procedure for fitting mixed linear models (experimental designs having both fixed and random effects). The estimated variance components were used as estimates of the population variances in the following equations. Estimated variances were obtained by incorporating the variance component into the expected mean square for the random effect of interest. We made statistical comparisons only within data sets, not between them, e.g., to determine which taxon is least variable, or which metric varies the most seasonally. Further, we did not transform any values or test assumptions that routinely underlie the analysis of variance (ANOVA). The analyzed data represent samples that are subject to uncontrolled influences such as weather. In the future, it

might be informative to use data collected over several years as independent experiments to address the issue of the effect of temporal variation on the estimates. We approximated the degrees of freedom (df_1) for obtaining F values by using Satterthwaite's effective df (Steel and Torrie 1980):

$$\text{Effective } df_1 = \frac{\left[\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2} \right]^2}{\left[\frac{(s_1^2 / n_1)^2}{(n_1 - 1)} \right] + \left[\frac{(s_2^2 / n_2)^2}{(n_2 - 1)} \right]},$$

where df_1 = effective degrees of freedom for the error term,
 s^2 = estimated variance with subscripts to identify the sample,
and
 n = sample size with subscripts to identify the sample.

We used two methods to calculate precision (i.e., detectable difference) over a range of sample sizes. The first equation provides an "optimistic" estimate for the difference between two means that is detectable at a given sample size. The second equation is more conservative and takes into account the assurance that the study has the desired precision (Steel and Torrie 1980):

$$P_{1_n} = t_{(\alpha, df_{2_n})} \sqrt{2 \frac{s^2}{r_n}},$$

where P_{1_n} = optimistic precision estimate for the n th sample,
 t = value from t -table,
 df = effective degrees of freedom for the error term for the n th sample,
 s^2 = estimated variance,
 r = number of replicates for the n th sample, and
 α = probability of a Type I error (falsely rejecting the null hypothesis).

Precision is defined as the absolute difference that is detectable. Thus, F_β provides greater assurance that the difference between means in future experiments will be no greater than the estimated ability to detect the specified difference in the means:

$$P_{2_n} = F_{(\beta, df_{2_n}, df_1)} t_{(\alpha, df_{2_n})} \sqrt{2 \frac{s^2}{r_n}},$$

where	P_{2n}	=	conservative precision estimate for the n th sample.
	F	=	value from F -table,
	df	=	effective degrees of freedom for the error term,
	t	=	value from t -table,
	s^2	=	estimated variance,
	r	=	replicate,
	α	=	probability of a Type I error (falsely rejecting the null hypothesis), and
	β	=	probability of a Type II error (accepting a false null hypothesis).

We present the results of applying the equations over a range of n values. Specifically, P_1 and P_2 were calculated by varying the replicates and subsequent degrees of freedom over a range of values. Curves of the calculated values of P_1 and P_2 were plotted on the same graph for each random-effect variable from each of six data sets (Figure 5). The upper curve on each plot represents the conservative precision estimates and the lower curve the more optimistic estimates. These curves make it possible to assess relationships between the number of replicates and the precision estimate. Because of the large number of curves generated, results have been summarized tabularly (Appendix M).

Readers should understand that the values presented in this document, while generally realistic, are not intended as exact estimates of requisite sample sizes. The requisite number of samples or resultant levels of precision could differ if sampling is done according to a design or under conditions (e.g., weather, season, wetland type, equipment) that differ from those upon which these estimates were based.

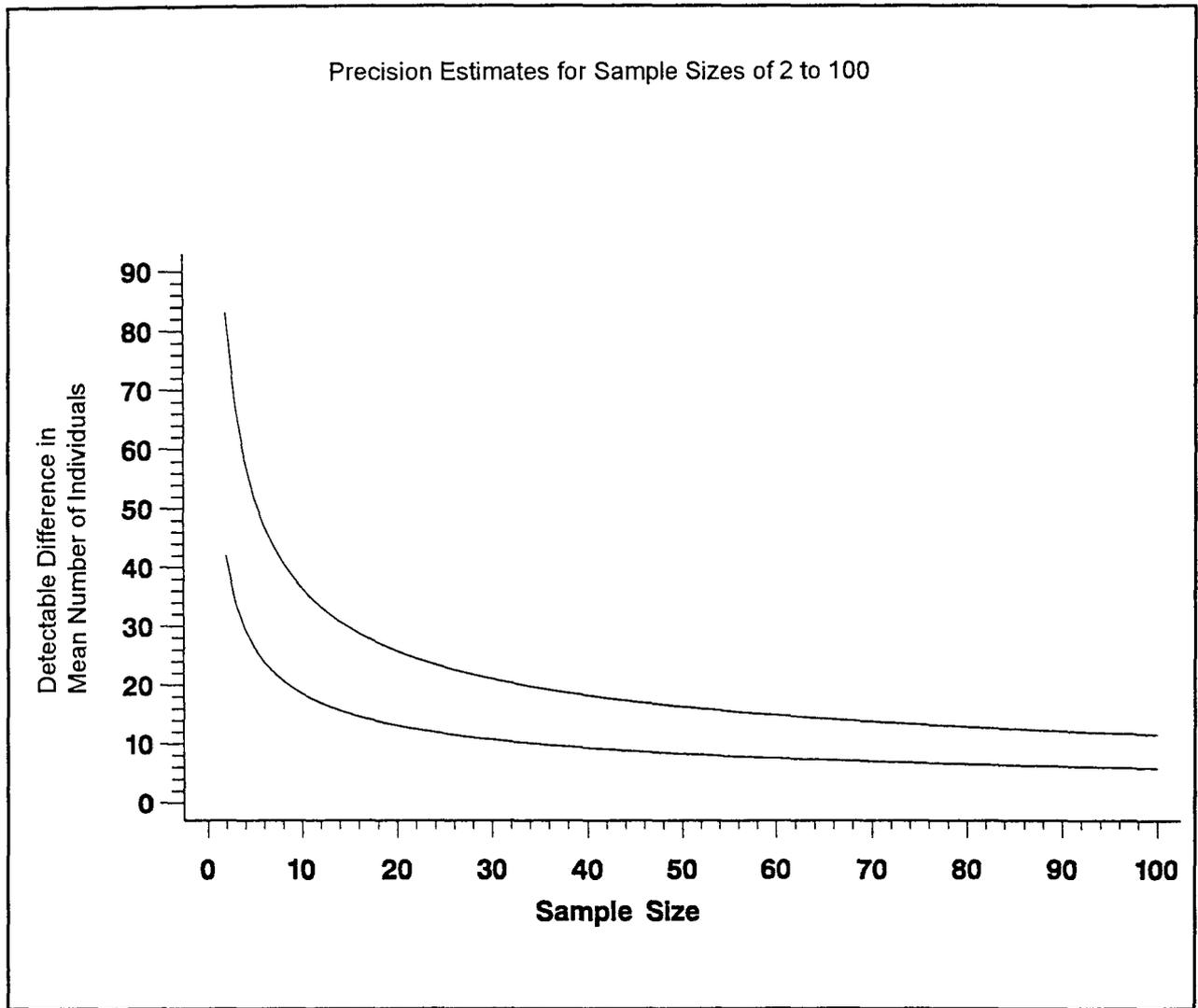


Figure 5. The difference between two means (of the number of Conchostraca in sweep nets) that can be detected by various sample sizes.

The upper curve is based on an equation that estimates precision conservatively, whereas the lower one estimates precision optimistically. See explanation on the preceding page. Results from curves for all major taxa and metrics are compiled tabularly in Appendix M.

2. Algal and Microbial Communities as Indicators of Prairie Wetland Integrity

2.1 Ecological Significance and Suitability as an Indicator

In prairie wetlands, four algal assemblages are commonly defined: phytoplankton (algae suspended in the water column), metaphyton (unattached and floating or loosely associated with substrata), and two assemblages of attached algae, namely epiphytic algae (attached to plants) and benthic algae [attached to sediments; however, the more specific meaning of benthic for wetlands is epipellic (attached to substrate rocks) (Crumpton 1989)]. The regional pool of species is probably largest for the benthic and phytoplanktonic forms, although individual samples of communities are usually taxonomically simple. Microbes prevalent in prairie wetlands include bacteria, viruses, yeasts, and microscopic fungi.

Because they form the basis of the food chains, algal and microbial communities are of critical ecological importance in prairie wetlands. The prairie wetland animal community depends on solar energy that has been converted by photosynthesis to biomass by algae or vascular plants (Neill and Cornwell 1992). The algae and vascular plants provide a substrate and food source for microbial communities, and the microbes in turn are consumed extensively by a wide variety of detritivorous invertebrates. Algae, and to a lesser degree vascular plants (Campeau et al. 1994), are also consumed directly by invertebrates. In some prairie wetlands, the levels of algal and microbial productivity approach those of vascular plants.

The relative importance of microbes vs. algae as supporters of prairie wetland invertebrates depends on interactions between microbes and algae, season and water regime, and chemistry of a particular wetland. Based on a statistical analysis of invertebrate biomass in submersed plant beds in 11 eastern Canadian lakes, Lalonde and Downing (1992) concluded that aquatic invertebrate biomass was highly correlated with the biomass of littoral phytoplankton, epiphyton, and vascular plants, a situation also noted in prairie wetlands (Murkin et al. 1991). However, Murkin et al. (1992) suggest that algae's role in determining the horizontal distribution of invertebrates within a prairie wetland might not be as great as it would seem. They based this conclusion on their failure to find spatial overlap between maximum density of acroinvertebrates and maximum epiphytic biomass (measured as chlorophyll) in Delta Marsh. Similarly, in most of the Cottonwood Lake wetlands that LaBaugh and Swanson (1993) studied, the seasonal and annual changes in microinvertebrate abundance were not statistically related to the abundance of algae. They speculated that microinvertebrates might be depending more on microbial biomass. Ducks which feed on wetland invertebrates also do not congregate in wetlands that have the most algae, although ducks that use algae-rich wetlands were found in a Saskatchewan study (Gloutney 1993) to spend more time feeding. Using isotope ratios to investigate food webs in Delta Marsh, Neill and Cornwell (1992) found that emergent vascular plants and the algae and microbes attached to them, rather than submersed macrophytes or metaphyton, were the most important sources of organic matter to the invertebrate consumers that were most abundant during June.

In addition to their value as food for wetland animals, algae influence invertebrates and vascular plants in at least five other ways: 1) phytoplankton and metaphyton reduce light penetration of the water column; 2) epiphytic and benthic algae mediate the levels and flux of nutrients,

contaminants, and oxygen across ecotones (e.g., sediment-water column) and plant surfaces; 3) all algae (when they respire, die, and decompose) can diminish dissolved oxygen in the water column and sediments; 4) some blue-green algae fix (add) gaseous nitrogen to the water column, thus enriching wetlands; 5) some blue-green algae are toxic to other organisms. Any of these phenomena can alter the basic habitat structure of prairie wetlands, and thus, the populations of invertebrates, amphibians, fish, and waterbirds. Moreover, microbial populations can alter the structure of habitat available to invertebrates because they are the major assemblage responsible for decomposition, and decomposition is usually the primary factor responsible for offsetting excessive accumulation of plant litter in prairie wetlands. Excessive plant litter accumulation with associated increase in microbial densities can create oxygen deficits in sediments and surface waters (Baird and Mathias 1979, Barica 1984, Barica et al. 1987), conditions that can impair seed germination, damage invertebrate communities, and further retard decomposition.

Certain microbial communities also can detoxify some chemicals. For example, microbes associated with wetland plants can detoxify some synthetic organic compounds (Hodson 1980) such as pentachlorophenol (Pignatello et al. 1985), the herbicides glyphosate (Goldsborough and Beck 1989) and atrazine (W. Crumpton, personal communication, Iowa State University, Ames, IA), as well as detergents (Federle and Schwab 1989).

Particular microbial communities also can reduce, via the process of denitrification, the overenrichment of wetlands. This is important at landscape and regional scales not only because wetlands are among the most effective ecosystems for removing nitrogen (Groffman and Tiedje 1989) but also because they intercept much of the runoff and groundwater before it reaches larger, more permanent waterbodies. Protection and enhancement of nitrogen removal functions of wetland microbial communities is important to maintain and restore important public uses of downslope lakes, rivers, and aquifers. In semipermanent wetlands of Iowa, Davis and van der Valk (1978b) reported removal of 86% of the runoff inputs of nitrate and 78% of the inputs of ammonia. Two open wetland complexes in North Dakota removed 13% and 58% of the tributary nitrate, as compared to a drained wetland complex in which there was a > 10-fold increase in nitrate (Malcolm 1979). An unvegetated wet basin in South Dakota that was loaded with municipal wastewater removed 1765 kg N/ha (White and Dornbush 1988). On a regional basis, Jones et al. (1976) in northwestern Iowa found that among 34 watersheds, those with a large percentage of land as wetlands had less nitrate in streamflow than those with a small percentage of wetlands.

Denitrification rates may be equal or greater at the beginning and end of the growing season than during mid-summer (Christensen 1985, Myrold 1988, Zak and Grigal 1991). Thus, denitrification functions in wetlands may be of greatest value in removing nitrate during years when runoff inputs occur early or late in the growing season. However, if runoff resulting from the spring melting of snow surrounding wetlands occurs prior to ice-out in wetlands, the runoff flows under the wetland ice, purging the basins of anoxic, ammonia-rich water which can subsequently be released into receiving waters (if seasonal connections exist) without being substantially denitrified, thus causing water quality problems (NDDHCL 1990). This adverse impact might be more likely to occur in landscapes dominated by semipermanent and permanent basins because they tend to remain frozen longer than temporarily flooded wetlands.

As indicators of wetland integrity, microbial communities have several characteristics usually considered to be advantages (Adamus and Brandt 1990):

- tight linkage to fundamental processes (e.g., decomposition, denitrification, respiration)
- easily collected and transported samples
- immediate response to contamination
- measurable in wetlands year-round and in wetlands that lack surface water
- sensitive to presence of some contaminants and available assay protocols (e.g., Ames test, Microtox test).

In addition, USEPA protocols are available (but may need adaptation to wetlands), and sufficient information is published to identify a few "indicator taxa" that clearly are associated with particular stresses.

Characteristics usually considered disadvantages when using microbial communities to determine wetland condition include:

- lack of an identifiably stressor-specific response
- laborious and slow identification (plate culture); process measurements difficult to interpret with regard to ecological significance
- rapid turnover of individuals of most species requires frequent sampling; microbes do not integrate conditions over time very well
- naturally great micro-spatial variation

Moreover, microbial communities are impractical for detecting bioaccumulation.

Algae have several characteristics usually considered advantages for monitoring ecosystem integrity (Adamus and Brandt 1990):

- pivotal in food webs, and tight linkage to fundamental processes (e.g., photosynthesis, respiration)
- rapid reproduction rates; short life cycles; sensitivity to short-term impacts
- generally immobile and thus reflect conditions at a particular site; useful for *in situ* exposure assessments and whole-effluent bioassays
- decay-resistant remains (diatom frustules and pigments) provide a means for establishing historical reference conditions in a wetland.

For researchers, standardized collection procedures are available and impact the monitored wetland only minimally. Also, tolerances and indicator value are well-known, particularly with regard to nutrients. Finally, USEPA has protocols, which may need modification to wetlands, that are available for sampling both structure and function of algal communities.

Characteristics usually considered disadvantages when using algae to determine wetland condition include:

- rapid turnover, requiring frequent sampling
- relative insensitivity to heavy metals and insecticides (Hellawell 1984)
- need for laborious identification of many taxa
- complicated interpretation of spatial patterns because of drifting cells of unattached species
- difficulty in linking responses of many algal taxa to a specific stressor
- poorly documented linkages to other components of the food chain in prairie wetlands.

2.2 Potential Indicator Metrics

Various measurements and metrics can be applied to algal or microbial samples, for use in characterizing conditions in reference wetlands, identifying the relative degree of past disturbance of a prairie wetland or assessing the current inhibition of key processes:

- richness of species and functional groups (per unit volume of sample, or per thousand randomly chosen individuals)
- number and biomass of cells per unit sample; chlorophyll-a ($C_{55}H_{72}MgN_4O_5$) concentration per unit volume
- proportional density and richness of species reputedly tolerant to a named stressor
- degree of temporal variability in richness, density, and/or biomass (expressed as coefficient of monthly or weekly variation)
- month during which maxima of richness, density, and/or biomass occur (generally, or for particular groups, e.g., blue-green algae, benthic algae)
- litter decomposition rate (or less precisely, the depth of fibric litter and proportional weight of dead vascular plant vegetation), used as an indicator of microbial activity
- denitrification enzyme activity (DEA)

The specific ways some of these metrics have been or could be interpreted as indicators of stressed conditions are described in Section 2.4.

In situ methods of measuring algal production (e.g., uptake rates of carbon radioisotope, oxygen production) are not considered here. These methods are described generally by Stevenson and Lowe (1986), and protocols applicable to prairie wetlands are given in Hooper and Robinson (1976), Britton and Greeson (1988), and Gurney and Robinson (1989b). Estimates of algal production are strongly influenced by the choice of method used to estimate production, and temporal variability of measurements can be enormous.

2.3 Previous and Ongoing Monitoring in the Region

Field studies of algae have been published in at least 19 papers covering at least 124 prairie wetlands. A survey of five wetlands by LaBaugh and Swanson (1988) sampled only the water column; surveys of about 50 wetlands by Kling (1975) and two wetlands by Shames et al. (1985) included benthic and epiphytic algae as well. Information on species composition of metaphytic, epiphytic, and benthic algal communities also is very limited. Apparently no published studies have described the species composition of microbial communities in prairie wetlands.

Most algal and microbial studies in the region have considered only the physiological processes (e.g., respiration, production) associated with algae or microbial communities in general. Almost without exception, these studies have been completed during a single year's growing season, and in nearly all cases in permanent prairie wetlands or lakes. In a few cases (Barica 1975, Hickman and Jenkerson 1978, Barica et al. 1980), these process-based studies have incidentally mentioned the dominant taxa that were found. Identified assemblages of microbes in the region apparently have not been cultured in the laboratory to determine their functional characteristics, e.g., potential as denitrifiers or their role in methanogenesis. Decomposition processes have been measured in a few instances, generally without identifying the microbial taxa responsible (e.g., Davis and van der Valk 1978a,b; Neely and Davis 1985b; Wrubleski et al. 1993). Two studies (LaVeglia and Dahm 1974, Johnson 1986) measured process rates in the microbial communities of a few prairie wetlands using various physiological and chemical indicators: respiration, monophosphatase activity, electronic transfer system (dehydrogenase) potential, glucose mineralization, ammonium production, nitrification, and sulfur oxidation. Species responsible for the measured processes were not identified.

Montana's water quality agency (State Department of Health and Environmental Sciences) currently uses epiphytic and benthic algae (specifically, diatoms) on a trial basis as an indicator of the condition of about five prairie wetlands that represent varying degrees of potential impairment. The information collected on species composition could yield valuable insights into sampling variability and habitat relationships. Ongoing research on algae in prairie wetlands by the National Hydrologic Research Institute in Saskatoon, Saskatchewan, is focusing on transfer of algal energy to zooplankton and effects of herbicides on algae (Appendix K).

2.4 Response to Stressors

The following subsections describe responses of the algal and microbial communities to hydrologic stressors, vegetative cover conditions, salinity, sedimentation/turbidity, excessive nutrient loads/anoxia, and pesticide and heavy metal contamination.

2.4.1 Algal and Microbial Communities as Indicators of Hydrologic Stressors

Declining water levels in a wetland often raise the water temperature and concentrate dissolved nutrients that exist in the water column as well as mobilize some of the nutrients from shoreline sediments and from plant litter that has become exposed. Such increases in nutrient concentration sometimes cause algal blooms in the remaining surface water (Schoenberg and Oliver 1988). Inundation can have the opposite effect, sometimes diluting and chemically binding nutrients to bottom sediments, cooling the water column, increasing algal competition with vascular plants, and thus reducing biomass of some algal taxa. However, inundation typically increases the leaf surface area available for colonization by some algae and provides increased opportunities for dispersal of some algal species into and out of a wetland.

Species Composition

Changes in the density of phytoplankton—as compared with metaphytic, epiphytic, and benthic algae—might suggest that water levels in a wetland have changed within recent days or perhaps weeks. Specifically, recent (within a year) inundation often decreases the ratio of phytoplankton biomass (per unit area) to biomass of the other algal community components (Hosseini and van der Valk 1989a,b). This occurs as higher water levels reduce canopy coverage of vascular plants, increase light penetration and the area of substrate available for colonization, and dilute the levels of nutrients that otherwise would support the proliferation of rapidly growing phytoplankton (Hooper and Robinson 1976, Gurney and Robinson 1988). Increased water levels can also differentially reduce phytoplankton density and productivity by creating habitat space for zooplankton, which graze selectively on the phytoplanktonic forms of algae. During the first year after flooding of one wetland, the biomass and productivity of both metaphyton and attached algae increased, whereas only the metaphyton continued this increase into the second year (Hosseini and van der Valk 1989a,b). Long-term changes in wetland water regimes might be inferred by collecting diatom remains using sediment cores (see Section 2.5.1) and determining what proportion of the found species (or pigments) are ones that are characteristically associated with drought or wet conditions, as inferred from salinity tolerances given for 143 taxa by Fritz et al. (1993) and for 62 taxa by Blinn (1993).

Species Richness

Data are insufficient to characterize the response of algal richness to changes in water levels of prairie wetlands. Sampling of five wetlands in the Cottonwood Lakes area identified 245 taxa in the two semipermanent wetlands, 159 in two seasonal wetlands, and 98 in a saline wetland (LaBaugh and Swanson 1988). The collective list from all five wetlands totalled 306 taxa, and 80% of these were present in the two semipermanent wetlands, 52% in the two seasonal wetlands, and 32% in the saline wetland.

Density, Biomass, and Productivity

Phytoplankton density and productivity can be lower in temporarily flooded wetlands than in persistently flooded prairie lakes (Robarts et al. 1995). Because flooding creates additional habitat space for both planktonic and epiphytic algae, the total biomass and production of algae in a wetland can increase in response to flooding, even if production of phytoplankton per unit area drops (Hosseini and van der Valk 1989a,b). Thus, total algal biomass or productivity of a prairie wetland, even if it could be measured accurately, would be a confusing indicator of water-level change.

Decomposition

One of the few studies conducted on this topic in a prairie wetland (Wrubleski et al. 1993) found that leaching and decomposition of aboveground litter was more rapid in six of eight plant species in a flooded wetland than in a dry wetland; only the litter of cat-tail (*Typha*) and common reed (*Phragmites*) showed no difference between wet and dry treatments. The depth of flooding was inconsequential, but there were important differences in decay rate among taxa, with *Chenopodium* decomposing the slowest and *Scolochloa* and *Scirpus lacustris* the fastest. The nutrient content of litter from *Phragmites* actually increased over time, probably indicating the flood-related development of a rapidly growing microbial and epiphytic algae community, a phenomenon noted in other prairie wetlands as well (e.g., Neely and Davis 1985b). The relative extent of plant litter, as estimated coarsely from low-altitude photographs, was found to be a poor indicator of past hydrologic conditions in one prairie wetland (van der Valk and Squires 1992).

Other Microbial Processes

Hydrologic conditions affect denitrification, a microbial process that is of considerable importance because it improves water quality by removing excessive amounts of dissolved nitrogen. Although effects of changing water levels on denitrification have not been studied in prairie wetlands, two recent landscape-scale studies of Saskatchewan fields (Elliott and de Jong 1992, van Kessel et al. 1993) highlight the key role of soil moisture:

Soil water content was the most dominant factor controlling denitrification activity, followed by the concentration of ammonium, total soil respiration, and nitrate (van Kessel et al. 1993).

Measurements of denitrification in a South Dakota wetland soil indicated that conditions of less than 22% volumetric soil moisture completely inhibit denitrification (Lemme 1988). A wetland does not have to be exposed to runoff for very long to reach these moisture levels and remove nitrate (i.e., convert and export nitrogen as a gas). Microbial communities that support denitrification develop rapidly in newly created wetlands (Duncan and Groffman 1994).

It remains unclear under which water regime denitrification is greatest. For example, Kantrud et al. (1989) state, "It would seem that temporary and seasonally flooded wetlands would be especially efficient in removal of excess nitrogen." There are at least two reasons why this might be so. First, fluctuating water levels that typify temporary and seasonal wetlands might be expected to enhance denitrification so long as 1) anaerobic conditions still occur, 2) moisture

levels in the upper soil layers are not too severely depleted (i.e., pore space is 30%–60% water-filled; Linn and Doran 1984, Lemme 1988), 3) carbon supplies also are not limiting (Fraser et al. 1988), and 4) salinity conditions are not extreme. Second, soil temperature might be expected to be warmer in temporary and seasonal wetlands during much of the year because of their shallow depths.

However, other logic suggests that semipermanent and permanent wetlands might be more effective than temporary and seasonal wetlands for removing nitrate. Because semipermanent and permanent wetlands are usually groundwater discharge or flow-through systems, they are less susceptible to drought, and by definition, they remain saturated and thus favorable to denitrification for longer periods. Prolonged drought in temporary wetlands not only results in moisture deficits inhospitable to denitrifying microbes but also can result in loss (via mineralization) of organic matter essential for sustaining denitrifiers. Organic matter content of soils in semipermanent and permanent wetlands generally seems to be greater than in temporary and seasonal wetlands [however, Loken (1991) reported less organic matter in soils of semipermanent groundwater discharge wetlands; he attributed this to high salinity of these basins inhibiting their productivity].

2.4.2 Algal and Microbial Communities as Indicators of Changes in Vegetative Cover

Species Composition

Algae and microbes respond quickly and persistently to changes in vegetative cover. As grazing, mowing, fire, and other factors decrease the amount of plant litter in prairie wetlands, the composition of algal and microbial communities can shift from characteristically epiphytic species to benthic or phytoplanktonic species. Long-term changes in plant cover of a wetland might be inferred by collecting diatom remains using sediment cores (see Section 2.5.1), and determining what proportion of the found species are ones that are characteristically shade-tolerant.

Species Richness

Species richness of algal communities sometimes declines with removal of vegetative cover (Seelbach and McDiffett 1983).

Density and Biomass

Algal and microbial biomass and density can either decrease (Rabe and Gibson 1984) or increase (Seelbach and McDiffett 1983) as vascular plant cover becomes sparser.

Decomposition, Other Microbial Processes

Decomposition rates can be retarded somewhat by wetland plant litter that has accumulated excessively (Godshalk and Wetzel 1978). However, microbial density and denitrification are generally greater in unplowed prairie soils than in plowed soils where plant litter is mostly removed (Linn and Doran 1984). Some rooted plants are capable of enhancing microbial populations and processes by 1) transferring nitrates from the sediment into aboveground tissues and eventually into the water column; 2) providing a carbon substrate (e.g., plant litter);

3) speeding the diffusion of oxygen (via roots) into otherwise anaerobic subsurface zones, especially during mid-growing season; and 4) increasing diurnal and seasonal fluctuations in the water table, and consequently the oxidation status, as a result of evapotranspiration. Densities of denitrifying microbes might be greatest where soil organic matter reaches a maximum just below the soil surface, but above the depth limit of the root zone (Parkin and Meisinger 1989). In this zone, impeded lateral flow increases the time available for nitrate loads to interact with prolific microbial populations present in the surrounding root masses.

2.4.3 Algal and Microbial Communities as Indicators of Wetland Salinity

Species Composition

In the prairie region, a salinity threshold of about 1000 mg/L separates algal species that are relatively salt-tolerant from ones that are not (Prepas and Trew 1983). Long-term changes in salinity of a wetland might be inferred by collecting diatom remains using sediment cores (see Section 2.5.1), and determining what proportion of the found species are ones that are characteristically salt-tolerant. However, this approach is unreliable in highly saline wetlands due to rapid dissolution of diatom and chrysophyte remains (Walker et al. 1995). Salinity limits and optima for 142 diatom taxa found in inland lakes of North America are presented by Fritz et al. (1993) and Blinn (1993) and these species might be used for reconstructing past salinity conditions in a wetland.

Species Richness

In saline lakes of western North America, the richness of diatom taxa is negatively correlated with specific conductance, with greatest richness corresponding to specific conductance of less than 45 mS (Blinn 1993). Diatom richness is greatest in waters where specific conductance is primarily the result of NaCl, or where concentrations of MgSO₄ are intermediate rather than where carbonate ions are dominant (Blinn 1993). Because local groundwater regimes play a major role in determining the ion chemistry of prairie wetlands, slight changes in groundwater flow might noticeably alter diatom species composition and richness.

Density, Biomass, and Production

Algal productivity increases with conductivity up to about 3000 µS/cm, and it decreases at higher salt concentrations (Reynolds 1979). Moreover, chlorophyll-a, an indicator of algal biomass, occurs at lower concentrations in highly saline prairie lakes than in fresher ones, i.e., ones with < 1000 mg/L total dissolved solids (Barica 1978). An empirical model is available for predicting the nutrient status of saline prairie lakes, given information on their conductivity and chlorophyll-a content, but the model is not accurate where the ratio of total nitrogen to total phosphorus is < 12 (Bierhuizen and Prepas 1985, Campbell and Prepas 1986, Evans et al. 1995).

Decomposition

Apparently no studies of the effects of salinity on decomposition have been conducted in prairie wetlands. One study elsewhere found that decomposition was slower in inland wetlands having greater salinity (Reice and Herbst 1982).

2.4.4 Algal and Microbial Communities as Indicators of Sedimentation and Turbidity

A relatively low biomass and density of algae and microbes can indicate wetlands that receive chronically elevated inputs of sediment. Even more indicative might be a shift in species composition.

2.4.5 Algal and Microbial Communities as Indicators of Excessive Nutrient Loads and Anoxia

Species Composition

Algae and microbes respond more quickly to nutrient additions than do submersed vascular plants (Crumpton 1989). Among algal assemblages in prairie wetlands, phytoplankton and epiphyton respond immediately to small, repeated nutrient additions, whereas metaphyton demonstrate a delayed but large and enduring response, and benthic algae respond hardly at all (Murkin et al. 1994b). When nutrients are added in only a single dose, phytoplankton show a stronger response than epiphyton (Gabor et al. 1994).

Species composition of algal communities (especially diatoms) has a long history of use as an indicator of the relative state of enrichment of a water body. Moreover, composition of algal species reflects not only the total level of nutrients but also the ratio of two nutrients, phosphorus and nitrogen. One study of a prairie wetland (Barica et al. 1980) showed that a large ratio of biomass of green algae (Chlorophyta) to blue-green algae (Cyanophyta) can indicate that an oversupply of nitrogen, relative to phosphorus, has occurred within a few months. This pattern has been supported by wetland studies in other regions (e.g., Michigan bogs, Hooper 1982) that found that Euglenophytes (one-celled, mobile green algae) in particular respond to increases in ammonium and Kjeldahl nitrogen (rather than to nitrate alone), as well as to other substances associated with decomposing organic matter. The indicator status of a large variety of algal taxa with regard to enrichment is cataloged in several publications (e.g., Prescott 1968, Lowe 1974, Richardson and Schwegler 1986, Leclercq and Maquet 1987, Descy and Coste 1990). From such listings, the long-term changes in nutrient status of a wetland might be inferred once diatom remains or pigments from sediment cores (see Section 2.5.1) are collected and analyzed to determine the proportion of the found species (or pigments) that are characteristically associated with eutrophication.

Among microbial assemblages, photosynthetic microbes appear to respond more immediately to nutrient additions than do most other microbes (Pratt and Cairns 1985). In some wetlands, enrichment increases the number of facultative-anaerobic bacteria (e.g., streptococci, enterobacteriaceae and aerobic spore forms, e.g., *Bacillus* spp., *Pseudomonas alcaligenes*, and *Aeromonas* spp.). Mesotrophic ponds can have elevated numbers of fluorescent pseudomonads, whereas oligotrophic waters can have more denitrifiers (*Pseudomonas fluorescens* and *Vibrio* spp.) (Schmider and Ottow 1985).

Species or Form Richness

Algal or microbial species-richness is not a precise indicator of enriched conditions because it can either increase (e.g., Pratt et al. 1985, Morgan 1988) or decrease (e.g., Hooper 1982, Schindler and Turner 1982) in response to nutrient addition.

Biomass or Density

Algal (Murkin et al. 1991) and microbial (Tate and Terry 1980, Schmider and Ottow 1985) biomass or density are strong indicators of a wetland's degree of enrichment. Increasing duration and frequency of algal blooms can be a sign of increasing enrichment of a wetland.

Decomposition

Microbial populations, and consequently decomposition, are at least temporarily accelerated by enrichment in some wetland types (e.g., Dierberg and Ewel 1984). However, it is not apparent that relatively high rates of decomposition are a sign of atypical enrichment in prairie wetlands, and over the long term, enrichment could reduce decomposition rates in a wetland if it results in anaerobic conditions becoming widespread.

Other Algal Indicators of Enrichment

In prairie wetlands, Hooper-Reid and Robinson (1978a) found statistical relationships between nutrient enrichment (or impoverishment) and various physiological indicators: alkaline phosphatase activity, nitrogenase activity, ratio of protein to carbohydrate and lipid, and silica uptake rate. The strength of these relationships varied within the growing season, and in contrast, Murkin et al. (1994b) found no such statistical relationships. Formation of polyphosphate bodies within algal cells has also been used as an indicator of phosphate oversupply (Stevenson and Lowe 1986). However, many anatomical and physiological approaches are relatively labor-intensive and are often more appropriate for use in research than in routine monitoring.

Other Microbial Processes

The activity of denitrifying microbes is probably greater in wetlands of greater fertility (e.g., moderately alkaline clays with adequate organic matter). For example, microbial biomass in soils of North Dakota was found to be greater in areas underlain by siltstone than in areas underlain by less fertile sandstone or shale parent material (Schimel et al. 1985). Denitrification rates might be greater in wetlands that have been exposed to nutrient runoff than in relatively pristine wetlands (personal communication, J. Kadlec, Utah State University, Logan). Tillage and fertilization of soils over time also might increase the suitability of remaining soil carbon as an energy source for denitrifying microbes (Groffman et al. 1992).

2.4.6 Algal and Microbial Communities as Indicators of Pesticide and Heavy Metal Contamination

Species Composition

Algal blooms commonly occur in wetlands following the application of herbicides to kill vascular plants. Benthic algae sometimes are the first to increase because they benefit from the opening of the canopy. By stabilizing bottom sediments somewhat and thus reducing turbidity, their establishment can pave the way for metaphyton such as *Chara*, which can reduce turbidity even further (Crawford 1981). A shift in community composition from large filamentous chlorophytes (green algae) to smaller diatom species and blue-green algal species—particularly those of the order Chaemaesiphonales—is another possible sign of herbicide effects on a wetland (Goldsborough and Robinson 1983, Herman et al. 1986, Hamilton et al. 1987, Gurney and Robinson 1989a). However, whether this occurs can depend on the particular herbicide that is applied. Limited data from laboratory assays (Peterson et al. 1995) suggest that 1) glyphosate might differentially inhibit diatoms, a key food of snails and midge larvae; 2) diquat might cause a shift from diatoms and blue-green algae to unicellular green algae; and 3) atrazine, hexazinone, simazine, and tebuthiuron might allow nuisance filamentous blue-green algae to become more dominant than other algal assemblages.

Effects of heavy metals and selenium on algae and microbes have been studied elsewhere (e.g., Crane et al. 1992), but they have received little study in prairie wetlands. Algal taxa that might be potential indicators of heavy metal contamination are identified in several studies from other regions (Lange-Bertalot 1979, Maeda et al. 1983, Deniseger et al. 1990), and microbial taxa that are potential indicators of contaminants are documented by Baath (1989) and Dean-Ross and Mills (1989).

Species Richness

Algal and microbial species richness is probably a weak indicator of wetland contamination with toxic substances, but species richness remains untested in prairie wetlands. Microbial diversity sometimes declines with exposure to hydrocarbon pollutants (Atlas et al. 1991) but not necessarily in response to heavy metals (Dean-Ross and Mills 1989).

Biomass and Density

In response to contaminants, the total biomass or density of algae and microbes can either decrease (e.g., Whitton 1971) or increase. Decreases are due generally to inhibition of reproduction and growth, whereas increases typically occur when contaminants are differentially toxic to animals that otherwise would graze on algae (e.g., Hurlbert et al. 1972), or to plants whose shading otherwise limits algal growth. Algae that inhabit sediments (benthic algae) appear to remain inhibited by some pesticides for a longer period than are algae that are attached to substrates above the sediment surface (Gurney and Robinson 1989a). This suggests that the ratio of benthic species to non-benthic species might be a useful indicator of persistent, sediment-adsorbed contaminants. However, the total biomass or density of algae and microbes is a poor indicator of contamination. This is especially true if the numbers of cells, rather than their volume, is the monitored indicator (Gurney and Robinson 1989a).

It cannot be assumed that contaminants that are harmless or harmful to vascular plants will usually have the same effect on algae. Many algal species are more sensitive than vascular plants to particular contaminants, especially those that inhibit photosynthesis (Fletcher 1990). Effects of contaminants on algal and microbial communities of prairie wetlands specifically have only recently been studied, beginning with a mesocosm study by Johnson (1986). A widely used herbicide, atrazine, was found to reduce algal productivity and growth by more than 40% when present at concentrations > 1 mg/L (Johnson 1986). Some evidence suggests that atrazine concentrations as low as 0.001 mg/L might be capable of altering algal species composition (deNoyelles et al. 1982) and biomass (Herman et al. 1986); effects may depend on duration of exposure (Jurgensen and Hoagland 1990). Some attached algae can develop resistance to atrazine after exposure to 0.050 mg/L (Detenbeck et al. 1993). Atrazine concentrations of up to 0.008 mg/L were found in a survey of 42 prairie wetlands in nine South Dakota counties (R. Ruelle, personal communication, FWS, Pierre, SD), and concentrations of 0.001–.005 mg/L occur most of the time in agricultural streams entering the Great Lakes (Frank et al. 1979). A concentration of 0.413 mg/L would be expected to occur immediately after a 0.5-ha prairie wetland is sprayed at recommended dosages (Sheehan et al. 1987). Reviewing other toxicity data, Sheehan et al. (1987) concluded that the expected in-wetland concentrations of 7 of 21 herbicides used in the prairie region could be toxic to algae if wetlands were sprayed directly.

Recent laboratory testing of 23 pesticides (20 herbicides, 2 insecticides, 1 fungicide) at realistic, environmentally expected concentrations resulted in impacts to a wide range of algal species from nine of the pesticides, five of which were triazine herbicides (Peterson et al. 1995). Least damaging to algae were the fungicide propiconazole and the herbicides picloram, bromoxynil, and triclopyr. Field assays indicate triclopyr might be relatively nontoxic to wetland vascular plants as well (Gabor et al. 1993). Johnson (1986) also found that two other herbicides (triallate and trifluralin) actually stimulated photosynthetic productivity by 20%–30% two weeks after application. However, triallate can be highly persistent under some conditions (Sheehan et al. 1987), and long-term effects were not determined. Carbofuran also mildly stimulated algal growth when present at concentrations of 10 and 100 mg/L. Phorate showed no effects, and fonofos inhibited algal growth only after 30 days, suggesting that a degradation product was responsible for toxicity. After applying another popular herbicide, glyphosate (Roundup), to prairie wetland mesocosms at a typical rate (2.5 L/ha), Shaw (1992) also reported a mild stimulatory effect on phytoplankton productivity at concentrations < 0.1 mg/L. However, greater concentrations depressed algal productivity (as measured by ¹⁴C uptake) in 3 of 4 wetlands, and the author noted that lower concentrations of glyphosate could be just as toxic to algae in waters of relatively low calcium and magnesium content. In a survey of 10 other Saskatchewan potholes, Shaw (1992) found a glyphosate concentration > 0.1 mg/L in only one.

Decomposition

Even when applied at concentrations 50 and 100 times normal field rates, one soil insecticide (AC 92,100) had no apparent effect on decomposition rates in a prairie hydric soil (LaVeglia and Dahm 1974). No data are available for other pesticides.

Other Microbial Processes

In a prairie wetland mesocosm, a dosing study of six herbicides (atrazine, fonofos, carbofuran, phorate, treflan, triallate) found that none had a significant impact on indicators of microbial functions (glucose mineralization, oxygen consumption, alkaline phosphatase activity, respiratory electron transfer system/dehydrogenase activity) (Johnson 1986). Similarly, an insecticide dosing study of an Iowa hydric soil found no impacts on some other indicators of microbial function (LaVeglia and Dahm 1974). Applying 50–100 potentially toxic contaminants to microbial communities and a variety of other organisms in laboratory bioassays, Blum and Speece (1991) found that chemicals that were highly toxic to a popular test organism—fathead minnow—were almost always toxic to a major denitrifier, *Nitrosomonas*. Two microbial assemblages responsible for decomposition (aerobic heterotrophs and methanogens) were less sensitive to the same contaminants.

Bioaccumulation

Apparently no studies have examined the role of algal and microbial communities as sinks for heavy metals or pesticides in prairie wetlands.

2.5 Monitoring Techniques

Methods for monitoring algal or microbial communities are described by Stevenson and Lowe (1986), Britton and Greeson (1988), and Aloï (1990). Microbial communities, especially assemblages of bacteria, are notoriously difficult to characterize because of the selectivity of culture techniques (Atlas 1984). Nonetheless, various bacterial strains can be placed in assemblages that likely have ecological significance (Mills and Wassel 1980).

Algae can be sampled at any season, but algal biomass is often greatest during the later part of the growing season (e.g., Hooper-Reid and Robinson 1978a, Crumpton 1989). In semipermanent wetlands, it may be advisable to sample metaphyton during sunny weather because sunlight makes the metaphyton mats more buoyant and thus easier to see and sample.

2.5.1 Direct Sampling

Chlorophyll-a is sometimes sampled from the water column as an indicator of algal biomass. Some studies in prairie wetlands (e.g., Hickman and Jenkerson 1978) show it being only weakly correlated with measures of algal biomass (dry weight) and productivity, while others (Hosseini and van der Valk 1989a,b, Labaugh 1995) report stronger correlation. Cell volume is also sometimes used as an indicator of production (e.g., Shames et al. 1985), but cell surface area seems to be a more accurate surrogate (Hooper-Reid and Robinson 1978b).

Algal communities in wetlands are generally collected using certain methods from sediment samples, water column samples, artificial substrates, or natural organic substrates.

Sediment sampling. Algae and microbes can be sampled from sediment surfaces in all types of prairie wetlands. Piston corers, plastic syringes, or other suction devices can be used. For example, Shames et al. (1985) used a plexiglass corer to remove the top 2 cm of sediment

when sampling benthic algae in a Manitoba wetland. In Florida cypress swamps, Dierberg and Brezonik (1982) sampled the nitrifying bacteria of surface sediments using a sterile piston corer and a plastic syringe with an attached tube.

Water column sampling. Whenever standing water is present for more than a few days, algae and microbes can be counted from samples of the water column of prairie wetlands (Robarts et al. 1992). Volumetric tube containers (Gurney and Robinson 1988) or fine-mesh nets have been used to collect samples. Vertically integrating, automated samplers also can be used (Schoenberg and Oliver 1988). Surface microlayers (top 250–440 μm) can be sampled using fine nets or screens mounted on a frame (Estep and Remsen 1985).

Artificial substrates. Artificial substrates (initially sterile materials placed in a wetland and subjected to natural colonization) sometimes integrate the algal and microbial assemblages from a large variety of microhabitats (Henebry and Cairns 1984, Goldsborough and Robinson 1986). Microbes or algae can be monitored by installing plexiglass plates or similar inert, sterile surfaces in prairie wetlands at any time when surface water is likely to be present for several days. The substrates are colonized by attached algae and microbes during this period, then retrieved for analysis. In prairie wetlands, cellulose acetate substrates roughened with sandpaper were used by Hooper and Robinson (1976), Hooper-Reid and Robinson (1978a,b), and Shames et al. (1985); acrylic rods were used by Hosseini and van der Valk (1989a,b), and Murkin et al. (1992).

Natural substrates. Epiphytic and benthic algae can be sampled using a quadrat approach, in which a frame is placed over a standard-sized area of bottom and substrates are scraped (Hooper and Robinson 1976). Frame sizes of 10 × 10 cm (Atchue et al. 1982) and 1–2 m² (Schoenberg and Oliver 1988) have been used in other regions.

To accurately estimate algal and microbial density, the surface area of substrate must be quantified. This can be a daunting task in the case of epiphytic algae, where plant surface areas need to be measured. Some investigators have approached this by measuring surface areas of a random sample of plants, sometimes with the use of a digital scanner, then measuring their volumes (by displacement) or dry weights and developing area-volume or area-weight calibration curves. The curves can be used to estimate plant surface area from future, simpler measurements of the volume or weight of other plants of the same species.

Bacterial and fungal abundance are usually estimated as colony forming units (CFU) using plate count techniques. However, concerns have been raised about the validity of this technique for monitoring fungi; use of low-nutrient culture media (rather than the typical enriched media) is also recommended (Baath 1989).

Use of more than one sampling method is recommended because different taxa occupy different habitats. For example, the data of Shames et al. (1985) indicate that species richness of one prairie wetland ranged from 18 to 35 species, and that of another ranged from 26 to 41 species, depending on which of five components of the algal community were sampled, and how they were sampled. The methods were acetate colonization substrate (smooth, roughened), epiphyton sampling (scraped from *Typha* stems), epipelon sampling (from core samples), and phytoplankton sampling (using a tube sampler). Each wetland was sampled 17 times during the growing season by each method. When results of sampling all five components were pooled,

species richness of the first wetland was 78 and that of the second was 80; thus, no single component of the algal community contained more than 45%–51% of the species. The number and proportion of species that were unique to one component of the algal community was smallest for the smooth substrate-colonizing component (1 endemic species, constituting 4% of all species on smooth substrate) and greatest for the phytoplankton component (17 endemic species, constituting 41% of all phytoplanktonic species).

2.5.2 Indirect Sampling Through Measurement of Processes

Algal and microbial communities can be monitored indirectly by monitoring processes such as decomposition and denitrification.

Decomposition

Procedures for measuring rates of decomposition in prairie wetlands are detailed by Murkin et al. (1989) and Davis and van der Valk (1978a,b). In the latter instance, the authors collected fresh standing-litter immediately after first frost. They clipped six, 1 × 1 m quadrats of each species, at 15-m intervals along a transect parallel to shore. They placed plant litter in nylon mesh bags on the sediment surface or suspended in the water column. They then removed a few bags periodically for about 1 year (more often at first). Silt and invertebrates were removed and samples were dried to a constant weight. The investigators noted that, by excluding litter-processing invertebrates, the bags might not precisely represent the natural rate of decomposition.

Denitrification Enzyme Activity (DEA)

A method for determining the relative activity level of important denitrifying bacteria in soils was applied to wetlands by Groffman and Tiedje (1989). Requirements include a laboratory with a gas chromatograph, a gas manifold (to make samples anaerobic), and facilities to do chloroform-incubation methods of carbon analysis, chloramphenicol microbial inhibition, and nitrogen gas measurement, from samples brought in from the field. The initial laboratory investment is approximately \$20K, and exclusive of the gas analysis tasks, one person can run 50–100 samples per day, with a cost of \$150/month for expendable supplies (P. Groffman, personal communication, Institute of Ecosystem Studies, Millbrook, NY).

Other Measures That May Reflect Microbial Processes

Respiration and other functional activities of microbial communities can be estimated by a variety of indirect methods. Methods for measuring respiration of entire ponds or wetlands are available (Madenjian et al. 1990). Probably the best-known microbial bioassay technique is the Microtox Standard Assay Procedure, which has been used to measure microbial stress in prairie wetlands potentially exposed to pesticides (Ruelle and Henry 1993). Measurements of the relative rates of lipid biosynthesis (Fairchild et al. 1984) are another expression of microbial function. Stressed microbial communities also sometimes have altered adenylate (ATP, ADP, AMP) energy charge ratios. Microbial biomass can be indirectly monitored by comparing levels of adenosine triphosphate (ATP) to ash-free dry weight (Meyer and Johnson 1983). The rates at which microbial communities colonize sterile substrates introduced to a wetland, and the characteristics

of the colonizing community, can also be used to indicate impacts from contaminant toxicity (Cairns et al. 1992).

2.5.3 Time-Integrating Methods

The nutrient status of a wetland during pre-settlement periods can sometimes be inferred by examining photosynthetic pigments or structural remains found in wetland sediments. In particular, levels of chlorophyll, chlorophyll derivatives, carotinoids, and myxoxanthin (a pigment associated with eutrophic blue-green algae) can be used to infer nutrient status, at least in permanent water bodies where past hydrological effects on species composition can be presumed to be insignificant. Samples are collected with corers; pigments can then be extracted with solvents and partitioned using chromatographic methods. Methods are described by Leclercq and Maquet (1987) and Agbeti and Dickman (1989). A baseline was established using such an approach in one prairie wetland (Begres 1971), and a project involving analyses of cores from 50 prairie wetlands is ongoing (S. Fritz, personal communication, Limnological Research Center, University of Minnesota, Minneapolis, MN).

2.5.4 Bioassay Methods

A review of laboratory, outdoor mesocosm, or *in situ* bioassay methods involving algae is beyond the scope of this document. Use of bioassays to explore contaminant toxicity to algae in prairie wetlands has been relatively limited. Examples include studies by Johnson (1986), Gurney and Robinson (1989a), Wayland and Boag (1990), and Ruelle and Henry (1993). Impacts of phorate, an organophosphate insecticide, on microbial populations were not detected using a culture test, the Microtox test (Dieter et al. 1994).

2.6 Variability and Reference Points

The following subsections deal with spatial and temporal variability of algal and microbial community characteristics in prairie wetlands.

2.6.1 Spatial Variability

Species Richness

One of the few studies to survey algal richness in prairie wetlands (Labaugh and Swanson 1988) sampled pothole wetlands representing five different hydrochemical environments in the Cottonwood Lakes area. When lists from all five wetlands and all six sampling dates (months) were pooled, the species total was 306—clearly more species than are usually found in any wetland's non-algal flora or fauna. Seventy-six algal species were found over the course of one season on three species of plants in a single shallow lake in Manitoba (Pip and Robinson 1982).

In other regions, studies that have compared protozoan communities among wetlands include Henebry et al. (1981) and Pratt et al. (1985). The former study, covering 13 Michigan wetlands over a 5-year period, found a range of 93 to 365 protozoan species. The latter study, covering 28 Florida ponds, found a range of 112 to 410 species, with a mean of 338 species in non-artificial ponds. Functional structure of the resident protozoan fauna changed slightly from year

to year, but wetlands in the same geographic region and experiencing similar climatic patterns had similar proportions of species in each functional group (Pratt et al. 1985).

Density, Biomass, and Production

Phytoplankton standing crop (biomass) is often expressed as chlorophyll-a, and can peak at 0.481 mg/L in some prairie lakes (Barica 1975, Barica et al. 1980). Phytoplankton averaged only 0.029 mg/L during the growing season in one Saskatchewan lake (Hickman and Jenkerson 1978), 0.002–0.006 mg/L in six prairie wetlands (Gloutney 1993), 0.001–0.380 mg/L in the Cottonwood Lake semipermanent wetlands (Labaugh and Swanson 1993), and 0.010 mg/L in a saline prairie lake (Campbell and Prepas 1986). Among 10 Northern Plains lakes and wetlands, algal volume ranged over 4 orders of magnitude (Labaugh 1995). Metaphyton standing crop in a prairie wetland that had been flooded for 2 years averaged 66 g/m² over a growing season (Hosseini and van der Valk 1989b) and peaked at 151 g/m² dry weight. Metaphyton in another prairie marsh was measured as 200 g/m² (van der Valk 1986). Among six Saskatchewan prairie wetlands, the biomass of epiphytic algae peaked at only 0.025 to 0.105 g/m² (Gloutney 1993). In Delta Marsh, epiphytic biomass was estimated to range from 2.3 to 32.3 g/m² (Hooper and Robinson 1976). Chlorophyll-a from Delta Marsh's epiphyton varied from < 0.01 to about 0.05 g/m², and it was greatest within a cat-tail stand, 3 m from the edge with open water (Murkin et al. 1992). Chlorophyll-a from epiphyton in some saline prairie lakes in Alberta averaged less than 0.07 g/m² (Campbell and Prepas 1986).

In shallow prairie lakes, phytoplankton densities can exceed 300,000 cells per ml (Hickman and Jenkerson 1978); bacterial densities can exceed 10,000,000/mL (Campbell and Prepas 1986). Phytoplankton primary productivity averaged 196.77 mg C·(m³)·hr⁻¹ and 196.77 mg C/hr/m² in a shallow prairie lake (Hickman and Jenkerson 1978), and in prairie wetlands the production of all algae combined generally ranges up to a few hundred g C·(m²)⁻¹·yr⁻¹ (Murkin and Batt 1987, Crumpton 1989). The production of epiphytic algae is perhaps greater on emergent than submersed vascular plants (Hooper and Robinson 1976) although it is difficult to standardize estimates of available surface area of plants. Within emergent plant communities, the level of epiphytic algal biomass varies largely with spatial and temporal variation in nutrient availability, e.g., from 2.3 to 32.3 g C/m² (Hooper and Robinson 1976, Hooper-Reid and Robinson 1978a).

Decomposition Rate

Few estimates are available to describe the variability of decomposition rates among and within wetlands. The half-life of fallen emergent plant litter in two Iowa prairie lakes ranged from 128 to 1011 days, depending on plant species, season, and other factors (Davis and van der Valk 1978a,b; Neely and Davis 1985b; Neely and Baker 1989).

Denitrification Enzyme Activity (DEA)

Measurements of DEA within a wetland vary somewhat spatially. Variability (coefficient of variation) of DEA measurements within wetlands ranges from 33%–89%, and the coefficient of variation among true replicates is about 10% (P. Groffman, personal communication, Institute for Ecosystem Studies, Millbrook, NY). Measurements of denitrification using alternative methods are highly variable and difficult to compare (et al. 1994), but DEA

measurements are believed to be generally less variable because they integrate conditions over time.

2.6.2 Temporal Variability

Species Richness

In the Cottonwood Lakes area, data indicate considerable monthly variability in algal richness (Table 1) (Labaugh and Swanson 1988). Species turnover rates have not been quantified for prairie wetlands.

Density

In a study of five wetlands in the Cottonwood Lakes area, four relationships were noted:

Table 1. Species diversity by basin type and month in the Cottonwood Lakes area (Labaugh and Swanson 1988).

Basin Type	Month(s) with the Most Peaking Species	Month(s) with the Fewest Peaking Taxa
Seasonal (n = 2 basins)	June (38 and 42 taxa)	May and July
Semipermanent (n = 2 basins)	October (32 and 55 taxa) and May (59 taxa)	June and September

Decomposition Rate

Interannual differences in decomposition rates and patterns of two species (*Scolochloa* and *Scirpus lacustris*) in a flooded prairie wetland were negligible (Wrubleski et al. 1993).

Denitrification Enzyme Activity (DEA)

No published information was found on interannual variation in prairie wetlands of DEA or other microbial functions.

2.7 Collection of Ancillary Data

It is easier to separate the anthropogenic from the natural causes of impairment of community structure if data are estimated or inferred simultaneously on the following features of particular importance to algae and microbes:

- age of the wetland and its successional status
- light penetration (water depth, turbidity, shade), temperature, sediment oxygen, general chemistry of waters (particularly pH and conductivity)

- leaf surface area and stand density of associated vascular plants
- density of grazing aquatic invertebrates
- moisture regime (e.g., time elapsed since last runoff, inundation, or desiccation event).

All of these features vary to a large degree naturally, as well as in response to human activities such as soil tillage, compaction, and erosion; fertilizer and pesticide application; and water regime modification.

2.8 Sampling Design and Required Level of Sampling Effort

For most algal communities, sample processing and species proportional counts (assuming 500 individuals) take 2–3 hours per sample (Stevenson and Lowe 1986).

2.9 Summary

The enormous diversity of algae (probably over 500 species in prairie wetlands) and the position of algae and microbes at the base of the food chain suggests their considerable ecological importance. It also highlights a need for monitoring key processes supported by algae and microbes and continued research to associate various rates of these processes (e.g., decomposition) with the seasonal sequencing and occurrence of particular species compositions. Published estimates of interwetland and interannual variability of algal and microbial taxonomic composition in prairie wetlands are nearly nonexistent.

Most algal and microbial communities recover quickly from acute disturbances. Because of this quick recovery, direct sampling of algal and microbial communities will fail to detect many acute disturbances. Alternatively, indirect examination of the pigment from just the portion of the diatom community that accumulates seasonally in sediment traps might provide some indication of current wetland conditions.

Algal species composition, and to a lesser degree species richness, demonstrates diagnostic responses to changes in vegetative cover, salinity, excessive nutrient loads, and sedimentation or turbidity (Table 2). Algae also respond sensitively to changing water regime and pesticide or heavy metal contamination, but existing information is too limited and confounding effects are too prevalent to currently allow widespread use of algae to diagnose impairment of prairie wetlands from these stressors.

Table 2. Summary evaluations of possible algal and microbial indicators of stressors in prairie wetlands. Evaluations are based on technical considerations, not cost or practicality. A rating of FAIR or POOR is assigned when too few data (FD) suggest potential as an indicator or when confounding effects (CE) of other variables often overshadow the effects of the listed stressor on the indicator.

Stressors	Possible Indicators	Evaluation
Hydrologic stressors	Species composition Richness Density, biomass, productivity Decomposition Denitrification	FAIR (CE) UNKNOWN FAIR (CE) POOR FAIR
Changes in vegetative cover	Species composition Richness Density, biomass, productivity Decomposition	GOOD FAIR (FD) POOR (CE) POOR (FD)
Salinity	Species composition Richness Density, biomass, productivity Decomposition	GOOD GOOD FAIR POOR (FD)
Sedimentation & turbidity	Species composition Richness Density, biomass, productivity Decomposition	FAIR (FD) FAIR (FD) FAIR (CE, FD) POOR (FD)
Excessive nutrients & anoxia	Species composition Richness Density, biomass, productivity Decomposition Denitrification	GOOD POOR GOOD POOR (CE) FAIR (CE)
Herbicides	Species composition Richness Density, biomass, productivity Decomposition	FAIR (FD) POOR (FD) FAIR (CE) POOR (FD)
Insecticides	Species composition Richness Density, biomass, productivity Decomposition	POOR POOR POOR POOR
Heavy Metals	Species composition Richness Density, biomass, productivity Decomposition	GOOD (CE) POOR POOR POOR

3. Vascular Plants as Indicators of Prairie Wetland Integrity

3.1 Ecological Significance and Suitability as an Indicator

In prairie wetlands, three growth forms of vascular plants usually are defined: emergent, floating-leaved, and submersed plants. To varying degrees, these form discrete zones within wetlands. Wetlands containing all three forms (and many subforms and species) are generally those with a core area that is flooded permanently, but with water levels that otherwise vary greatly from year to year. In such wetlands, nutrients are more available and support substantial invertebrate densities and waterbird use.

The foliage and/or seeds of several species of vascular plants are consumed regularly by waterfowl (see Appendix A). The food values of wetland vascular plants are attributable both to their being consumed directly (mainly by microinvertebrates and tadpoles) and to their serving as an attachment surface and secondary energy source for algae, amphibian larvae, and invertebrates.

Also, vascular plants as well as algae influence the fertility of prairie wetlands by harnessing solar energy through photosynthesis. In contrast to algae which release the stored solar energy almost immediately after their death, the energy from vascular plants is made available by microbes slowly, over a period ranging from weeks to months. Thus, during seasons when algal populations are at a minimum, the energy originally trapped by vascular plants could well be a significant source of energy for invertebrates that later in the growing season are consumed by waterbirds (Nelson and Kadlec 1984). Of particular importance is the value of vascular plants in prairie wetlands as a substrate for growth of attached algae and microbes (Campeau et al. 1994) and as habitat cover (shelter) that protects invertebrates, amphibians, and birds from predators and severe weather (Murkin et al. 1992). Evidence from other regions (Hanson and Swanson 1989) suggests that the type of wetland plant can influence the size structure of invertebrate communities (the ratio of large to small individuals), and thus perhaps influence the value of the invertebrate community to waterbirds. Using isotope ratios to investigate food webs in Delta Marsh, Neill and Cornwell (1992) found emergent vascular plants and the algae and microbes attached to them, rather than submersed macrophytes or metaphyton, to be the most important sources of organic matter to the invertebrate consumers that were most abundant during June.

Vascular plants are also important because they influence the amount, rate, and seasonal timing of nutrient and contaminant cycling across the water column–sediment ecotone. Some plants remove nutrients directly from the water column, thus seasonally tying up some of the nutrients that otherwise might support nuisance algal blooms. In the sediment, plant roots can take up nutrients (and contaminants). In some cases, plant roots can transfer both nutrients and harmful substances to plant foliage, making these substances more available to food chains. Wetland plants also help maintain wetland water quality by stabilizing shorelines and reducing wind-driven resuspension of sediments that otherwise impair light penetration and reduce primary productivity.

Characteristics of vascular plants usually considered advantages for indicating wetland integrity include:

- immobility (plants reflect site conditions and are practical for use in *in situ* exposure assessments)
- interpretability of gross patterns of spatial distribution as indicators of condition (patterns are interpretable from a distance without requiring permission for access to private property, e.g., through interpretation of aerial imagery)
- sensitivity to a wide variety of stressors (especially hydrology, salinity, and changes in vegetative cover); sensitivities of individual species are relatively well known; submersed plants are especially sensitive (e.g., to turbidity, overenrichment)
- known taxonomy and straightforward identification to genera
- well-developed sampling techniques and community metrics.

Vascular plant characteristics usually considered disadvantages for indicating wetland integrity include:

- lagged response to stressors (episodic stresses may not be reflected)
- relative insensitivity to insecticides and heavy metals (but can bioaccumulate these)
- difficulty sampling some assemblages (e.g., submersed species)
- laborious identification of some assemblages
- difficulty characterizing communities during the dormant season.

3.2 Potential Indicator Metrics

As applied to plant communities, the following measurements and metrics were considered for characterizing conditions in reference wetlands, identifying the relative degree of past disturbance of a prairie wetland, or assessing the current inhibition of key processes:

- richness of species, functional assemblages, and rare species (per unit area, or per thousand randomly chosen individuals) in extant communities and in seed banks
- number and biomass of stems per unit area, or cumulative shoot length, or canopy cover per unit area (when measured at the time of its annual maximum, this is commonly used as a proxy for annual plant production)
- relative dominance and richness of species, particularly of species reputedly tolerant or intolerant to a named stressor (measured both in extant communities and seed banks)

- interannual variability in richness, density, and/or biomass
- germination rate of seeds in sediment samples
- bioaccumulation of contaminants.

The specific ways some of these metrics have been or could be interpreted as an indication of stressed conditions are described in Section 3.4.1. Various *in situ* methods of measuring macrophyte production (e.g., uptake rates of carbon radioisotopes, oxygen production, photosynthetic rate, respiration) are not considered in this document. In some instances, whole-system respiration rates, integrated over a 24-hour period, might usefully indicate wetland functional integrity.

3.3 Previous and Ongoing Monitoring in the Region

Virtually all studies of prairie wetlands mention plant species that are dominant in the studied wetland. Studies focusing primarily on vascular plants number at least 59 and cover in excess of 2200 prairie wetlands (Appendix J). The metrics most commonly measured in plant surveys are relative abundance and percent cover (canopy density). Although long-term monitoring of mature vegetation has been conducted in the Cottonwood Lakes wetlands and perhaps other areas, apparently only eight long-term studies (> 7 years of data) have been published.

No State agencies responsible for prairie wetlands currently monitor plants as indicators of wetland ecological integrity. At a regional level, USEPA's EMAP has documented plant communities in 30–40 wetlands that span gradients of water regime, probable disturbance, and geography. Variables that are being measured include species richness, areal cover, cover ratio, and amount of standing dead litter.

At more local levels, species composition and density of plants are being tested for possible use as indicators of the success of wetland restoration efforts in Iowa (Galatowitch 1993a,b), Minnesota (Madsen 1988, Sewell 1989), and perhaps elsewhere. Research on ecological relationships affecting plant communities continues to be conducted at NPSC and at the State universities. Personnel from The Nature Conservancy and/or State Natural Heritage programs conduct botanical surveys of prairie wetlands in relatively undisturbed localities, and periodically they check a few of the wetlands known to contain plant species of State, regional, or national importance because of their rarity. In wetlands known to contain regionally rare species, monitoring of these species can be used to indicate long-term integrity of the wetland. In prairie pothole wetlands of the United States, examples of regionally rare species are *Napaea dioica*, *Carex formosa*, *Eleocharis wolfii*, and *Astragalus neglectus* (interpreted from information provided to USEPA in 1992 by The Nature Conservancy).

3.4 Response to Stressors

The following subsections describe responses of the vascular plant communities to hydrologic stressors, vegetative cover conditions, salinity, sedimentation/turbidity, excessive nutrient loads/anoxia, and pesticide and heavy metal contamination.

3.4.1 Vascular Plants as Indicators of Hydrologic Stressors

Species Composition

The species composition of vascular plant communities, especially the submersed forms, is an excellent indicator of hydrologic conditions that have occurred in prairie wetlands as recently as within the past 2–4 years (Stewart and Kantrud 1972b, Millar 1973, Weller and Voigts 1983, Weller et al. 1991, Squires and van der Valk 1992). As little as 2 cm of standing water in a wetland can result in development of a plant community that differs by 75% from the one that develops when surface water is absent (van der Valk and Davis 1978).

In general, a shift over a 2–3 year period toward increased dominance by submersed species and a decline in emergent species (both adult plants and propagules) can indicate recent periods of wetter-than-usual conditions (Weller and Spatcher 1965, Millar 1973, Weller and Fredrickson 1974, van der Valk 1981, van der Valk and Squires 1992, van der Valk et al. 1994). Conversely, reduced dominance of submersed and other "obligate" wetland species (or species that typify semipermanent wetlands, see Appendix F), in association with increased dominance of emergent and "facultative" wetland species that are often annuals or biennials (or species that typify temporary wetlands, see Appendix F), can be used to determine if relative drought conditions have occurred recently (van der Valk and Davis 1978, Pederson 1981, Poiani and Johnson 1989, Poiani et al. 1995). For a few species indexed in Appendix F, quantitative data on water-depth ranges and related hydrologic variables also are available.

More specifically, van der Valk (1981) noted that recent hydrologic conditions of a prairie wetland can often be surmised from current species composition, and less recent conditions can be inferred from the seed bank. Certain conditions can be deduced retrospectively by knowing the proportion of extant species that characteristically 1) are annuals, perennials, or vegetative reproducers (see Appendix A), 2) are mudflat-germinators vs. standing-water germinators, and 3) have seeds that remain viable for long periods of time, vs. those with short-lived seeds which depend highly on dispersal. For example, if few mud flat annuals (species that have long-lived seeds and can only become established when there is no standing water) are found in seed banks underlying open water, it suggests that standing water has probably persisted for many years. Under ideal conditions, seed bank analysis can be used to infer conditions that existed up to 70 years ago (Wienhold and van der Valk 1989). Unfortunately, autecological knowledge is insufficient to assign many prairie wetland species to the predictive categories (especially 2 and 3 above) or to describe their typical seed longevity. This limits the utility of this deductive approach.

If an attempt were made to define hydrologic criteria for protecting various kinds of prairie wetland plant communities or assemblages of species, definitions of optimal heights and durations of water levels (and their variability) would be two important components. Using data on surrounding landscape factors (runoff potential) and anthropogenic uses, quantitative estimates of expected water heights and durations could be made for wetlands within particular landscapes, and from this, the types of plant communities that should be present could be predicted (e.g., Poiani and Johnson 1993a,b,c; Poiani et al. 1995). However, a major limitation of implementing this approach currently is that numeric estimates of adult plant and seed tolerances for water depth and duration have been made for only a few prairie wetland species. Prairie

wetland species with the most information on hydrologic tolerances include *Typha* spp. (>30 references), *Scolochloa festucacea* (21 references), and *Phragmites australis* (18 references). See Appendix E for lists of references, by plant species, on water-depth tolerances in prairie wetlands. Complicating this further is the fact that hydrologic tolerance thresholds vary somewhat within species as a result of genetic variation and confounding influences of seasonal (Meredito et al. 1990, 1991) and chemical conditions at the time that flooding or drawdown occurred.

In general, flooding of prairie wetlands tends to have a greater effect on community composition than does occasional drought (van der Valk and Squires 1992). The specific effects of hydrologic alteration depend on flooding depth, season, frequency, duration, initial water levels, sediment type, dominant plant species, and other factors. Nonetheless, vascular plants are potent indicators of long-term hydrologic change in wetlands.

Community Zone Locations

The presence, position, and heterogeneity of vegetation zones, each representing a recognizable plant community, can be used as an indicator of wetland integrity. For example, if a particular zone is absent but was expected based on knowledge of a wetland's hydrology, then some perturbation can cautiously be assumed to have occurred (van der Valk and Welling 1988).

When wet conditions continue for at least 2 years in wetlands that formerly had a temporary or seasonal moisture regime (i.e., they become semipermanent or permanently inundated), the shallower zones of emergent vegetation often die off first, leaving a central deep zone of emergents surrounded by open water (Millar 1976). At least in situations where water level increases are large and abrupt, emergent communities disappear rather than shift to shallower areas (van der Valk 1994). During droughts, emergents can become re-established in the center of a wetland basin. Overall, hydrologic changes usually cause only slight shifts in the position of the submersed, emergent, and meadow zones (Harris and Marshall 1963, van der Valk and Davis 1976, Johnson et al. 1987). Boundaries of vegetation zones in larger prairie wetlands tend to be less distinct than in small wetlands (Johnson et al. 1987), so their use as an indicator can be restricted in large wetlands.

Species Richness (of Mature Plants)

Plant species richness is a complex indicator of prior hydrologic conditions. Low richness can indicate prior periods of drought (Driver 1977, Galatowitsch 1993a,b), partly because prolonged dehydration facilitates the formation of homogeneous stands by a very few species. Perhaps less often, low richness can indicate prior periods of inundation when floods drown many emergent species. In some instances, prairie plant communities that are initially species-rich tend to suffer less loss of productivity as a result of drought than communities that initially are species-poor (Tilman and Downing 1994). Semipermanent wetlands generally have greater plant species richness than temporary or seasonal wetlands of the same size. This is partly because water persistence and depth is sufficient to allow submersed and floating-leaved plants, as well as terrestrial forms, to exist and successfully reproduce.

When increased species richness results from wetter conditions, it is often because increased water levels tend to fragment the monotypic stands of emergent species (especially cat-tail, bulrush, pickerelweed) and allow partial invasion by submersed and floating-leaved species that add to diversity (Botts and Cowell 1988, McIntyre et al. 1988). Wetlands that are more persistently wet also can have higher usage by birds and other mobile animals that introduce seeds, thus diversifying the wetland plant community (Pip 1987b).

In part of Delta Marsh, reflooding of various units to different depths after a drawdown that had lasted 1 or 2 years had several effects (van der Valk and Squires 1992, van der Valk et al. 1994). Reflooding to original water levels greatly increased the variety of vegetation forms (class richness, and number of classes containing multiple species), whereas reflooding to water levels that were 1 m above the original resulted in a halving of species richness. Reflooding to intermediate water levels gave unclear results.

After a drawdown and gradual reflooding of an Iowa semipermanent wetland, the peak in total species richness occurred 3 years after the drawdown, whereas the richness of just the submersed and floating-leaved species did not peak until 4–5 years after drought. In their Delta Marsh studies, van der Valk and Squires (1992) reported a lag time of at least 3 years before small (< 1 m) changes in wetland water level could be detected using wetland plants.

Species Richness (of Seed Banks)

The species richness of seeds in wetland seed banks (seeds that lie dormant in soils and sediments) can be used to crudely approximate the time elapsed since hydrologic alteration occurred in wetlands that now are dry. This requires calibration to conditions currently present in similar but hydrologically unaltered wetlands. For example, Wienhold and van der Valk (1989) found that mean species richness in a series of unaltered wetlands was 12.3, but fell to 7.5, 5.4, 5.0, 3.2, and 2.1 species in potholes drained up to 5, 10, 20, 40, and 70 years ago, respectively. After wetlands had been drained for more than 20 years, about 60% of the species disappeared from the seed bank.

Biomass, Production, and Cover Ratio

For emergent plants, decreases in areal cover can signify recent inundation events (van der Valk and Squires 1992), and increases in areal cover can indicate recent drought events. If periods of dehydration are brief (a few hours or days), the areal extent of emergent plants, especially those with rigid stems (e.g., cat-tail, common reed) is unlikely to change. The presence of "hemi-marsh" conditions—relatively equal proportions of open water and emergent wetland—indicates that a wetland has probably undergone a wet-dry cycle of about 2–7 years. During such a cycle, droughts lasting more than 1 year (Welling et al. 1988a) and flooded conditions lasting at least one winter (Millar 1973, McKee et al. 1989) can damage or impair the recruitment of many wetland emergent plants. For submersed plants, flood conditions lasting at least 2 years are required for establishment of some species (e.g., *Potamogeton pusillus*, Millar 1976). In Iowa, prairie wetlands restored within the last year had significantly more floating-leaved plants than those restored 2 years previously (Hemesath 1991). Emergent plant cover was less, and open

water area was greater, on restored wetlands that had been drained > 30 years ago than on restored wetlands that had been drained more recently.

Models are available for predicting the duration of floods and droughts within a wet-dry cycle that likely produced a particular number and configuration of wetland types (Woo et al. 1993, Larson 1995) and associated species assemblages (e.g., Poiani and Johnson 1993a,b,c; Poiani et al. 1995). Similarly, models are available for predicting future plant species composition, given a particular hydrologic change (Poiani and Johnson 1993a,b,c, van der Valk et al. 1989, FWS 1993, de Swart et al. 1994). A computer simulation by Poiani and Johnson (1993a,b,c) indicated that for a particular depth class, the ratio of open water to wetland vegetation (the cover ratio) was always greater when water levels were held constant for 5 years than when they fluctuated during that time. Thus, a relatively large cover ratio might be considered potentially indicative of a relatively sustained input of water to a wetland.

Areal cover of submersed and floating-leaved plants changes less with increased inundation than does the areal cover of emergent vegetation. However, cover of non-emergent species is usually reduced somewhat because of increased wave action and turbidity (van der Valk and Davis 1978). For example, although flooding to 1 m above normal in Delta Marsh, allowed bladderwort (*Utricularia vulgaris*) to invade stands of cat-tail, whitetop, and common reed, the flooding completely eliminated beds of sago pondweed (*Potamogeton pectinatus*) (Murkin et al. 1991). At the opposite extreme, complete drawdown lasting for much of a growing season has catastrophic effects on most submersed species. However, a few submersed and floating leaved species can survive up to 1 year of dessication e.g., *Callitriche palustris*, *Potamogeton gramineus*, *Myriophyllum spicatum*, *Lemna minor*, *Spirodela*, *Najas*, *Potamogeton pectinatus*, *Marsilea mucronata*, and *Ceratophyllum demersum* (Stewart and Kantrud 1972b, van der Valk and Davis 1978, Cooke 1980, Davis and Brinson 1980, Nichols et al. 1989).

Most studies of flooding effects in prairie wetlands have focused on semipermanent wetlands. A study of seasonal wetlands found that production (especially aboveground production) of one emergent species, *Scholochloa festucacea*, increased in response to 10 weeks of flooding to a depth of 25 cm in the spring (Neill 1994).

Seed Density

The density of seeds in wetland seed banks can be used to crudely approximate the time elapsed since hydrologic alteration occurred in wetlands that now are dry. This requires calibration to conditions currently present in similar but hydrologically unaltered wetlands. For example, Wienhold and van der Valk (1989) found that mean total seed density in a series of unaltered or recently (within 5 years) altered wetlands was 3600–7000/m², but declined to 1400, 1200, 600, 300, and 160 seeds/m² for the 10, 20, 30, 40, and 70 years after drainage, respectively.

Germination Rate

Seedlings of most emergent species in prairie wetlands fail to germinate from areas where sediments are not exposed until after early July. Germination is particularly hindered if the previous growing season has been especially wet, thus supporting exceptional biomass of

submersed plants (as well as of emergent species) whose residual litter can smother emergent seedlings (van der Valk 1986). Consequently, the presence of viable emergent plant seeds, but not seedlings, sometimes can indicate that a drawdown, if it occurred, did not occur until late in the preceding growing season (Welling et al. 1988b, Meredino et al. 1990); that the wetland was overgrown with submersed plants and filamentous algae the preceding year; and/or that conditions prior to the growing season dramatically affected microbial and invertebrate communities that normally decompose most litter.

3.4.2 Vascular Plants as Indicators of Changes in Vegetative Cover Condition

Species Composition

Species composition can be used loosely to indicate the severity and type of process that has removed vegetation in a prairie wetland. A dominance of relatively tall and robust, adventive species or their hybrids, as opposed to shorter emergent species, is one possible sign of a lack of periodic disturbance from livestock, burning, mowing, or cultivation (Stewart and Kantrud 1972a). A relative scarcity of highly palatable (to cattle) plant species can also signify that intensified grazing has occurred during drier years. Plants that are annuals (see Appendix A) tend to be the most affected by early-season mowing (Stewart and Kantrud 1972a). Other emergent species tend to be affected differentially by herbicides and fire (Thompson and Shay 1985). Information on shifts in community composition that can result from grazing and other removal processes is summarized by Kantrud et al. (1986a) and Kirby et al. (1992). Indicator species that suggest the occurrence of previous grazing, tillage, or mowing in prairie wetlands are shown in Appendix A, which was expanded from Stewart and Kantrud (1972), Millar (1973), and Kantrud et al. (1989). However, both Walker and Coupland (1970) and Stewart and Kantrud (1972 a,b) concluded that land use factors (e.g., intensity of grazing, mowing, tillage), as compared with hydrologic and water chemistry influences, are less important determinants of species composition, except during drought years.

Community Zone Locations

Cover removal differentially affects the outermost zones of prairie wetlands because these are the most accessible to equipment and grazing livestock. Presence of a deep emergent zone surrounded by open water can be a sign of intense grazing (Stewart and Kantrud 1972a).

Species Richness

Decreases in wetland plant richness are sometimes a reflection of reduced intensity of disturbance from grazing, fire, or other removal processes in prairie wetlands. Conversely, long-term increases in species richness can indicate periodic occurrence of these disturbances. However, species richness is a poor indicator of vegetation removal because in some instances, the thinning of stands of native vegetation allows new plant species to obtain a competitive foothold. Although this can increase the species richness initially, species richness can decline over the long term if new species, as is often the case, are ones that are highly aggressive and tend to form homogeneous stands. Even at 3 years after being restored, several Iowa wetlands had significantly lower species richness within each of their vegetative zones (except the deepwater zone) than did natural wetlands (Galatowitsch 1993 a,b).

Biomass, Areal Cover, and Cover Ratio

Decreases in areal cover and biomass of wetland vegetation, and increase in the cover ratio, are a strong indicator of recent fire, haying, excessive grazing, or contamination with herbicides have occurred recently or for a prolonged period. Conversely, long-term increases in areal cover can indicate relative absence of these disturbances. A survey of restored prairie wetlands in Iowa (Galatowitsch 1993 a,b) found that shoot densities of emergent vegetation were less in restored wetlands than nearby natural wetlands.

3.4.3 Vascular Plants as Indicators of Wetland Salinity

Species Composition

The species composition of vascular plants is an excellent indicator of wetland salinity in prairie wetlands (Stewart and Kantrud 1972b, Looman 1981). Most freshwater macrophytes cannot tolerate more than 10 ppt dissolved salts (Reimold and Queen 1974, Ungar 1974). Of the 195 major plant species in prairie wetlands, the general categories of salinity that describe the occurrence of 157 (79%) species are known (Appendix A), and quantified tolerance (or preference) ranges are available for 120 species based on presence or absence in wetlands spanning a salinity gradient (Appendix D). For many species, exact thresholds of salinity tolerance vary by the type of salt (Mg, Na, etc.), life stage, genetic population, duration of exposure, temperature, and other factors (Liefers and Shay 1983).

Species Richness

Diminished species richness can be a sign of hypersaline conditions (Reynolds and Reynolds 1975).

Biomass and Cover Ratio

Compared to most prairie wetlands, hypersaline wetlands probably have lower areal cover and biomass of emergent vegetation, but only a few data are available (e.g., Wali 1976). An analysis of experimental data from the Delta Marsh suggested that, at least for some species, the influence of salinity on vascular plant production can overshadow the effects of other factors associated with water level change (Neill 1993). In Australia, saline lakes sometimes support greater densities of submerged macrophytes (and consequently waterfowl) because salts enhance the flocculation of suspended clay particles, thus increasing water clarity and light penetration (Kingsford and Porter 1994). In contrast, in England increases in lake salinity may cause shifts in plant community dominance from submerged plants to phytoplankton, at least at intermediate levels of nutrient loading (Moss 1994). In Ohio, fertilization of salt-tolerant inland wetland plants with nitrogen spurred their production (Loveland and Ungar 1983). In a study of four prairie wetlands, Fulton et al. (1979) and Fulton and Barker (1981) found that "vegetation density was a better indicator of soil properties than was the type of vegetation."

Germination Rate

Seed germination rates are inhibited by high (> 2 mS/cm) salinities, as occurs most often during drawdown events (Smith 1972, Galinato and van der Valk 1986).

Biomarkers

Preliminary experiments by Mendelsohn and McKee (1992) suggest that the concentration of proline, an amino acid, in plant tissue can indicate the occurrence of salt stress that occurred during the previous five days.

3.4.4 Vascular Plants as Indicators of Sedimentation and Turbidity

Species Composition and Community Zone Locations

A shift in community composition from submersed species to emergent and floating-leaved species can signal recent increases in turbidity, which could be attributed to suspended sediment and/or phytoplankton (Niemeier and Hubert 1986, Hough and Forwall 1988). Submersed plants in northern latitudes often have only a brief period during which they must grow sufficiently to reach the water surface. If turbidity or other factors inhibit their growth during this period, or if a late winter shortens the growth period, submersed plants may not reach the water surface before their growth is stunted by algal blooms that begin in early summer (Engel and Nichols 1994). Submersed plants are generally less tolerant of increased turbidity than are benthic algae and phytoplankton (Dennison et al. 1993).

Some of the submersed plant species of greatest value to waterfowl and invertebrates do not persist when the Secchi disk depth is less than about 0.3 m (Chambers and Kalff 1985, Kantrud 1990). Secchi transparencies of 0.2–0.4 m are common for brief periods during algal blooms in prairie wetlands (Barica 1975). Tolerances of submersed plants to turbidity are defined more accurately by the light compensation point of each species, the point where its photosynthesis equals its respiration (Dennison et al. 1993, Kahl 1993). In Wisconsin, submersed species that are most tolerant of turbidity are characterized by rapid growth during the early spring, summer leaf canopies, and winter tubers or rhizomes (Engel and Nichols 1994). Data on relative depth maxima and ranges for many submersed species are compiled in Davis and Brinson (1980), and a more refined database is currently being developed by USEPA's Wetlands Research Program (N. Detenbeck, personal communication, USEPA Environmental Research Laboratory, Duluth, MN). The more shade-tolerant submersed species can perhaps be identified from information on turbidity in Appendix A.

The relative extent of submersed species also declines with increasing sediment input when the sediment so fills a depression that water depths become too shallow and standing water fails to persist through the growing season (Edwards 1969). Similarly, in very shallow, temporary wetlands, sedimentation probably increases the dominance of non-wetland species.

The species composition of emergent species might also indicate relative degree of sedimentation because mature plants, and perhaps their seeds, differ with respect to tolerance to burial (van der Valk et al. 1981). Although stiff-stemmed emergents (e.g., cat-tail, common reed)

seem least affected by sedimentation, comparative data for other prairie species are mostly lacking.

Species Richness

Turbidity and sedimentation can reduce the species richness of wetland plants (e.g., Engel and Nichols 1994), both directly and because of secondary hydrologic impacts. If the effects of sediment on germinating seeds can be assumed to be similar to the effects of accumulating plant litter, then the results of van der Valk's (1986) experiments in a prairie wetland suggest that the species richness of adult emergent wetland plants would be reduced by increased sediment. The viability of the seeds of most of these plants, however, is relatively unaffected when covered by up to 35 cm of sediment (van der Valk and Davis 1979); removal of overlying sediment should allow them to germinate.

Biomass and Cover Ratio

As described above, turbidity definitely decreases the areal cover of submersed macrophytes. Decreases in areal cover or biomass of emergent species are probably not as strong an indicator of sedimentation, but data are lacking. If the effects on germinating seeds of sediment can be assumed similar to the effects of accumulating plant litter, then the results of van der Valk's (1986) experiments in a prairie wetland suggest that the shoot density of emergent wetland plants would be reduced. In deep permanent basins, sedimentation can increase the area of substrate within the euphotic zone that can be colonized by wetland plants thereby supporting increased areal cover of emergents.

Germination Rate

Repeated burial by as little as 5 cm of sediment per year can be detrimental to seedlings of some emergent species (van der Valk et al. 1981). In Delta Marsh, a sediment layer of at least 1 cm significantly reduced seed germination of many emergent species, and a layer of 4–5 cm prevented germination of most of the species tested (Galinato and van der Valk 1986).

3.4.5 Vascular Plants as Indicators of Excessive Nutrient Loads and Anoxia

Species Composition and Community Zone Locations

Declines in submersed plants, and increases in emergent and especially floating-leaved plants (e.g., *Nuphar*, *Lemna*, *Wolffia*), are one sign of increased inputs of nutrients to wetlands, especially in wetlands that initially had been nutrient-poor. Although a few submersed species (*Ceratophyllum demersum*, *Utricularia vulgaris*) appear to tolerate moderate nutrient additions, most submersed species decline. This is because algae respond more quickly than vascular plants to nutrients; consequently they proliferate in open water areas in the form of light-obscuring blooms that limit submersed macrophyte growth and reproduction (Mulligan et al. 1976, Phillips et al. 1978). Most emergent species (except those capable of forming floating mats) increase less rapidly in response to waterborne nutrient inputs than do floating-leaved species (Ozimek 1978, Shimoda 1984, Graneli and Solander 1989) because the emergent plants obtain nutrients mainly from the sediment, whereas most floating-leaved plants obtain nutrients

directly from the water column. Enrichment can also stress individual emergent plants, if it causes algal blooms that, upon collapse, deprive sediments of oxygen for prolonged periods as they decay (e.g., McDonald 1955, Barica and Mathias 1979, Barica 1984, Hartog et al. 1989).

Of the emergent species, various annual species, as well as cat-tail and common reed, often dominate enriched wetlands (Kadlec 1979, Hartland-Rowe and Wright 1975, Finlayson et al. 1986, Kadlec 1990, Kadlec and Bevis 1990). Species that are most efficient in transferring oxygen to their roots might flourish the most under highly enriched conditions, when sediment oxygen levels decline (Barko and Smart 1983). Cat-tail, for example, effectively transfers oxygen and also requires only trace amounts of dissolved oxygen for germination (Leck and Graveline 1979). Chemical conditions appear to have a greater influence on the presence of rarer, perennial species than on the occurrence of aggressive, common species (Pip 1979). Many reports, especially in the European literature, have categorized individual wetland plant species according to their nutrient-level preferences, and thus as to their potential as indicators of eutrophication (Moyle 1945, Swindale and Curtis 1957, Stewart and Kantrud 1972b, Pip 1979, Wiegleb 1981, Zoltai and Johnson 1988, Husak et al. 1989). Plant species composition is less effective in reflecting moderate enrichment than severe enrichment. This is because algal and microbial communities are often initially more effective than vascular plants in assimilating inputs of nutrients (Richardson and Marshall 1986).

Species Richness

Increasing species richness of herbaceous plants, particularly of emergent species, can signal moderate increases in the fertility of a wetland (Pip 1987a,b; Graneli and Solander 1988). However, severe enrichment can decrease species richness for reasons given above (Lind and Cottam 1969, Lachavanne 1985, Tilman 1987, Hough et al. 1989, Toivonen and Back 1989). Although plant richness is correlated positively with nutrient levels (especially phosphorus) in prairie wetlands, water chemistry variables explain less than half the total variability in plant richness (Pip 1987a).

Biomass and Cover Ratio

Increases in areal cover and biomass of non-submersed wetland plants are commonly one sign of enrichment. Variables that describe plant characteristics having short turnover times, such as aboveground biomass and leaf area of annual plants, can be relatively sensitive indicators of enrichment. Cat-tail biomass and production respond to annual fluctuations in nitrate, making cat-tail a possible indicator of erratic inputs of nutrients (Davis 1989). In one fertilization experiment in a prairie wetland, the aboveground production of cat-tail (*Typha glauca*) increased 19% and that of burreed (*Sparganium eurycarpum*) increased 57% (Neely and Davis 1985a). However, enrichment of prairie wetlands does not always increase plant biomass and production in the long run. This is because the shading and smothering effect of litter that remains following 1 year's excessive production can inhibit the germination and growth of individuals in successive years (Nelson and Anderson 1983, Neill 1990).

Biomarkers

Experiments by Mendelssohn and McKee (1992) and others suggest that the concentration of alcohol dehydrogenase in plant roots can indicate the occurrence of oxygen stress, perhaps from overenrichment, within the previous five days.

3.4.6 Vascular Plants as Indicators of Pesticide and Heavy Metal Contamination

Limited data on the effects of pesticides and metals on prairie wetland plants are contained in tables published by Sheehan et al. (1987), and in USEPA's PhytoTox database. A host of factors associated with actual applications influence contaminant toxicity and plant mortality (Doust et al. 1994), and these factors can include:

Environmental Factors: water temperature, organic content, pH, alkalinity, suspended solids.

Dose Factors: concentration, the specific compound or formulation (inert ingredients), frequency of application or exposure, duration of exposure.

Biotic Factors: the plant species, its life stage (season), degree of simultaneous stress from other factors.

Species Composition

Wetland plant species differ in their tolerances of various heavy metals, selenium, and herbicides. Consequently, changes in species composition can indicate past and ongoing incidents of exposure to these contaminants.

Although few studies have compared relative sensitivities of various wetland plants to a particular contaminant, data compiled from many single-species experiments involving heavy metals suggest that emergent plants might be generally more tolerant of heavy metals than submersed plants, which in turn might be more tolerant than algae (Outridge and Noller 1991). Duckweed (*Lemna*) appears to be particularly sensitive to the heavy metals cadmium and nickel, and chromium concentrations of 10 mg/L are inhibitory (Huffman and Allaway 1973). Cat-tail can tolerate lead, copper, and chromium accumulations of at least 10 µg/g (dry weight) of aboveground biomass; zinc accumulations in cat-tail can reach 25 µg/g (dry weight) without apparent ill effects (Mudroch and Capobianco 1979). Common reed can tolerate industrial wastewater with high levels of heavy metals (e.g., up to 250 micrograms/g sediment copper concentrations), as do bulrushes (Seidel 1966). In an Ontario river, submersed species (*Elodea*, *Ceratophyllum*, and *Myriophyllum*) appeared to be less tolerant of industrial wastes than floating-leaved and short, rooted aquatic plants (*Potamogeton*, *Nuphar*, and *Nymphaea*), which were in turn less tolerant than cat-tail and common reed (Dickman et al. 1980, 1983; Dickman 1988).

By knowing the characteristic sensitivities of various plant species, plant community composition can be used cautiously to infer past exposure to particular contaminants. For example, application of one herbicide resulted in reduced dominance of *Phragmites australis* but increased dominance of particular *Lemna*, *Callitriche*, and *Potamogeton* species. Floating-leaved

herbaceous plants are sensitive to the physical effects of oil compounds associated with herbicide applications, and growth of the duckweed *Spirodela oligorhiza* is affected by PCB concentrations of 5 mg/L (Mahanty 1975). Cat-tail can tolerate petroleum oil concentrations of 1 g/L (Merezhko 1973) and, along with common reed (*Phragmites*), appeared to be the most tolerant macrophyte downstream from an industrial effluent source in Ontario (Dickman 1988). Bulrushes are killed by phenol concentrations of 100 mg/L and abnormalities occur at large phenol concentrations, but new shoots form quickly (Seidel 1966).

Species Richness

Species richness of vascular plants in prairie wetlands would be expected to decline in response to atypically high loadings of certain heavy metals and especially to chronic exposure to herbicides. However, documenting data are lacking.

Biomass and Cover Ratio

Herbicides have an obviously direct effect in reducing the areal coverage of selected vascular plants to which they are applied directly, especially early in the growing season. Indirect effects also can occur. Emergent cat-tails can be killed by herbicides applied for control of submersed plants (Newbold et al. 1974). Low concentrations of the popular herbicide, glyphosate sometimes stimulated growth of sago pondweed, *Potamogeton pectinatus*, a major submersed plant in prairie wetlands (Hartman and Martin 1985), but glyphosate is lethal to most emergent wetland plants at typical application levels (Sheehan et al. 1987).

Relatively few chemical assays have been conducted in field mesocosms in the prairie region. The commonly used herbicide, atrazine, has been examined the most. A review of published literature on atrazine effects found a wide range of concentrations (0.050–1.310 mg/L) associated with adverse effects on one prairie wetland species, *Potamogeton perfoliatus* (Hofmann and Winkler 1990, Swanson et al. 1991). The concentration that would be expected to occur immediately after a 0.5-ha prairie wetland is directly sprayed with atrazine would be about 0.413 mg/L (Sheehan et al. 1987), and chronic levels in prairie wetlands appear to be much lower than this (Ruelle and Henry 1993). A concentration of 1 mg/L atrazine caused a 50% decline in biomass of three macrophytes (*Lemna*, *Ceratophyllum*, and *Elodea*) over a 30 day period in a prairie wetland mesocosm (Johnson 1986). The pesticides carbofuran, fonofos, phorate, treflan, and triallate had no statistically significant effect on biomass of the plants mentioned above (Johnson 1986). Effects of herbicides on flowering of plants and storage of energy reserves (as seeds or tubers) has received little study in prairie wetlands, and could have important implications for waterfowl that feed extensively on these items and their associated invertebrates just prior to migration in late summer and early fall. Effects of heavy metals and selenium on prairie wetland plants are mostly unstudied. Selenium is toxic to some wetland plants at concentrations greater than 1.25 mg/L (Ornes et al. 1991).

Bioaccumulation

Wetland plants rapidly take up selenium (Ornes et al. 1991) and bioaccumulate many other heavy metals (Freemark et al. 1990). Cat-tails are among the few plant species that have been analyzed for bioaccumulation of contaminants in prairie wetlands (True and Dornbush 1984).

Biomarkers

Activity of the enzyme, peroxidase, has demonstrated usefulness in aquatic plants as a marker of previous exposure to several metals and organic contaminants (Byl 1994).

3.5 Monitoring Techniques

Many documents provide detailed guidance on sampling herbaceous vegetation (e.g., Higgins et al. 1994). Some address wetlands specifically, for example, Phillips (1959), Schwoerbel (1970), Mueller-Dombois and Ellenberg (1974), Woods (1975), Dennis and Isom (1983), Downing and Anderson (1985), Moore and Chapman (1986), Fredrickson and Reid (1988a), van der Valk (1989). Methods for measuring production of prairie species are described by Neill (1993).

3.5.1 Ground-based Sampling

If wetlands can be sampled only once, mid-growing season is usually the recommended time. However, many plants are apparent and/or identifiable only for a few weeks of the growing season. Thus, if the aim is to estimate annual production or quantify community composition accurately, repetitive visits that account for the diverse phenologies of wetland species should be implemented (Dickerman et al. 1986, Smith and Kadlec 1985). Ideally, annual visits should be timed to coincide with year-specific weather conditions, rather than calendar dates. Trampling of herbaceous vegetation and compaction of saturated soils during even a single site visit can induce community changes detectable in subsequent visits. Thus, field crews should be as small as possible and follow the same path in and out of a wetland. In deeper wetlands, use of underwater SCUBA transects is sometimes appropriate (Schmid 1965). If herbaceous wetland vegetation must be sampled destructively in order to obtain specimens for identification (e.g., in very turbid or deep waters), then equipment such as dredges, oyster tongs, plant grappling hooks, and steel garden rakes can be used (Britton and Greeson 1988). Equipment designed specifically for sampling submersed macrophytes is described by Macan (1949), Woods (1975), Dromgoole and Brown (1976), Satake (1987), and others. However, whenever possible, plants should be identified in the field rather than collected.

3.5.2 Aerial Methods

The EMAP monitoring effort is using low-altitude aerial metric photographs (scale about 1:2400) to measure vegetation cover annually on each of 48 wetlands. Low-altitude imagery has been used successfully in several previous studies in the prairies (Kreil and Crawford 1986, Welling et al. 1988b, van der Valk and Squires 1992, van der Valk et al. 1994) to differentiate emergent plant communities, stem density classes, and in some cases, species. Filters and image-processing techniques can also be used to highlight various spectra, such as those sensitive to chlorophyll-a (Patience and Klemas 1993). Under ideal circumstances, such an approach might be used to indicate the presence, relative biomass, and condition of particular wetland species.

When communities or species can be distinguished reliably from aerial images, the relative spatial extent (percent cover) of the communities or species can be measured more accurately and cost-effectively than from ground transects. In the van der Valk and Squires (1992) study, false infrared imagery from an altitude of 610 m was used. Aerial videography is also being used

more frequently for measuring wetland structure and determining wetland integrity (Cowardin and Sklebar 1993), and can delineate cover types covering as little as 1 m² in some instances (Olson 1992). As demonstrated by Welling et al. (1988b) and described by Caldwell and van der Valk (1989), aerial imagery can be used to make maps which, when overlaid with bathymetric maps derived from field transects, are useful for measuring the depth ranges of various plant species and provide essential information that cannot be derived from quadrat data. Although aircraft are commonly used for obtaining the photographs, tethered balloons with remotely triggered cameras also show some potential for use in monitoring prairie wetlands (Edwards and Brown 1960), and cost about \$20 each, excluding the camera (Davis and Johnson 1991).

3.5.3 Potential or Historical Vegetation

Because the vegetation in prairie wetlands shows such tremendous interannual variability, seeds lying in wetland soils or sediments (the "seed bank") are often sampled in lieu of or in addition to mature plants. The assumption is that, because seeds decompose much more slowly than foliage (over decades rather than months), they indicate conditions not necessarily shown by live vegetation. Similarly, pollen from prairie wetlands remains intact in anaerobic sediments for long periods, and under some circumstances can be identified to species under a microscope, potentially yielding information on the nutrient status and water levels present historically in the wetland (Watts and Bright 1968, Vance and Mattewes 1994).

The simplest and most economical method of analyzing the seed bank is known as the seedling emergence (or seedling assay) method. Procedures are described by van der Valk and Davis (1978) and Galatowitsch and van der Valk (1994). Each sample consists of about 1000 cm² of substrate removed to a depth of 5 cm. Large organic matter (leaves, etc.) is removed, sometimes using a coarse sieve, and the sample is distributed in a shallow (<cm) layer in two pans. In one pan the sediment is kept covered with a centimeter of water, and in the other it is merely kept moist. The pans are kept in a greenhouse or sunlit shelter for the duration of the growing season, or longer in a heated facility if necessary. As seeds sprout, they are removed, enumerated, and identified by comparison with a reference collection of seedlings. An adjacent pan containing sterile soil is also tracked to ensure that seedlings in the other two pans are the result of seeds contained in the sediment, rather than airborne. Results of using this method with samples from prairie wetlands are reported by van der Valk and Davis (1979), Pederson (1981), Poiani and Johnson (1988, 1989), Welling et al. (1988a,b), Merendino and Smith (1991), Galatowitsch (1993 a,b).

The seedling-emergence method requires time to establish a reference collection by taking seeds from known (fully identified) mature plants and growing the seeds to the seedling stage. Because seedlings of many species (e.g., *Carex*, *Cyperus*, *Bidens*) can seldom be identified until they are fully mature, they must at first be tentatively grouped by appearance and counted with representatives of each taxa grown to maturity for identification. The success of the seedling emergence method hinges on the veracity of the assumption that seedling density is proportional to seed density when in fact, there are frequently situations where mature plants are obvious in a wetland but use of the seedling emergence method produces none of their seedlings (van der Valk and Davis 1978; Welling et al. 1988a,b; Wienhold and van der Valk 1989). This situation arises because 1) appropriate incubation conditions are unknown for some species; 2) some wetland plants produce seeds only rarely (they normally reproduce vegetatively); and 3) there is

often considerable inter- and intraspecific variation in seed set, seed viability, and seedling competition. For these reasons, seed bank analysis techniques are inadvisable where the objective is to inventory rare species. In particular, some seedbank assays tend to overestimate annual "weedy" species (those with readily geminable seeds) while underestimating certain species that are especially sensitive to competition and/or which inhabit the drier zones of wetlands (S. Galatowitsch, personal communication, University Minnesota, St. Paul, MN). Nonetheless, other evidence (Poiani and Johnson 1988) suggests that the influence of such variation on the overall ability to characterize a wetland's past or potential vegetation is, at least some of the time, probably minor.

3.5.4 Bioassay Methods

A review of laboratory, outdoor mesocosm, or *in situ* bioassay methods involving vascular plants is beyond the scope of this document. Use of bioassays to explore contaminant toxicity to plants in prairie wetlands has been relatively limited. Examples include studies by Johnson (1986), Wayland and Boag (1990), and Ruelle and Henry (1993). Both mature plants and seeds have been assayed. Test species and protocols for assaying the effects of pesticides or other contaminants on wetland plants are proposed by Freemark et al. (1990), Smith (1991), Swanson et al. (1991), and Doust et al. (1994).

3.5.5 Bioaccumulation

Methods for collecting wetland plants and assessing bioaccumulation of contaminants in plant tissues are described in Moser and Rope (1993b).

3.6 Variability and Reference Points

The following subsections address spatial and temporal variability of vascular plant community composition in prairie wetlands.

3.6.1 Spatial Variability

Spatial variability has been quantified for species richness, biomass and germination rate.

Species Richness

As a point of reference, approximately 922 herbaceous vascular plant species characteristic of pothole or riverine wetlands have been recorded in the North Plains region, which includes the Dakotas and eastern parts of Montana, Wyoming, and Colorado, but not parts of the prairie region in Minnesota and Iowa (Reed 1988). In prairie counties of North Dakota, about 135 (15%) of the North Plains region's wetland species are considered "rare" by the State's Natural Heritage Inventory (Appendix I); this represents 55% of the 245 rare plant species occurring in any habitat in North Dakota. Most of the rare wetland plants are associated with riverine, bog, or forested wetlands rather than with typical prairie pothole basins.

About 191 (21%) of all the North Plains wetland species occur frequently as dominants in prairie pothole wetlands (Appendix A). Among the dominant species are 17 species (9%) officially

considered to be "introduced" (Reed 1988), of which five are annuals. These are: *Acorus calamus*, *Agropyron repens*, *Artemisia biennis*, *Bidens cernua*, *Cirsium arvense*, *Cirsium floodmannii*, *Echinochloa crusgalli*, *Echinochloa muricata*, *Glaux maritima*, *Glyceria maxima*, *Kochia scoparia*, *Lysimachia thyrsoflora*, *Lythrum salicaria*, *Plantago major*, *Sonchus arvensis*, *Spirodela polyrhiza*, and *Stachys palustris*. The total number of annuals that sometimes dominate prairie wetlands is 33 (17% of all dominant species in prairie wetlands) (Appendix A).

In a survey of 112 prairie potholes in southern Manitoba, Pip (1979) found more than 47 vascular plant species. In a subset of 39 of the sites, she found 32 species with a mean of 5.3 species per wetland, and noted that even wetlands that were adjacent seldom had similar floras. In a larger ($n = 177$) set of Manitoba wetlands, she found a mean of 4.9 species per wetland. In a survey of 261 vegetation stands in 82 prairie potholes in northeastern Montana, Lesica (1993) found a total of 173 species, of which 12 were exotics. Among seven wetland complexes ranging in size from 7 to 15 ha, he found between 48 and 74 species per complex, and between 6 and 11 plant communities. Species richness was not strongly correlated with community richness. From five natural wetlands in eastern North Dakota, Kreil and Crawford (1986) reported a total of 64 vascular plant species. From 140 quadrats (each 1.0×0.5 m) in temporary and seasonal wetlands of eastern North Dakota, Hubbard et al. (1988) collectively found 41, 38, 20, and 16 species (on Tetonka, Parnell, Worthing, and Southam soil types, respectively). In part of the Delta Marsh, 65 plant species were found during four growing seasons, although no more than 48 of these were present in any single year (Squires and van der Valk 1992).

In a survey of 20 semipermanent wetlands in prairie regions of Iowa, Galatowitsch (1993 a,b) found a total of 158 species, of which 106 were species that typify wetlands. Collectively, there were 133 species in ten natural wetlands (range 41–63 per wetland, mean = 45.8) and 83 species in ten restored wetlands (range 24–52 per wetland, mean = 26.9). There were 75 species (48 of them "wetland" species) that occurred only in the natural wetlands, and 25 species (10 of them "wetland" species) that occurred only in the restored wetlands; most of the latter species were submersed plants or species planted for erosion control. Between 1 and 22 species were in the driest parts of each wetland, 7 and 49 species in the sedge meadow zone, and 7 and 19 species in the shallow emergent zone. Seedbanks of the natural wetlands contained 15 species, as compared with eight species in the restored wetlands.

Number of species in the Delta Marsh seed bank varied from 8 to 20/m², depending on the zone and depth from which the samples were collected (van der Valk and Davis 1979, van der Valk 1986). Over 90% of the seeds in most seed banks consists of fewer species than this (Wienhold and van der Valk 1989). However, germination of all seed bank samples from the Delta Marsh resulted in a cumulative total of over 40 species. Up to 14 species were found in seed banks in 11 wetlands in Iowa (Wienhold and van der Valk 1989) but in Iowa's Eagle Lake wetlands, van der Valk and Davis (1978) found 45 species. Cumulatively, from a sample of eight smaller Iowa wetlands, van der Valk and Davis (1976) found 29 species. One seedbank survey of 35 Iowa wetlands found an average of 16 species per wetland (Clambey 1975) and another survey of four Iowa wetlands found 24 species per wetland (LaGrange and Dinsmore 1989b).

Within individual semipermanent wetlands, richness is generally less in the wetter marsh zones than in drier meadow zones upslope (Nelson and Anderson 1983). From a survey of 246 stands of wetland vegetation in southern Saskatchewan, Walker and Coupland (1970) found the

greatest richness in the wet meadow and marsh meadow portions of wetlands that were slightly saline and lightly grazed and mowed (43 species in nine wet meadow stands, 37 species in seven marsh meadow stands). Across a gradient of moisture, salinity, and disturbance from grazing and mowing, the stands that had the most unique floras were those that were the wettest, the most saline, and the most disturbed.

Biomass, Density, Cover Ratio

Biomass also varies greatly by species and spatially. In the Canadian prairies, aboveground biomass varied from 425 g/m² for one emergent species (*Scirpus lacustris*) to 1750 g/m² for another (*Typha latifolia*) (Shay and Shay 1986). For just a single species (cat-tail), aboveground biomass among several prairie wetlands can range 0 to 2106 g/m² (van der Valk and Davis 1978, Shay and Shay 1986), and up to 2400 g/m² under conditions of artificial enrichment (Neill 1990). Among seven communities of emergent wetland vegetation in eastern North Dakota, the net production was reported to range from 0.30 to 0.97 g/m²/day (Hadley and Buccos 1967). Submersed and floating-leaved species tend to be less productive than emergent species.

In describing their seed bank analyses, van der Valk and Davis (1978) noted, "Among replicate samples within a vegetation type, there [is] a great deal of variation in the number of individuals of a given species ... standard deviations are larger than the means in many cases." Moreover, some of the species currently present in a wetland will be absent from seed banks, whereas others may have seed densities in the sediment of several thousand per square meter. In the Delta Marsh, total seed densities in 250 sediment samples averaged 4582/m², and ranged from 140/m² in open water areas, to 2230/m² in common reed stands and 5810/m² in cat-tail stands (Pederson 1981). However, van der Valk and Davis (1979) found the number of viable seeds in the upper 5 cm of the Delta Marsh seed bank to vary from 7363 to 56,289/m², depending on the zone and sediment depth from which the samples were collected (analyses of cores extending from the surface down to 35 cm revealed a maximum of 255,000 seeds/m²). In a North Dakota wetland, seed densities across wetland zones varied by a factor of about 8; a maximum of 9370/m² was found (Poiani and Johnson 1989). In Iowa 10 natural (undrained, seasonally or semipermanently flooded) wetlands had an average seed density of 7369/m² whereas 10 nearby restored wetlands (previously drained, now permanently flooded) wetlands had an average of 3019/m² (Galatowitsch 1993 a,b). In contrast, in Minnesota a series of 30 undrained wetlands had an average seed density of 3600/m², whereas five recently drained wetlands had an average as high as 8000/m² (Wienhold and van der Valk 1989).

Cover ratio varies tremendously among prairie wetlands, but few estimates of spatial variability are available.

Germination Rate

Even within a single species, germination rates can vary considerably, e.g., 14%–51% for cat-tail (Weller 1975).

3.6.2 Temporal Variability

Species Richness

Over a 7-year period, species richness of plants in a single, 1.7-ha, semipermanent wetland in Iowa varied from 7 to 19 species per year (Weller and Voigts 1983). Only one species was present all 7 years of the wet-dry cycle; two of 23 common species occurred during only 1 or 2 years. Over an 85-year period, vascular plant richness in an Iowa prairie lake varied from about 11 to 29 taxa (Niemeier and Hubert 1986). Apparently only one of 52 species was found throughout the period.

Biomass and Cover Ratio

Over a 5-year period, the biomass of one plant species (*Scirpus validus*) in an Iowa wetland varied from 0 to 486 g/m²; that of another (*Sparganium eurycarpum*) varied from 271 to 543 g/m²; and that of a third (*Typha glauca*) varied from 772 to 1075 g/m² (van der Valk and Davis 1980). Within a single year, the aboveground biomass of *Carex rostrata* in a single Minnesota wetland varied from 114 to 852 g/m², whereas the belowground biomass varied from 150 g/m² to 328 g/m². Production peaked at 11 g·(m²)⁻¹·da⁻¹ (Bernard 1974). High interannual variation in the cover ratio typifies prairie wetlands. In a 10-year study of 71 Manitoba wetlands, Millar (1973) found that areal cover changed in 32 (46%) of the wetlands, and of these, a complete conversion to open water occurred in 17 wetlands. In a North Dakota prairie wetland, seed densities in sediments varied from 2840/m² one year to 9370/m² the next (Poiani and Johnson 1989).

3.7 Collection of Ancillary Data

It is easier to separate the anthropogenic from the natural causes of impairment of community structure if data are collected or inferred simultaneously on the following variables of particular importance to vascular plants:

- age of wetland and its successional status
- light penetration (particularly for submersed species)
- water or saturation depth
- conductivity and general chemistry of waters and sediments
- abundance of herbivores (particularly muskrat, geese, grazing cattle, crayfish)
- sediment type
- duration, frequency, and seasonal timing of regular inundation
- time elapsed since the last severe inundation, drought, or fire.

All of these features vary to a large degree naturally as well as in response to human activities such as soil tillage, compaction, and erosion; fertilizer and pesticide application; introduction of exotic species; and water regime modification.

3.8 Sampling Design and Required Level of Sampling Effort

If the sole objective is to inventory the presence or absence of plant species within wetlands, then a timed search method that covers all the obviously recognizable zones may be appropriate (e.g., Pip 1979, 1987a,b,c), but if vegetative cover and dominance are to be quantified, decisions must be made concerning the physical layout of transects and/or quadrats within a wetland. This task can pose a particular challenge if the objective is to characterize a wetland as a whole unit rather than describe ecological relationships within just one zone or biological community. Choices involve deciding whether to place quadrats in a purely random manner, or randomly along transects, or at regular intervals along transects, or randomly/regularly within delimited vegetation zones. If transects are used, there are further choices regarding whether to locate the transects randomly (van der Valk and Davis 1978, Gurney and Robinson 1988, LaGrange and Dinsmore 1989b), evenly (gridded), or in relation to vegetation zones. Statistical methods are available for defining zones somewhat objectively as demonstrated in prairie wetlands by Johnson et al. (1987). The alignment of transects is usually perpendicular to shore (Wienhold and van der Valk 1989), but can be parallel to the long axis of the wetland (Niemeier and Hubert 1986), or follow the four compass axes (e.g., Poiani and Johnson 1988). When transects are used, vegetation can be enumerated using point counts (plants intercepted by the transect at specified intervals are counted), intervals (simple presence/absence of plants intercepted by the transect at specified intervals is noted), or intercepts (all plants intercepted are counted). Based on data from one prairie wetland, Weller and Voigts (1983) concluded that the intercepts method was least cost-effective, and the other two methods gave similar results. The EMAP effort has characterized species composition, relative dominance, and richness of prairie wetlands by randomly locating five 0.25-m² quadrats within each vegetation zone of each wetland while walking through the center of the zone parallel to its long axis. The rationale given for choice of this method is that vegetation in prairie wetlands tends to occur as concentric zones, which would not be well-sampled by transects perpendicular to the shore or center of the wetland. Species not found by this method, but observed while walking between quadrats, are also recorded.

3.8.1 General Considerations

To characterize vegetation at sampling points, investigators (e.g., Wienhold and van der Valk 1989) have used the Relève method (Mueller-Dombois and Ellenberg 1974), the Daubenmire approach (Daubenmire 1959), or the Braun-Blanquet (1932) approach (e.g., LaGrange and Dinsmore 1989a). In some situations the midpoint values of cover classes can be averaged to obtain mean cover values for each species within a zone (vegetation community), and these values can be weighted by area to obtain total cover values for species within zones.

If the sole purpose is to assess the habitat value of vegetation as shelter for wildlife (i.e., cover of individual species does not need to be determined), then visual obstruction readings (Robel et al. 1970) and profile board methods (Jones 1968) can be used (Duebber and Lokemoen 1976, Nudds 1982, Higgins 1986, Barker et al. 1990). Cover estimates can be made once at the peak

of vegetation development and/or during bird nesting, but additional estimates may be desirable earlier in springtime (to estimate residual matter) and at other dates if livestock are grazing the wetland. Cover estimations made by the EMAP effort featured the Daubenmire approach; cover was estimated as the portion of water surface (for emergents) or bottom (for submersed plants) shaded by each species.

One factor that affects sampling costs is the desired level of taxonomic identification. Identifying plants to the species level usually allows investigators to make more refined statements about the condition of a wetland, but this identification can increase the required time and requires them to have advanced training and experience. There are no data to indicate whether, and under what conditions, identification of plants only to the genus or family level would be sufficient to define the ecological integrity of a prairie wetland.

Sampling costs are determined not only by the time required to identify plants, but also by the number of quadrats examined. This number should depend on expected variability (coefficient of variation), wetland size, and the desired precision. Larger wetlands require more transects or quadrats, usually spaced farther apart, to accurately characterize overall community composition. More linear wetlands (e.g., narrow fringe marshes along lakes) usually require more tightly spaced sampling points, as do ecotone areas along transects. In a Wisconsin lake, the number of samples needed to adequately quantify the biomass of the entire submersed plant community within various plant community zones, given a goal of maintaining a probability of 95% of being within 15% of the mean, ranged from seven to 200, depending on the zone (Nichols 1984).

3.8.2 Asymptotic Richness: Results of Analyses

If the goal is not just to quantify species richness within samples but for the whole wetland (or complex), then considerably more samples are required. The number will be determined not through examination of coefficients of variation but by plotting species accumulation curves (the cumulative number of species vs. number of samples, or vs. wetland area). Based on their Saskatchewan wetland data, Walker and Coupland (1968) reported that up to 30 quadrats (0.5 m × 0.5 m) per vegetation zone were necessary to characterize the species composition of the zone, i.e., to level off the species accumulation curve. Apparently no similar analyses have been prepared for other prairie wetlands.

For this document, we analyzed plant taxonomic richness from two data sets from prairie wetlands. The database descriptions that follow are generalized. For a detailed description of monitoring design and data structure of each data set, see Appendix L. One was from quadrats along transects in a series of 20 semipermanent wetlands in Iowa (Galatowitsch 1993 a,b). Data from the quadrats and transects were pooled into a single list for each wetland. Our calculations of asymptotic richness indicated that, for example, only 11 wetlands would need to be sampled to capture 99% of the taxa present in all 20 wetlands (Appendix O). The statistical approach used to determine this was described in Section 1.5.

The 20 Iowa wetlands consisted of 10 restored and 10 natural wetlands. When the restored wetlands were compared with the natural wetlands, restored wetlands were found to accumulate taxa at a slightly more rapid rate. This was likely due to the greater homogeneity of species composition among the restored wetlands. Thus, for monitoring designs and conditions similar to

those of Galatowitsch (1993 a,b), restored wetlands would not have to be sampled quite as extensively as natural wetlands. About eight wetlands would be a sufficient number to detect 90% of the plants in the combined population of 20 wetlands.

The other data set consisted of vegetation quadrats from the Marsh Ecology Research Program (MERP) experimental units of Delta Marsh, Manitoba (Squires 1991). Data involving multiple quadrat collections per year were combined from two wetlands to create a single taxa list for each of four treatments:

DD1LOW: One year of drawdown, followed by reflooding to the original depth ($n = 385$ quadrats covering four post-treatment years)

DD2LOW: Two years of drawdown, followed by reflooding to the original depth ($n = 362$ quadrats covering four post-treatment years)

DD2MED: Two years of drawdown, followed by reflooding to an intermediate depth ($n = 387$ quadrats covering four post-treatment years)

DD2HIGH: Two years of drawdown, followed by reflooding to a high (deep) depth ($n = 351$ quadrats covering four post-treatment years).

Our calculations of asymptotic richness revealed the following ordering of species accumulation rates by treatment (Appendix O):

DD2MED (fastest) > DD1LOW > DD2HIGH > DD2 LOW.

The differences in accumulation rate among treatments were not great, and the results suggest species composition was most homogeneous among the quadrats in the DD2MED treatment. In most cases an average of about 37 quadrats was adequate to detect 99% of the taxa that were cumulatively present in the full 351–387 quadrats, although for any particular treatment, as few as 20 and as many as 40 quadrats could be needed, depending on the sequence in which the quadrats are collected. Had all 3281 quadrats been considered regardless of treatment (a situation that more closely approximates sampling of natural wetlands with varied water regimes), about 448 quadrats would be needed to detect 99% of the species found in all 3281 quadrats.

3.8.3 Power of Detection: Results of Analyses

The Components of Variance approach, as described in Section 1.5, was applied to just the Squires data set. As tabulated in Appendix M, the mean species richness per quadrat levels off at a sample size of about 20 quadrats in wetland situations and monitoring designs similar to those of Squires. To distinguish interannual (or between-wetland) changes of (for example) two plant species, data from a total of between 9 (minimal) and 12 (maximum) quadrats would need to be collected. Conversely, if budget or other considerations limited sample size to five quadrats per wetland, then one would be able to detect a mean difference of 2.9 to 3.6 species between wetlands or years. In both instances, we are assuming there is an 80% certainty of being correct at the 5% level. From the same data set, if the objective is to estimate mean seedling density per quadrat, then more than about 12 quadrats are needed in wetland situations and monitoring designs similar to those of Squires. To distinguish interannual (or between-wetland) changes in seedling density of (say) 60 seedlings per quadrat, one would need to

sample using only about four quadrats, but to detect a change of only 20 seedlings one would need to use 25–30 quadrats.

3.9 Summary

The species composition of vascular plant communities, and to a lesser degree their species richness, can indicate changes in prairie wetland salinity, water regime, and (among submersed species) sedimentation or turbidity (Table 3). Thresholds for responses are well-documented with extensive published field data from the region. Vascular plants also respond sensitively to changing nutrient levels, grazing, and presence of some contaminants, but existing information is too limited and confounding effects are too prevalent to currently allow widespread use of vascular plants to diagnose impairment of prairie wetlands from these stressors.

Vascular plant communities are exceptionally valuable indicators of conditions in individual prairie wetlands because they are immobile and because they integrate stresses that have occurred intermittently or chronically over months and years. This is especially true when wetland seed banks can be analyzed. Analysis of seed banks, although time-consuming and containing some biases, also can provide one piece of data useful towards defining appropriate "reference" conditions for a wetland resource. Compared with use of other indicators, monitoring of plant species composition causes minimal disturbance, involves (for most taxa) relatively simple identification procedures, and does not require frequent sampling or tight scheduling within a season. Where access to private property is restricted, the gross spatial patterns of vascular plants within a wetland can be characterized using widely available aerial photography, and then used cautiously to infer wetland ecological condition.

Individual prairie wetlands generally contain about 40–60 species of vascular plants, whereas the cumulative total of wetland plant species in the prairie region exceeds 900. There are relatively few quantified, published estimates of interwetland and interannual variability of vascular plant richness and biomass in prairie wetlands. Plant species composition varies considerably among otherwise apparently similar wetlands and varies among years as well according to a distinctive wet-dry cycle.

Additional research is needed to improve the potential for using vascular plants as indicators of prairie wetland condition. Descriptive investigations of life histories of many species are needed so that the daunting number of species can be grouped into fewer functional assemblages (such as defined by van der Valk 1981 or Boutin and Keddy 1993). Responses to various stressors of such streamlined groupings can then be investigated more efficiently (and the results can be generalized more accurately) than if responses of purported indicator species were the sole focus of research and extrapolation. Research is also particularly needed to document the threshold responses of different vascular plant seeds, seedlings, and mature plants to sedimentation.

Table 3. Summary evaluations of vascular plant indicators of stressors in prairie wetlands. Evaluations are based on technical considerations, not cost or practicality. A rating of FAIR or POOR is assigned when too few data (FD) suggest potential as an indicator, or when confounding effects (CE) of other variables often overshadow the effects of the listed stressor on the indicator.

Stressors	Possible Indicators	Evaluation
Hydrologic stressors	Species composition Community zone locations Richness (mature plants) Richness (seed banks) Biomass, cover ratio Seed density Germination rate	GOOD GOOD FAIR (CE) FAIR (CE) GOOD FAIR (CE) POOR (CE)
Changes in vegetative cover conditions	Species composition Community zone locations Richness Biomass, cover ratio	GOOD GOOD POOR (CE) GOOD
Salinity	Species composition Richness Biomass, cover ratio Germination rate Biomarkers	GOOD FAIR (CE) FAIR (FD) FAIR (CE) FAIR (FD)
Sedimentation & turbidity	Species composition Richness Biomass, cover ratio Germination rate	GOOD POOR (FD) FAIR (CE) POOR (CE)
Excessive nutrients & anoxia	Species composition Richness Biomass, cover ratio Biomarkers	GOOD POOR (CE) FAIR (CE) POOR (CE, FD)
Herbicides	Species composition Richness Density, biomass, productivity Decomposition	FAIR (FD) POOR (FD) FAIR (CE) POOR (FD)
Insecticides	Species composition Richness Density, biomass, productivity Decomposition	POOR POOR POOR POOR
Heavy metals	Species composition Richness Density, biomass, productivity Decomposition	FAIR (CE) POOR (FD) POOR (FD) POOR (FD)

4. Invertebrates as Indicators of Prairie Wetland Integrity

4.1 Ecological Significance and Suitability as an Indicator

Invertebrates include aquatic insects, freshwater crustaceans (e.g., amphipods, crayfish), aquatic annelids (worms), zooplankton, and immature stages of certain terrestrial insects (e.g., Lepidoptera) that occur mainly in wetlands. The term "macroinvertebrate" or "macrofauna" refers to the larger organisms clearly visible to the unaided eye, as opposed to microinvertebrates, which include most smaller zooplankton, such as rotifers. Although invertebrates occur in wetlands everywhere, prairie wetlands support notably great numbers. This is because prairie wetlands have especially rich soils, slow water turnover times, and seasonally fluctuating water tables, all of which support the high levels of algal production and spatially complex vegetative stands that are important to invertebrates.

Invertebrates are the vital link that makes algal production and emergent plant material available as an energy source for waterbirds and other animals. Invertebrates do this by consuming algae and decaying plant material and then by being consumed by higher order animals (Driver et al. 1974). Invertebrates represent grazing, filtering, detritivore, and predator trophic pathways of energy flow, and thus should reflect the status of these fundamental processes in a wetland. Planktonic invertebrates (e.g., cladocerans) are potentially able to consume more than an entire day's production of algae (Porter 1977). In doing so, they considerably improve and maintain light penetration of the water column during the growing season. This in turn gives submersed aquatic plants a chance to flourish (Hanson and Butler 1990), and these macrophytes serve as a substrate that supports an even greater density of invertebrates, as well as a food source for many organisms.

In some cases, waterbirds appear to select wetlands having the greatest densities of invertebrates (Talent et al. 1982). Even where they do not, waterbirds spend more time foraging in wetlands that have greater abundance of macroinvertebrates (Kaminski and Prince 1981a,b, 1984). Whereas larvae are eaten mainly by ducks, emerging insects are consumed by many songbird species. The nutritional requirements of growing ducklings and breeding hens can be fulfilled only by an invertebrate diet (Swanson et al. 1974, 1977). However, the degree to which food supply—as opposed to vegetative cover and predation—truly limits the breeding and reproductive success of waterfowl populations at a regional scale is unknown. The variety (species richness) of invertebrates might be at least as important as the quantity because waterfowl require or use different invertebrate species from different parts of the wetland at different seasons (Swanson and Meyer 1973, Kaminski and Prince 1981a,b, 1984). Invertebrate richness supports elevated waterbird richness because different waterbirds use different invertebrate assemblages. If changes in hydrologic regime or turbidity cause changes in a key habitat component of invertebrates (e.g., submersed plants), the invertebrate species associated with that habitat could be reduced or eliminated from the wetland, even if the wetland remains well-vegetated with other types of plants. If the affected invertebrates are critical to waterbirds, waterbird productivity could suffer.

Soil macroinvertebrates (especially earthworms and certain midge larvae) also dominate the diet of several shorebird species that stop to feed in prairie wetlands during migration. Yet, soil invertebrates have seldom been monitored in temporary and seasonal wetlands, especially

during portions of the growing season when surface water is lacking. Nematodes are one abundant, diverse, and sensitive invertebrate assemblage that has been found by many European studies to be a useful indicator of soil condition (Bongers 1990, Freckman and Ettema 1993), and might find similar application here. Quantitative sampling methods are relatively well-developed (Schouten and Arp 1991), and additional research could document the relative importance of nematodes to ecosystem processes.

Invertebrates are also important because they influence the amount of contaminants that are available to other components of the food web, and the rate of contaminant cycling across several ecotones (e.g., sediment-water column, wetland-upland). In the sediment, burrowing invertebrates can make more generally available the nutrients (or contaminants) contained in decaying plant roots. Nutrients released to the water column by invertebrates help sustain algal productivity.

Several characteristics of invertebrates usually considered advantages for monitoring ecosystem integrity (Adamus and Brandt 1990) include:

- documented, characteristic responses to all major wetland stressors (hydroperiod, sediment, nutrients, contaminants); many "indicator taxa" identified
- varied larval lifespans, ranging from short (e.g., cladocerans) to long (e.g., dragonflies), allowing use of invertebrates as indicators of both chronic and acute disturbances
- noninsect invertebrates reflect the quality of the wetland itself (they usually complete their entire life cycle within a single wetland)
- invertebrates can be confined for whole-effluent bioassays or *in situ* assessments
- decay-resistant remains (e.g., shells) provide a means for establishing historical reference conditions in a wetland
- can be sampled with inexpensive equipment
- invertebrate sampling protocols available from USEPA.

Certain characteristics of invertebrates usually considered disadvantages for monitoring wetland integrity include:

- requires excessive time to adequately isolate organisms from debris in many types of samples
- laborious identification beyond the family level
- difficulty in interpreting the occurrence of a particular species in an individual prairie pothole wetland (it is unknown whether occurrence is related to conditions within the wetland, proximity to sources of effective colonizers, or ephemeral conditions such as

favorable winds that facilitated colonization; this is true mostly of insects and is less true of invertebrates that do not emerge from the wetland as adults)

- difficulty in measuring precisely the true densities in dense stands of vegetation
- requires many techniques and sampling tools to sample all important invertebrate assemblages present in a wetland.

4.2 Potential Indicator Metrics

When applied to invertebrate communities, the following measurements and metrics can be used to characterize conditions in reference wetlands, identify the relative degree of past disturbance of a prairie wetland, or assess the current inhibition of key processes:

- richness of species and functional groups (per unit of area or volume, or per a specified number of randomly chosen individuals)
- number and biomass of individuals per unit of area or volume
- relative dominance and richness of species reputedly tolerant to a named stressor
- interannual variability in richness, density, and/or biomass
- homogeneity of size or biomass classes within a species population
- levels of tissue contaminants (biaccumulation)
- density of dormant but viable life stages.

The specific ways some of these metrics have been or could be interpreted as an indication of stressed conditions are described in Section 4.4.1. However, apparently no studies in prairie wetlands have systematically examined correlations among these metrics or their merits relative to one another. A few studies of this type that have been completed in streams (Barbour et al. 1992, Kerans et al. 1992, Resh and Jackson 1993, Niemi et al. 1993) might be used as a model.

4.3 Previous and Ongoing Monitoring in the Region

Aquatic invertebrates have been the focus of at least 35 published studies, covering over 200 prairie wetlands (Appendix J). All of these studies measured numbers of individuals (or density) and at least four measured biomass. Apparently only Duffy and Birkelo (1993) have attempted to measure annual production. In most studies the invertebrates were identified only to family. Seven studies have sampled a wetland for more than 2 years, and only one-third of the studies involved sampling of more than five wetlands.

Montana's water quality agency (Department of Health and Environmental Sciences) is currently using benthic (bottom-dwelling) invertebrates on a trial basis as an indicator of the condition of five prairie wetlands. USEPA's EMAP has not yet undertaken monitoring of prairie wetland

invertebrates at a regional scale, but EMAP has investigated various sampling methods in dozens of North Dakota wetlands. Variables that are being estimated include species richness and relative abundance. At a localized level, invertebrates have been used as possible indicators of the success of wetland restoration efforts in Iowa (Hemesath 1993) and Minnesota (Sewell 1989, Sewell and Higgins 1991). Research on ecological relationships of invertebrates, especially as affecting waterfowl, continues to be conducted by scientists at the NPSC, by the Minnesota Department of Natural Resources, and by universities.

To draw conclusions about wetland integrity from samples of invertebrates, it is essential to have species-specific information on their tolerances and life histories. Appendix B indexes invertebrate taxa according to water regime, and more detailed databases of this type have been assembled by Euliss (personal communication, NPSC, Jamestown, ND). Also, a database that classifies prairie wetland invertebrates by feeding type and waterfowl food importance is maintained at North Dakota State University (Overland et al. 1993). The recent book by Rosenberg and Resh (1993) categorizes over 200 invertebrate species, many of them prairie wetland species, according to their tolerances to acidic conditions and organic pollution.

4.4 Response to Stressors

The following subsections describe responses of the invertebrate communities to hydrologic stressors, vegetative cover conditions, salinity, sedimentation/turbidity, excessive nutrient loads/anoxia, and pesticide and heavy metal contamination.

4.4.1 Invertebrates as Indicators of Hydrologic Stressors

Species Composition

The usefulness of species composition for inferring hydrologic conditions of prairie wetlands has been demonstrated with midges (Driver 1977, Euliss et al. 1993), water beetles (Hanson and Swanson 1989), and macroinvertebrates generally (Neckles et al. 1990, Bataille and Baldassarre 1993). Species composition can indicate how long and in what seasons a wetland has contained surface water. This requires that each species first be classified as to its hydrological requirements, a relatively simple procedure using life history categories such as defined by Hartland-Rowe (1966), McLachlan (1970, 1975, 1985), Wiggins et al. (1980), Jeffries (1989), and Eyre et al. (1991). Appendix B contains such information for dominant prairie wetland invertebrates.

In general, prairie wetlands can be cautiously deduced to be of greater hydrologic permanence when they contain a higher density and richness of longer-lived and/or relatively immobile species (e.g., snails, mollusks, amphipods, worms, leeches, crayfish), as compared with the density and richness of short-lived species (e.g., anostracans, conchostracans), species that survive the winter as drought-resistant eggs (e.g., *Daphnia*), and/or species that are relatively mobile (e.g., chironomid midges). This is probably due to the likelihood that drought and drawdown render the less mobile species more vulnerable to predation, as well as causing their direct loss because of desiccation and saline toxicity. From season-long, weekly activity-trap sampling of three pothole wetlands 80 miles west of the Delta Marsh, Bataille and Baldassarre (1993) found that a permanent wetland was dominated by cladocerans, a semipermanent

wetland by ostracods, and a seasonal wetland by copepods. Considering just the emerging aquatic insect component, the permanent wetland was dominated by midges; the semipermanent wetland by water beetles (early season) and midges and other fly species (mid- and late-season); and the seasonal wetland by midges (mid-season) and other fly species (late season).

Some evidence (Neckles et al. 1990), however, suggests that wetland water regime in particular situations has little effect on the dominance of several major taxa that characteristically overwinter as adults or larvae (species of Dytiscidae, Corixidae, Ceratopogonidae, Ephydriidae, and some Chironomidae). Caution is required in interpreting data because anecdotal evidence suggests that some species with supposedly minimal dispersal abilities are frequently carried passively into new areas by mobile waterbirds (Swanson 1984).

A shift from herbivorous to detritivorous species of macroinvertebrates, and in the ratio of open-water forms (e.g., zooplankton, water striders) to forms that characteristically dwell in vegetation (e.g., some mayflies), can suggest that a prairie wetland has recently undergone inundation (Murkin and Kadlec 1986 a,b; Murkin et al. 1991). In particular, densities of non-predatory midges (Chironomidae) increase greatly during the first year after flooding, and within this family, species characterized by the greatest tolerance for low oxygen levels increase the most (Murkin and Kadlec 1986b). Densities of swimming (nektonic) and bottom-dwelling (benthic) predatory invertebrates do not increase with flooding as much as do numbers of nektonic and benthic herbivores and detritivores. Predatory species can even decrease after flooding (Murkin et al. 1991), and they often increase as drought or drawdown progresses (Table 4).

Long-term changes in wetland hydrology might be inferred by collecting decay-resistant remains of invertebrates from sediment cores or settling traps, and determining if the species present are ones that occur mostly in semipermanent, seasonal, or temporary wetlands.

Species Richness

Data from North Dakota indicate that even the wetlands that are flooded only temporarily have many more species than non-wetland areas (Euliss et al. 1993). Within wetlands, flooding can increase invertebrate richness somewhat, but perhaps only during the initial year of flooding (Table 5). For example, flooding of Manitoba marshes containing cat-tail, hardstem bulrush, and common reed to a level 1 m above normal increased the variety of both nektonic and benthic invertebrates in vegetation but not in open water (Murkin et al. 1991). The increase in benthic taxa persisted for only a short period after flooding (Murkin and Kadlec 1986b). A similar pattern was noted for midge diversity in other Manitoba wetlands by Driver (1977). When inundation persists for years with little fluctuation in water level, sediments often become anoxic and light deficits can reduce the amount and variety of aquatic plants available as invertebrate habitats, thus reducing invertebrate richness (Neckles et al. 1990). In drained wetlands whose water regime is restored, richness increases during the first few years following restoration (Nilsson and Danell 1981, Hemesath 1991).

Table 4. Response of nektonic and benthic invertebrate herbivores/detritivores and predators to water level manipulations in the Delta Marsh.

Unflooded = the normal water level of the experimental wetland. Flooded = the water level in an otherwise similar wetland that had been raised about 1 m above normal at the beginning of the growing season and maintained at that level throughout the season. The invertebrate response was measured at the beginning of the growing season 1 year after the water level had been raised in the flooded wetland (Murkin and Kadlec 1986b, Neckles et al. 1990, Murkin et al. 1991, 1992).

Invertebrate Component and Its Response			
Habitat	Condition	Nekton (swimming invertebrates)	Benthos (bottom dwelling invertebrates)
Open water	Unflooded	Herbivores/detritivores surpass predators.	Herbivore/detritivore numbers are about equal to those of predators.
	Flooded	Herbivores/detritivores greater than in unflooded habitat.	Predators and herbivores/detritivores are about equal, and both are about equal to their numbers in unflooded habitat.
Emergent vegetation	Unflooded	Predators surpass or equal herbivores/detritivores.	Herbivore/detritivore numbers are about equal to those of predators.
	Flooded	Predator and herbivore/detritivore numbers are about equal to their numbers in unflooded habitat.	Numbers of herbivores/detritivores are greater than in unflooded habitat.

Table 5. Response of taxonomic richness of nektonic and benthic invertebrates to water level manipulations in the Delta Marsh.

Unflooded = the normal water level of the experimental wetland. Flooded = the water level in an otherwise similar wetland that had been raised about 1 m above normal at the beginning of the growing season and maintained at that level throughout the season. The invertebrate response was measured at the beginning of the growing season 1 year after the water level had been raised in the flooded wetland (Murkin and Kadlec 1986b, Neckles et al. 1990, Murkin et al. 1991, 1992).

Invertebrate Component and Its Response		
Condition	Nekton: Taxonomic Richness	Benthos: Taxonomic Richness
Unflooded	About equal in open water habitats to levels in emergent vegetation.	About equal in open water habitats to levels in emergent vegetation.
Flooded	About equal in flooded open water habitat to levels in unflooded open water, but in flooded emergent vegetation is greater than in unflooded emergent vegetation.	About equal in flooded open water to levels in unflooded open water, but in flooded emergent vegetation is greater than in unflooded emergent vegetation (except for cat-tail stands of emergents, where richness is unchanged from unflooded condition).

Species richness of midges tends to be greater in wetlands having longer durations of standing water during the growing season (Driver 1977, Nelson and Butler 1987). This is partly because wetlands with longer hydroperiods generally are deeper and more likely to contain submersed and floating-leaved plants that diversify the range of habitats available to this assemblage of invertebrates. Also, wetlands with longer durations of flooding are less likely to experience deep freezing of sediments and types of human activities (e.g., soil compaction, cultivation) that reduce habitat quality for invertebrates (Swanson et al. 1974). However, in a study of five wetlands in the Cottonwood Lakes area, 47 species of water beetle were found in seasonal wetlands whereas 38 were found in semipermanent wetlands (Hanson and Swanson 1989). The seasonal wetlands had 18 exclusive species whereas the semipermanent wetlands had only 11.

Density and Biomass

Flooding generally increases invertebrate densities in wetlands, but perhaps only for about a year after initiation of flooding (Table 6). For example, flooding of Manitoba marshes containing cattail, hardstem bulrush, and common reed to a level 1 m above normal caused a major year-long increase in numbers of nektonic invertebrates in both the vegetation and in open water areas. Biomass of nektonic invertebrates increased only in the vegetated areas. Densities of benthic invertebrates increased in flooded vegetation but not in open areas. On a year-round basis, invertebrate biomass and production is probably greatest in semipermanent wetlands (Nelson 1983, 1989, Bataille and Baldassarre 1993, Duffy and Birkelo 1993), but sometimes can reach greater seasonal peaks in temporary and permanent wetlands.

It has been suggested that the density and viability of dormant stages of some invertebrates could be used to determine in advance whether (and how rapidly) the restoration of a drained wetland will restore its functional characteristics, e.g., part of its invertebrate community (N. Euliss, personal communication, NPSC, Jamestown, ND). The eggs or other dormant stages of several invertebrate taxa—notably the cladocerans, anostracans, and conchostracans—may hatch in response to certain conditions during an 8-week incubation in laboratory aquaria. If incubated sediment samples from a farmed wetland fail to produce such hatchings, it could be assumed that degradation has been so severe as to make full functional restoration impractical, just as lack of seed viability of seed banks is sometimes interpreted.

4.4.2 Invertebrates as Indicators of Changes in Vegetative Cover

Species Composition

An increase in the ratio of algae-consuming species (e.g., certain mayflies) to detritivorous species (e.g., certain worms, isopods, amphipods) and in the ratio of open-water forms (e.g., zooplankton, water striders, midges) to vegetation-dwelling forms (e.g., amphipods, snails, some mayflies) is expected if a wetland has been exposed to herbicides, grazing, fire, flooding, or other vegetation removal processes. This is because such disturbances, by thinning the canopy of emergent plants, create open water areas where algae and submersed macrophytes proliferate (Overland et al. 1993).

Data from the Delta Marsh (Murkin et al. 1991) suggest that the ratio of predatory to herbivorous-detritivorous invertebrates might be used to indicate changes in cover conditions. Predatory

Table 6. Response of density and biomass of nektonic and benthic invertebrates to water level manipulations in in the Delta Marsh.

Unflooded = the normal water level of the experimental wetland. Flooded = the water level in an otherwise similar wetland that had been raised about 1 m above normal at the beginning of the growing season and maintained at that level throughout the season. The invertebrate response was measured at the beginning of the growing season 1 year after the water level had been raised in the flooded wetland (Murkin and Kadlec 1986b, Neckles et al. 1990, Murkin et al. 1991, 1992).

Season	Condition	Invertebrate Component and Its Response	
		Nekton: Density and Biomass	Benthos: Density and Biomass
Spring	Unflooded	Greater in emergent than open water habitats.	About equal in emergent and open water habitats.
Spring	Flooded	Greater in emergent than open water habitats. Density in both habitats is greater than it is in these habitats in unflooded wetland, but only density (not biomass) is greater in flooded open water than in unflooded open water.	Greater in emergent than in open water habitats, and in emergents is also greater than in unflooded condition. Density and biomass in flooded open water are no greater than in unflooded open water.
Summer	Unflooded	Greater in open water than emergent habitat.	About equal to levels in open water and emergent habitat.
Summer	Flooded (year 1)	Mostly greater than in unflooded condition in both habitats, but biomass in flooded open water differs little from biomass in unflooded open water.	Greater in emergent than in open water habitats.
Summer	Flooded (year 2)	In emergent habitat, a decline as compared to levels in this habitat the first year after flooding. Decline results in levels similar to those in unflooded emergent habitat (except in stands of <i>Sclochloa</i>).	Increased densities continue from first post-flooding year in some emergent habitat (common reed and hardstem bulrush), but density changes only slightly in open water habitat.

species tend to dwell in emergent vegetation, but invade open water areas to some degree in midsummer as submersed and floating-leaved plants develop. At that time, densities of predatory species are similar to their densities in emergent stands in the springtime. Field studies that have examined invertebrate responses to changes in vegetation cover are indexed in Appendix F. Field studies that have examined invertebrate responses to changes in water regime are indexed in Appendix G.

Species Richness

High invertebrate richness in prairie wetlands is associated with presence of vegetation. Beyond some point, however, vegetation stands can become so dense that invertebrate richness declines (e.g., Broschart and Linder 1986, Kaminski and Prince 1981a,b), perhaps because of developing anoxic conditions.

The variety of invertebrate families, especially of larger invertebrates, can be greater in wetlands that have been mowed than in otherwise similar wetlands that have not, especially if the hay is not removed (Kaminski and Prince 1981a,b, Beck et al. 1987). Surprisingly, a wetland whose emergent cover had been rototilled had a greater variety of invertebrates than an otherwise similar undisturbed wetland (Kaminski and Prince 1981b). This may have been a short-term response attributable to more rapid warming of the disturbed soils in the spring (H. Murkin, Institute for Wetland and Waterfowl Research, Oak Hammock Marsh, Manitoba).

Data from the Delta Marsh also suggest that during normal springtime conditions, open water and emergent vegetation differ little with regard to their variety of nektonic and benthic invertebrates. By midsummer, open water sites contain submersed plants, and consequently can support a greater variety of nektonic invertebrates. If nearby emergent vegetation has been flooded within the last 1–2 years, it can support as many or more nektonic and benthic species (Murkin et al. 1991). An exception might be stands of cat-tail. In these, flooding seems to have little effect on the variety of benthic invertebrates (Murkin and Kadlec 1986b). Other data from the Delta Marsh (Kaminski and Prince 1981a,b) suggest that invertebrate richness is not always greater in wetland units that have relatively equal proportions of open water and emergent vegetation, compared with those that do not.

Density and Biomass

Invertebrate biomass in prairie wetlands is strongly linked to plant biomass (McCrary et al. 1986) and thus to areal cover of vegetation. This is because vegetation provides submersed habitat space that macroinvertebrates colonize at a far greater density than open water areas (Engel 1990). Throughout the early growing season, an undisturbed seasonal wetland in North Dakota had much greater densities of invertebrates than a flooded summer-fallow wetland (Swanson et al. 1974). Invertebrates also were more abundant in the undisturbed wetland than in a flooded grain-stubble wetland, except in late June.

Beyond some point, however, vegetation stands in prairie wetlands can become so dense that invertebrate density and biomass decline (e.g., Kaminski and Prince 1981a,b, Murkin et al. 1982, Broschart and Linder 1986, Murkin and Kadlec 1986b). At least in some prairie wetlands, the paucity of some invertebrates in dense stands is due more to the lack of oxygen in sediments of

these habitats, than to cooler temperatures or lack of algal foods in the stands, both of which are due to shading (Wrubleski 1987, Murkin et al. 1992). A common amphipod in prairie wetlands, *Hyalella azteca*, requires an oxygen concentration of at least 1.2 mg/L over the span of a month to achieve full reproductive and growth potential (Nebeker et al. 1992).

Wetlands containing open-water areas interspersed with relatively equal areas of dense vegetation often have the greatest invertebrate biomass (Kaminski and Prince 1981a,b). However, a study in one prairie wetland (Murkin et al. 1992) indicated that peaks in the horizontal distribution of invertebrates do not generally occur at the ecotone between the open water patches and stands of macrophytes, as was commonly assumed (e.g., Voigts 1975). Rather, peaks in invertebrate abundance seem to occur wherever the greatest variety of substrates occurs, and this variety is not always greatest along the ecotone between open water and vegetation.

Data from the Delta Marsh (Kaminski and Prince 1981a,b) indicate that cover ratio has less influence on invertebrate abundance and biomass than does the type of cover manipulation that has occurred (mowing, rototilling, etc.). In particular, practices that allow large amounts of plant litter to decay over the winter seem to support exceptional abundance and biomass of invertebrates the following spring (Kaminski and Prince 1981a,b; Ball and Nudds 1989). Thus, the degree to which vegetation removal has a neutral or beneficial effect on invertebrates seems to depend partly on the type of removal process (e.g., mechanical thinning, ditching, burning, herbicides, herbivore introduction), the type of vegetation, and the particular spatial patterns that are created (Nelson and Kadlec 1984).

Invertebrate association with open water or emergent vegetation varies by season. Data from the Delta Marsh suggest that early in the growing season, numbers of nektonic and benthic invertebrates are greater within emergent vegetation stands than in open water areas (Murkin et al. 1992). By midsummer, open water sites containing submersed plants have more nektonic invertebrates and about equal numbers of benthic invertebrates when compared to sites with emergent vegetation. However, if the emergent vegetation has been recently flooded, densities of benthic invertebrates (but not necessarily nektonic invertebrates) will be greater in emergent vegetation than in open water areas (Murkin and Kadlec 1986b, Murkin et al. 1991). Also, data from four North Dakota semipermanent wetlands show that stands of cat-tails supported greater densities of invertebrates than open water patches, whether natural or created by herbicide application (Solberg and Higgins 1993a).

Macroinvertebrate densities in a South Dakota prairie wetland (McCrary et al. 1986) were greatest in *Ceratophyllum demersum*, and zooplankton densities were greatest in *Lemna* minor, compared with other species of non-emergent plants. Higher summertime densities of invertebrates in open water areas (containing submersed plants) than in stands of emergent vegetation have also been documented in an Iowa marsh (Voigts 1975).

The occurrence of consistently high densities of invertebrates throughout a wetland is likely a sign of hydrologic and vegetational conditions that are spatially diverse. This is because different invertebrates (and different life stages of the same invertebrate species) require different cover densities, hydroperiods, and types of vegetation at different seasons (Murkin et al. 1992).

Interannual Variability of Density and Biomass

In the Delta Marsh, interannual changes in abundance and biomass of invertebrates were least in areas that had been disturbed by mowing (Kaminski and Prince 1981b).

4.4.3 Invertebrates as Indicators of Wetland Salinity

Species Composition

Certain invertebrates are characteristic of hypersaline prairie wetlands. These include the Anostracan brine shrimp (*Artemia*), brine flies (*Ephydra*), ostracods, and a few species of midges and aquatic worms. Other taxa known to be relatively tolerant (up to < 30,000 mg/L salt) include certain species of midges, mosquitoes, aquatic worms, dragonflies, water beetles (especially the Dytiscidae and Hydrophilidae), and water bugs (Kreis and Johnson 1968, Swanson et al. 1974). Although some of the salt-tolerant species in these groups also occur in less saline wetlands, their abundance is typically greater in saline wetlands. Thresholds of 80 and 5000 $\mu\text{S}/\text{cm}$ specific conductance might be of ecophysiological significance for some wetland invertebrates because these levels seem to represent disjunct points in the spatial distribution of water beetle (Coleoptera) species-distribution in Canada (Lancaster and Scudder 1987). Above a salinity of 50 g/L, the usual numerical dominance of chironomid midges in prairie lakes shifts to dominance by dolichopodids and ephydrids ("brine flies") (Timms et al. 1986). Composition of midge species provides excellent evidence of salinity gradients in the 0-10 g/L range, but not above (Walker et al. 1995). Among semipermanent wetlands, most gastropods occur only where specific conductance is less than about 5000 $\mu\text{S}/\text{cm}$ (Swanson et al. 1988) or 3 g/L (Timms et al. 1986). Salinity ranges of dozens of prairie benthic invertebrates, as determined from their distribution among many lakes, are reported by Larson (1975), Timms et al. (1986), Timms and Hammer (1988), and Walker et al. (1995); these data have largely been incorporated into Appendix B.

Species Richness

The variety of invertebrate species within major taxonomic assemblages generally declines in prairie wetlands with increasing salinity and/or with increasing specific conductance, in part because the biomass of most submersed plants decreases (Hartland-Rowe 1966, Timms et al. 1986, Lancaster and Scudder 1987) and in part because fewer taxa are physiologically adapted to higher salinity levels. However, the range of tolerances is likely to be wide, as evidenced by studies of aquatic beetles, for which the correlation between salinity and species richness is not strong (Timms and Hammer 1988).

Density and Biomass

The few species that tolerate highly saline conditions often occur at very great densities in prairie wetlands. This is partly attributable to reduced pressures from competition and predation. Water beetle populations in highly saline lakes also appear to have smaller body sizes and fewer class sizes (Lancaster and Scudder 1987).

4.4.4 Invertebrates as Indicators of Sedimentation and Turbidity

Species Composition

A shift from herbivorous and filter-feeding species (many midges, zooplankters, and mayflies) to sediment-burrowing species (many aquatic worms) can indicate that major turbidity and sedimentation incidents have occurred or are continuing. This is because a reduction in light penetration kills submersed plants and attached algae, and these plants contain a characteristic assemblage of herbivorous species. Burrowing species meanwhile can continue to exploit soft sediments. Excessive sedimentation might be expected to have different effects on species that overwinter in wetland sediments as eggs, as opposed to overwintering as diapausing adults, but this apparently has not been investigated. Long-term changes in wetland turbidity and sedimentation might be inferred by collecting decay-resistant remains of invertebrates from sediment cores or settling traps, and determining if the historical species are ones that characteristically prefer turbid, silty, anoxic environments (see Section 4.6.2).

Species Richness

A diminished variety of invertebrates is another sign that turbidity and sedimentation conditions have been severe for the reasons just given. Species richness is particularly likely to decline in semipermanent and permanent wetlands, where sediments are most likely to become anoxic.

Density and Biomass

Total density or biomass of invertebrates is a poor indicator of sedimentation because either increases or decreases can occur in response to increased sedimentation. Increases often occur when some species of burrowing aquatic worms that tolerate low oxygen conditions are able to proliferate and in the absence of intense predation come to dominate the aquatic community.

4.4.5 Invertebrates as Indicators of Excessive Nutrient Loads and Anoxia

Species Composition

Particular assemblages of invertebrate species have commonly been reported to be useful indicators of lake trophic state [as categorized in the book by Rosenberg and Resh (1993)] and might be similarly useful for signaling wetlands that have received excessive nutrients. In general, the proportion of "scrapers" (species that characteristically graze on algae) increases with eutrophication, at least during the early stages of enrichment. Specifically, increases in the ratios of 1) tubificid worms to sedentary aquatic insects, 2) the midge subfamilies Tanypodinae and/or Chironomini to the subfamily Orthoclaadiinae, 3) non-burrowing mayflies to burrowing invertebrates, and/or 4) cladocerans to rotifers, have been reported to indicate excessive nutrient loading of wetlands or other water bodies (Wiederholm 1980, Kansanen et al. 1984, Rosenberg et al. 1984, Jones and Clark 1987, Ferrington and Crisp 1989, Radwan and Popiolek 1989). Over 600 invertebrate species are categorized according to their association with a particular water body's nutrient status in a literature-based table in Rosenberg and Resh (1993). Long-term changes in nutrient status of a wetland might be inferred by collecting decay-resistant remains of

invertebrates from sediment cores or settling traps, and using the Rosenberg and Resh (1993) table to determine what proportion of the found species are ones that characteristically prefer enriched environments.

Species Richness

Species richness of invertebrates can decrease (Wiederholm and Eriksson 1979, Sedana 1987) or increase (Tucker 1958) in response to enrichment. In lakes, zooplankton richness initially increases with increasing phytoplankton production, then it decreases as production continues to rise (Dodson 1992).

Density and Biomass

Density and/or biomass of invertebrates, especially midges, increase with larger increases in wetland fertility (Johnson and McNeil 1988, Ferrington and Crisp 1989, Murkin et al. 1991). Indeed, the density of midges (as measured using emergence traps) has been recommended as an efficient indicator in some situations of secondary production in lakes (Welch et al. 1988). Although data from other regions show invertebrate density increasing in response to increased nutrients (e.g., Tucker 1958, Sedana 1987, Belanger and Couture 1988, Cyr and Downing 1988), substantial and chronic nutrient additions are needed to cause this response (Gabor et al. 1994, Murkin et al. 1994b), and at some point the response of the whole invertebrate community changes from an increase to a decrease in density. This occurs as plant litter accumulates faster than it can be processed effectively and oxygen is depleted from the sediments and water column (Almazan and Boyd 1978), causing a reduction in densities of many invertebrates (Hartland-Rowe and Wright 1975, Schwartz and Gruending 1985, Pezeshik 1987). Based on results of two experimental nutrient loading studies in prairie wetlands, Murkin et al. (1994b) suggest that "ideal nutrient addition levels" which balance positive fertilization effects against adverse oxygen depletion are between 60 and 200 mg/L for phosphorus and between 1600 and 2400 mg/L for nitrogen, added biweekly during summer.

4.4.6 Invertebrates as Indicators of Pesticide and Heavy Metal Contamination

Species Composition

In general, herbicides are not as acutely lethal to invertebrates as are insecticides (e.g., Buhl and Faerber 1990). Perhaps the most toxic herbicides are the triazines, including the commonly used herbicide atrazine. Atrazine has been shown to cause shifts in community composition and emergence times of aquatic insects at a concentration of 2 mg/L (Dewey 1986), and as little as 0.230 mg/L reduced the development of midges (Macek et al. 1976). The herbicide triallate is also quite toxic to prairie invertebrates (Johnson 1986, Arts et al. 1995). Other herbicides used in wetlands have been shown to increase the dominance of invertebrates (e.g., many aquatic worms) that are tolerant of low dissolved oxygen, a result related to the large oxygen deficit caused by decay of massive amounts of plants (Scorgie 1980). Herbicides can also increase the dominance of open-water forms (e.g., cladocerans) as their algal food base blooms after the reduction of shading aquatic vegetation.

Among insecticides, the synthetic pyrethroids (especially deltamethrin) are generally more toxic to invertebrates than the organochlorine, organophosphorus, and carbamate pesticides (Sheehan et al. 1987). Mollusks are a possible exception to this ranking. Mayflies and amphipods tend to be more sensitive to most insecticides than are midges and adult water beetles. In one of the few bioassays conducted in a prairie wetland, Johnson (1986) found the insecticides carbofuran, fonofos, and phorate to be highly toxic to two invertebrates (*Daphnia* and a midge species). Carbofuran's toxicity to aquatic invertebrates was corroborated in Wayland and Boag's (1990, 1995) prairie wetland bioassays. Phorate's toxicity was corroborated in prairie wetland mesocosms by Dieter et al. (1996). When applied to a Minnesota wetland at typical field concentrations, the pesticides temephos, chlorpyrifos, and dursban killed copepods, cladocerans, and phantom midges (Helgen et al. 1988).

A host of factors influence toxicity and mortality, and are sufficient to change the generic rankings of insecticide toxicity as well as lethal thresholds. For example:

Environmental factors: water temperature, organic content, pH, alkalinity, suspended solids.

Dose factors: concentration, the specific formulation (inert ingredients), frequency of application, duration of exposure.

Biotic factors: the invertebrate species, its life stage, proximity of unexposed microhabitat patches, degree of simultaneous stress from other factors that may be related (e.g., oxygen stress and enrichment from plant decomposition and photosynthetic inhibition for 1–2 weeks after herbicide application) or unrelated (e.g., drought).

The availability of vegetation can be particularly important to invertebrate survival in wetlands having sediments that are contaminated chemically or that are persistently anoxic or saline. In such situations vegetation provides a colonization surface isolated from the sediments, where contaminants often are concentrated (Nebeker et al. 1988). Richness and abundance of epiphytic and nektonic (swimming) invertebrate groups can thus remain high in some contaminated but well-vegetated wetlands (McLachlan 1975).

In other regions and in sediments exposed to some herbicides or severely contaminated by heavy metals, investigators have noted a shift from a community of aquatic insects and toward a community dominated by certain oligochaetes (aquatic worms) (e.g., Howmiller and Scott 1977, Wentzel et al. 1978, Winner et al. 1980). In non-wetland water bodies, areas that are at least moderately contaminated often are dominated by chironomid midges (Winner et al. 1980, Cushman and Goyert 1984, Rosas et al. 1985, Waterhouse and Farrell 1985, McCarthy and Henry 1993) and other aquatic invertebrate species whose adults have wings and short life cycles, e.g., water bugs (Hemiptera) and water beetles (Coleoptera) (Borthwick 1988, Courtemanch and Gibbs 1979, Gibbs et al. 1981). Wetland amphipods (*Gammarus*, *Hyalella*), clam shrimp (*Lynceus brachyurus*), and many zooplankton species, appear to be very sensitive to certain pesticides, whereas most aquatic snails and worms are less sensitive (Sheehan et al. 1987, McCarthy and Henry 1993). Amphipods are especially useful as indicators of contamination because they are relatively stationary (i.e., because they do not emerge and fly away like aquatic insects, their presence can be more indicative of the longer-term conditions of

a wetland). Dosed populations may require at least a year to recover (Gibbs et al. 1981, McCarthy and Henry 1993). Amphipods occur in most wetlands with relatively persistent standing water, and their response to pesticides has been documented in prairie pothole wetlands specifically (Borthwick 1988). Pesticide bioassays in prairie wetlands by Ruelle and Henry (1993) indicated greater sensitivity of *Daphnia magna* than *Hyaella azteca* and greater sensitivity among younger than older individuals of both.

In wetlands that lack surface water, nematodes can be particularly sensitive indicators of contaminant toxicity; those of the subclass Adenophorea tend to be more sensitive than those of the subclass Secernentea (Platt et al. 1984, Zullini and Peretti 1986, Bongers 1990). The nematode suborder Dorylaimina, oribatid mites, and many ground beetles (Carabiidae) are highly sensitive. Apparently the least sensitive organisms in such habitats are the soft-bodied invertebrates such as earthworms, terrestrial herbivores such as ants and weevils, and invertebrates that inhabit the upper soil layers such as springtails (Collembola) (Bengtsson and Tranvik 1989).

For protecting soil invertebrates, Bengtsson and Tranvik (1989) suggest maximum allowable concentrations for lead of less than 100–200 mg/kg; less than 100 mg/kg for copper; less than 500 mg/kg for zinc, and less than 10–50 mg/kg for cadmium. Concentrations of metals, pesticides, and other substances toxic to invertebrates are tabulated rather comprehensively in USEPA's AQUIRE database.

Species Richness, Density, and Biomass

Depressed richness and density of aquatic invertebrates is sometimes suggestive of past or ongoing exposure to pesticides, heavy metals, or other contaminants in permanently flooded (Ferrington et al. 1988, Krueger et al. 1988, Winner et al. 1975, Marshall and Rutschky 1974) and drier (Bengtsson and Tranvik 1989) habitats. Richness of a pond invertebrate community was reduced following application of the herbicide linuron (Stephenson and Kane 1984). Richness and density of invertebrates can decline even at levels of phenols and oil-water ratios not known to be toxic in laboratory studies (Cushman and Goyert 1984).

Bioaccumulation

Bioaccumulation of some substances appears to be greater among sand-dwelling invertebrates than mud-dwelling invertebrates (Muir et al. 1983). Several aquatic invertebrate species effectively accumulate certain heavy metals in lakes (e.g., Hare et al. 1991) and probably wetlands, but few data are available specifically from prairie wetlands.

Physical and Genetic Deformities

Physical deformities of individuals often accompany severe pollution. For example, midges with deformed mouth parts were noted in areas of synthetic-coal-derived oil pollution (Cushman and Goyert 1984). This indicator is difficult to recognize objectively, and it has not been examined in prairie wetlands. Perhaps more objective would be the application of electrophoresis techniques in genetic analysis. Such an approach might be able to rapidly detect past exposure of an invertebrate population to a pesticide, assuming that surviving organisms have a different gene

frequency than the parent population (M. Brinkman and W. Duffy, personal communication, South Dakota State University, Brookings, SD).

4.5 Monitoring Techniques

Methods and equipment for field-sampling of invertebrates, including wetland taxa, are reviewed comprehensively by Murkin et al. (1994a). Other useful summaries are provided by Edmondson and Winberg (1971), Downing and Rigler (1984), Isom (1986), Fredrickson and Reid (1988b), Ross and Murkin (1989), Staley and Rope (1993), and Rosenberg and Resh (1993).

4.5.1 General Considerations

Larvae of most aquatic invertebrates can be found in wetlands throughout the year. However, particular groups (e.g., midges, mayflies) are more evident from about May until September, whereas others (e.g., Anostraca, some Trichoptera) are more abundant earlier in the spring and can be found outside the usual growing season (Swanson et al. 1974). If wetlands can be sampled only once, then the late wet season or beginning of the dry season is usually the recommended time because density and richness in many wetlands tend to be greatest then (Marchant 1982). Depending on the study objective, the sampling schedule may need to be adjusted to coincide with phenologies of particular taxa (Resh 1979, Sklar 1985). For example, one might want to avoid sampling immediately after a synchronous emergence of the usually dominant species (i.e., a day or week when nearly all individuals of a species emerge at once). Maximum information is often obtained when most invertebrates are within a size range (later, larger instars) retained by nets used to sample them, and can be identified with greatest confidence. Estimates of macroinvertebrate production or seasonal change in standing crop generally require that samples be collected at least monthly and preferably biweekly.

4.5.2 Sampling Equipment

The choice of equipment depends largely on the wetland microhabitat to be sampled. Different assemblages of wetland invertebrates inhabit sediments (benthos), rooted plants or algae (phytomacrofauna), open water (nekton), and the surface film (neuston).

A significant problem in analyzing wetland invertebrate data arises from difficulties in determining the spatial dimensions of the area from which a sample was drawn. Accurate estimates of density (individuals per unit area) are difficult to achieve because of difficulties in accurately measuring the complex wetland substrate (submersed plants, emergent plant stems, etc.). To address this, some investigators have removed the substrate along with the collected sample, measured both, and reported density as weight (or number of organisms) per unit of weight or area of substrate (e.g., Mrachek 1966). In some cases regression coefficients have been calculated to convert plant weights to plant area, which can be further converted to estimates of invertebrate density using previously determined empirical relationships (Downing and Cyr 1985, Downing 1986).

The most common method of sampling invertebrates in prairie wetlands has involved use of sweep nets (or modified dip nets). These are the familiar long-handled, inexpensive insect nets. They can be used in water or air, except in mostly robust or dense stands of vegetation. They

are either swept through a standard length of vegetation, or placed on the bottom and hauled vertically through the water column in a rapid stroke. User variability can be a concern, but sweep nets are convenient to use and are particularly suited for capturing large (e.g., crayfish) or quick-moving species such as adult dragonflies and water striders that are not collected by other methods. Samples are not strictly quantitative because the unit of area swept is difficult to determine accurately (Adamus 1984, Plafkin et al. 1989). Also, measured species composition is strongly influenced by mesh size. In trial comparisons against a modified Gerking sampler (see below), Kaminski and Murkin (1981) found sweep nets to be just as effective in sampling water-column species. Other researchers who have described results from use of sweep nets in prairie wetlands include Hanson (1952), Voigts (1975), Swanson (1984), McCrady et al. (1986), Kreil and Crawford (1986), Lancaster and Scudder (1987), LaGrange and Dinsmore (1989b). Sweep nets are one device being used to sample prairie wetlands in the EMAP effort as well as in the effort conducted by the State of Montana.

A method suitable anywhere the water is at least a few inches deep involves use of activity (funnel) traps. Most trap designs follow descriptions of Murkin et al. (1983) and Swanson (1978b). Traps are positioned below the water level or on the bottom for hours or days, and nektonic invertebrates that enter the funnel-shaped trap cannot escape. Traps can be positioned vertically (more likely to capture emerging insects) or horizontally. Collections contain only nektonic invertebrates or, if placed on the bottom, only non-sedentary invertebrates (e.g., adult clams are usually missed). Non-insect invertebrates (e.g., *Hyalella*) as well as aquatic insects are passively collected. Traps can be fitted with lights to increase their attraction to some insects (Lancaster and Scudder 1987). Use is limited to wetlands with standing water, and traps are probably more effective when placed in open water areas or at the edge of vegetation patches, than if placed within dense stands. If traps are set for more than a few days, it sometimes is necessary to move them as water levels recede. Samples are not strictly quantitative because it is impossible to tell what size area the organisms came from and because some invertebrates and fish caught in the trap prey on other captives during the holding time (Murkin et al. 1983). However, the samplers are lightweight and inexpensive, and sample processing time is less than for some other methods because samples are mostly free of plant material and sediment. Because activity traps accumulate organisms over time, fewer species are missed as a result of ephemeral factors that cause avoidance.

From two years of biweekly samples from 10 wetland sites, Murkin et al. (1983) found significant correlation between the total numbers of invertebrates collected in activity traps and the number collected using sweep nets, although species composition differed somewhat (e.g., traps attract predatory invertebrates disproportionately). In a study of midges, Welch et al. (1988) found no difference in total catch between 0.142-m² and 0.283-m² trap sizes. Traps with inverted funnels inserted in the jar necks caught more pupae than traps without funnels, and total catch in the traps without funnels was 58% of the catch in traps with funnels. Other researchers who describe results of using activity or funnel traps in prairie wetlands include Armstrong and Nudds (1985), Kreil and Crawford (1986), Helgen et al. (1988), Hanson and Swanson (1989), Neckles et al. (1990), Murkin et al. (1991), and Bataille and Baldassarre (1993). Activity traps are also being used in the EMAP effort.

If the objective is to sample invertebrates that inhabit mainly the water column, tube samplers (e.g., "water column samplers," "Swanson samplers," "Gerking samplers", "stovepipes", "box

samplers") can be used. These are plexiglass cylinders about 6 cm wide that enclose a standard area of bottom substrate. Like corers (see below) they may sample some benthic organisms, but they are not designed to effectively penetrate the sediment [the device described by Euliss et al. (1993) is possibly an exception]. In some (e.g., the Gerking sampler), the bottom can be sealed off with a sliding door, plug, or similar feature once the sampler is in place. Some have been fitted with a reinforced cutting edge on the bottom. Tube samplers are not effective in dense vegetation or for catching quick-moving organisms, burrowing species, very large organisms, or many epiphytic species. A major advantage is that, by usually sampling the entire vertical extent of the water column, they capture diurnally migrating species that are missed by samplers that can sample only at a particular depth.

The most popular type of tube sampler for use in prairie wetlands seems to be the design of Swanson (1978a, 1983), which has been used by Neckles et al. (1990), Broschart and Linder (1986), and LaBaugh and Swanson (1988). A refined and expanded version of this sampler is described by Euliss et al. (1993). Also, Gates et al. (1987) described a type of tube sampler that simultaneously collects invertebrates on plants and in the sediment. They found this to give results for plant invertebrates at least as precise and sometimes more accurate than obtained by clipping macrophytes (see below). Other designs are described by Gerking (1957), Korinkova (1971), Mackay and Qadri (1971), Legner et al. (1975), Martin and Shireman (1976), Minto (1977), Hiley et al. (1981), Freeman et al. (1984).

Emergence and areal light traps are another option. They consist of floating nets or funnels, covering an area of 0.1 or 0.5 m², that are anchored at and just above the water surface or (rarely) are submerged. They are left in place for a specified period of time, during which they are checked daily. They passively collect aquatic insects that are developing from larval to winged adult stage (i.e., emerging from the water column), and thus do not collect non-emerging invertebrates which sometimes are a dominant component of the invertebrate community. Use of emergence traps is limited to wetlands containing open patches of surface water during the growing season when most insects emerge. They can be placed over either open water or short vegetation. If traps are set for more than a few days, they should be moved periodically to avoid altering the sampled habitat by shading it (H. Murkin, personal communication, Institute for Wetland and Waterfowl Research, Oak Hammock Marsh, Manitoba). Traps also must sometimes be moved as water levels recede. A popular type of emergence trap appears to be the design of LeSage and Harrison (1979), as modified by Wrubleski (1984). A design by M. Butler, described by Nelson and Butler (1987) is also used. Results of using emergence traps in prairie wetlands are described by Driver (1977), Nelson and Butler (1987), Wrubleski (1989), Neckles et al. (1990), Bataille and Baldassarre (1993), and Ross and Murkin (1993 a,b).

Because emergence traps are left in place for up to several weeks, they reduce the problem encountered by other samplers of missing key species because of an inappropriate time of visit. Sample processing times are favorable because organisms do not have to be separated from sediments and plant material. Because emerging insects come from a variety of microenvironments, emergence and areal light traps can integrate well the extreme spatial heterogeneity within many wetlands. On the other hand, the traps make it impossible to standardize or determine the unit of area from which the organisms originated. Moreover, some trapped organisms may prey upon each other, confounding any quantitative estimates, if traps are not emptied often. Initial purchase and maintenance of traps can be costly, and vandalism

can be a problem. If emergence traps are unavailable, many species of emerging aquatic insects can be identified and densities grossly estimated from exuviae (shed remains) that are easily sieved from the water surface (McGill et al. 1979).

Another choice for sampling invertebrates that normally attach to wetland plants involves use of artificial substrates. Plants are not sampled directly, but rather, plastic plants or other sterile surfaces (e.g., Hester-Dendy plate samplers) are totally submersed in the wetland water column and allowed to be colonized over a period of at least a month (Macan and Kitching 1972, Cairns 1982). Because such substrates standardize the surface area and texture, collections from substrate samplers are highly comparable to each other, making them attractive for use in monitoring of water-column water quality. They also are lightweight, and sample processing is relatively easy. However, disadvantages include the fact that a return trip to the wetland is required, vandalism can be a problem, their use is limited to wetlands with surface water, they sample only epiphytic species, and representativeness is sometimes uncertain (Adamus 1984). In stands of submersed vegetation, Gerrish and Bristow (1979) used plastic mimics of the pondweed, *Potamogeton richardsonii*, interspersed among live experimental plants. Although this yielded no significantly different numbers of invertebrates or species per unit of surface area than were found on real plants, aquatic worms were significantly more common on the artificial substrates. Natural substrates initially devoid of organisms can also be used as colonization substrates. For example, plant litter of measured area or volume can be placed in wetlands to allow colonization by detritivorous species over a specified period of time.

If the objective is to sample invertebrate communities inhabiting relatively unvegetated wetland sediments, then dredges—also called grab samplers (Ekman, Ponar, etc.)—are often used. They consist of a box with jaws that is lowered onto the sediment. The jaws enclose a specified area of bottom, and retrieve sediments and associated organisms to a sediment depth of about 5 cm. Dredges are used only where surface waters of at least 0.5 m in depth are present, and they are not effective where there are aquatic plants that jam the jaws and prevent full closure. Because an unknown number of organisms subsequently escape and the exact area and sediment depth of the spot being sampled is never certain, estimates of density are only crudely quantitative. Large organisms (e.g., crayfish), organisms in the water column, and fast-moving species in particular are sampled poorly. Dredges are cumbersome and relatively expensive, and their samples are time-consuming to sort, but they have been used in prairie studies by Driver (1977) and Timms et al. (1986).

Another option for sampling sediments is core samplers. Unlike grabs, corers do not have jaws, and instead rely on compactive force or suction to retrieve sediments, sometimes to a depth of about 15 cm. They suffer the same disadvantages as dredges. Samples usually are more precisely quantitative, but the mean size of organisms effectively captured is often smaller because of the narrow dimensions of corers. Core samplers are sometimes the only option for quantitatively sampling sediment organisms in wetlands that lack surface water. Where aquatic plants interfere with core sampler operation, some investigators have suggested welding a saw blade to the leading edge of the corer, for clipping heavy roots and stems (Murkin and Kadlec 1986b). The corer design that seems to have been used the most in prairie wetlands is that of Swanson (1978c); a similar design is proposed by Bay and Caton (1969). Results of using corers in prairie wetlands are described by Murkin et al. (1982), Talent et al. (1982), Murkin and

Kadlec (1985a), Broschart and Linder (1986), Kreil and Crawford (1986), Murkin and Kadlec (1986b) Nelson and Butler (1987), Neckles et al. (1990).

During periods when sediments or soils are not covered by water, pitfall traps and soil extraction techniques can be used, and sometimes yield the highest densities and species richness (Coulson and Butterfield 1985). If only plant-dwelling invertebrates need to be sampled, another approach involves directly clipping the vegetation while confining it in an enclosed box. Clipped vegetation is then carefully examined for invertebrates in the laboratory. This might provide more precise quantification than does use of sweep nets, although nektonic invertebrates are seldom captured. Downing and Cyr (1985) found the most cost-effective quadrat size for clipping to be 500 cm². Plants were enclosed in a 6-liter plastic box. Clipping aquatic macrophytes in quadrats of varying sizes yielded five times higher populations of invertebrates than did sampling with some tube samplers (Gerking, Macan, or Minto samplers). Vacuum suction also can be used to help remove small invertebrates from foliage in the field (Southwood 1981).

4.5.3 Time-Integrating Methods

The above methods are used primarily to sample living organisms. Sometimes, the sclerotized, decay-resistant remains of particular invertebrate groups (chironomid head capsules, and exoskeletons and eggs of snails, ostracods, daphnids, and conchostracans) persist in an undecomposed condition in wetland sediments for months, years, or even decades and centuries. Settled remains can be collected by a variety of devices (e.g., "sediment traps") and sieved to separate the remains. This, along with identification and enumeration of body parts, can be a difficult, laborious, and somewhat subjective process, but the resulting data on species composition provide clues to the wetland's previous long-term environmental conditions. Examples are demonstrated generally by Walker et al. (1991), Walker (1993), and Streever and Crisman (1993), and in prairie lakes specifically by Synerholm (1979) and Euliss et al. (1993). In wetlands exposed to mixing winds, the resulting resuspension of decay-resistant remains from many time periods can complicate data interpretation, unless samples are collected for comparison simultaneously using other methods (N. Euliss, personal communication, NPSC, Jamestown, ND). As part of 1992–1994 pilot studies in the prairie region, EMAP sponsored the development and testing of methods for accurately sampling decay-resistant remains of invertebrates.

4.5.4 Bioassay Methods

A review of laboratory, outdoor mesocosm, or *in situ* bioassay methods involving invertebrates is beyond the scope of this report. Use of bioassays to explore toxicity in prairie wetlands has been relatively limited. Examples include studies by Johnson (1986), Borthwick (1988), Helgen et al. (1988), Wayland and Boag (1990), and Ruelle and Henry (1993).

4.5.5 Bioaccumulation

Methods for collecting wetland invertebrates and assessing bioaccumulation of contaminants in their tissues are described in Staley and Rope (1993).

4.6 Variability and Reference Points

Numerical estimates cited in the following sections are difficult or impossible to compare with one another because they are based on samples collected with a variety of equipment and methods, in different wetlands, and for different time periods. They are cited only to provide order-of-magnitude illustration of levels of various parameters that have been encountered in prairie wetlands. For a specific listing of the methods behind each cited value and study, see Appendix J.

4.6.1 Spatial Variability

Species Richness

The true species richness of invertebrates in prairie wetlands is generally unknown because taxonomic knowledge and resources have nearly always been insufficient to make species-level determinations of specimens (Table 7). This is suggested by the fact that a collection from four prairie wetlands of 2594 individuals representing just a single invertebrate assemblage—the aquatic Coleoptera—yielded 57 species. Remarkably, these four wetlands contained almost half the aquatic Coleopteran species ever found in North Dakota (Hanson and Swanson 1989). Similarly, weekly collections of midges emerging from a single pond within the Delta Marsh yielded a total of 84 species (Wrubleski and Rosenberg 1990).

Although comparisons among studies are hindered by the fact that levels of resolution in taxonomic determinations have varied greatly, some results are presented (Table 7).

Richness varies significantly within wetlands as well. When Delta Marsh samples from various periods and years were pooled, the zone with cumulatively the most families was the hardstem bulrush (*Scirpus acutus*) zone (47 families), followed by the open water zone (44 families), and cat-tail zone (36 families). When manipulated parts of the Delta Marsh were included as well, the zone with cumulatively the most families was the whitetop (*Scolochloa festucacea*) zone (50 families), followed by cat-tail (48 families), hardstem bulrush and red goosefoot (47 families), softstem bulrush (*Scirpus validus*) (46 families), rayless aster (*Aster brachyactis*) (45 families), and the open water zone (44 families) (H. Murkin, personal communication, Institute for Wetland and Waterfowl Research, Oak Hammock Marsh, Manitoba). However, in both manipulated and unmanipulated wetlands, the relative rankings of zones based on their species richness varied by season and year.

Species Composition

Species that are most numerous or constitute the greatest biomass are often the most ecologically influential. The species of invertebrates that dominate prairie wetlands vary from wetland to wetland; those invertebrates reported in the literature to dominate at least one prairie wetland are shown in Appendix B. Species that are most sensitive to environmental change are often those with the narrowest habitat requirements, and species with narrow habitat requirements can often be identified as those with locally restricted distributions. Of the cumulative total of 74 taxa found by biweekly core sampling of four South Dakota semipermanent

Table 7. Comparison of studies on species richness.

Location, citation	Basin type	Sampling approach	Number of taxa
Nebraska, Rainwater Basin (Gordon et al. 1990)	Seasonal (<i>n</i> = 8), 1 field season	One field season	39 (cumulative)
South Dakota (Duffy & Birkelo 1993)	Semipermanent	Cores, biweekly	74 (mean = 44/basin, range = 31–58)
South Dakota (Broschart & Linder 1986)	Lakeside marsh	Tube samples and cores, one field season	Benthos: 5.7 families/basin Nekton: 7.0 families/basin
Iowa (Voigts 1975)	Lakeside marsh	No information	20–32 families
Iowa (LaGrange & Dinsmore 1989a, b)	Restored semipermanent	Sweep net samples, single visit	4–16 families/basin
Iowa (Hemesath 1991)	Restored semipermanent (<i>n</i> = 17)	Sweep net samples, 3/basin in June	7–17 families/basin, cumulative richness of 32 families
North Dakota (Euliss, pers. comm., NPSC, Jamestown, ND)	Semipermanent (<i>n</i> = 18)	Sweep net samples, 2 field seasons	29 families cumulatively, median = 18/basin (range, 9–25)
North Dakota, Cottonwood Lakes (LaBaugh & Swanson 1988)	Semipermanent & Seasonal (<i>n</i> = 5)	No information	9–19 species/basin (rotifers, copepods, and cladocerans only)
Manitoba, Delta Marsh (Neckles et al. 1990, Murkin et al. 1991)	Lakeside, unmanipulated	Funnel trap (24-hr sets, 5 years)	54 families; max./sample = 18; median = 8
Manitoba, Delta Marsh (Kaminski & Prince 1981a,b)	Lakeside	No information	1.2 families/m ³ (1 year); 1.9 families/m ³ (another year)
Manitoba (Bataille & Baldassarre 1993)	Semipermanent (<i>n</i> = 3)	Activity traps, weekly	26 families/500 samples
		Emergence traps, weekly	50 families/500 samples

wetlands, 33 taxa were found in only a single wetland (W. Duffy, personal communication, South Dakota St. University, Brookings, SD). Of 32 families found collectively in the 17 restored prairie wetlands in Iowa, five families were found only in one wetland (Hemesath 1991, Hemesath and Dinsmore 1993). Of 54 families found in funnel traps in four differently manipulated units of the Delta Marsh over a 5-year period, 19 were present in only one unit. The hydrologically unmanipulated unit had the most taxa (8) that occurred nowhere else; these included several mayflies (H. Murkin, personal communication, Institute for Wetland and Waterfowl Research, Oak Hammock Marsh, Manitoba). In the survey of 18 semipermanent wetlands in North Dakota which found 29 invertebrate groups, the groups with the most restricted distribution (as sampled with a sweep net) were fairy shrimp, amphipods (2 wetlands each), and broad-shouldered water striders (1 wetland) (N. Euliss, personal communication, NPSC, Jamestown, ND).

Density and Biomass

Macroinvertebrate densities as high as 36,000/m² are reported from the Delta Marsh by Neckles et al. (1990), although densities of just the benthic invertebrates reached a seasonal peak of about 1200/m² (Murkin et al. 1982, Murkin and Kadlec 1986b). In a lakeside prairie marsh in South Dakota, Broschart and Linder (1986) reported summertime means of 3534 and 7898/m² in ditched and unditched areas respectively. Another lakeside marsh in Iowa had a maximum of about 15,000/m², mainly associated with submersed plants (Voigts 1975). Midge larvae alone were present at densities of up to 10,092/m² in prairie wetlands used by mallards with broods, whereas samples from 16 randomly selected wetlands had a maximum of 5337/m² (Talent et al. 1982). When a corer was used, the mean densities from eight seasonal wetlands in the Rainwater Basin of Nebraska ranged from only 28/m² in one wetland to 86/m² in the most productive wetland (Gordon et al. 1990).

In sweep net samples from undisturbed seasonal wetlands in North Dakota, Swanson et al. (1974) reported a mean of about 6500 individuals per m³, whereas densities in water column samples from eight seasonal wetlands in the Rainwater Basin of Nebraska ranged from 106,000/m³ in one wetland to 1,636,000/m³ in another (Gordon et al. 1990).

After core-sampling five natural wetlands of eastern North Dakota bimonthly in summer for 2 years, Kreil and Crawford (1986) reported a mean of 2686 individuals/m³ in the poorest wetland and a mean of 89,460 individuals/m³ per wetland. Using a core-type sampler in the Delta Marsh, Kaminski and Prince (1981a,b) reported mean densities of benthic invertebrates of 6993 and 18,906/m³ (depending on year), whereas Neckles et al. (1990), using vertical activity traps, reported a mean of 210,000/m³ from the Delta Marsh. The following year, the mean invertebrate density was only 6993/m³. Mean density of core-sampled invertebrates among four South Dakota semipermanent wetlands ranged from 4782/m² to 20,063/m² (Duffy and Birkelo 1993). Using a corer elsewhere in North Dakota, Nelson (1989) found densities of 78,000/m² in semipermanent wetlands and 2400/m² in seasonal wetlands.

In tube samples from the lakeside prairie marsh in South Dakota, Broschart and Linder (1986) reported means of macroinvertebrates from tube samples of 9687 and 15,194/m³ in unditched and ditched areas respectively. Zooplankton densities averaged about 700,000/m³. In a shallow prairie lake in Minnesota, zooplankton densities peaked at over 450,000/m³ in early autumn (Hanson and Butler 1990). In seasonal wetlands in North Dakota, they peaked at > 2,000,000/m³

but peaked at $< 1,000,000/m^3$ in semipermanent wetlands in the same area (LaBaugh and Swanson 1993). Zooplankton densities also exceed $1,000,000/m^3$ in the Delta Marsh (Collias and Collias 1963) and in seasonal wetlands of the Rainwater Basin of Nebraska (Gordon et al. 1990).

In five natural wetlands in eastern North Dakota, Kreil and Crawford (1986) found an average of between 53 and > 3000 individuals per trap. Activity traps in three prairie wetlands in western Minnesota caught an average of between 156 and 1323 individuals, with seasonal peaks of between 738 and 4448 individuals (per sample per wetland per sampling period). Wetlands that had the largest numbers 1 year did not necessarily have similar rank among the three wetlands the following year (M. Hanson, personal communication, Minnesota Department of Natural Resources, Bemidji, MN).

Numbers of nektonic invertebrates found in part of the Delta Marsh during 24-hr sets of each underwater funnel trap ranged up to about 180 (Murkin et al. 1991), whereas in nearby potholes, Bataille and Baldassarre (1993) found up to 2094 per trap. In unmanipulated parts of the Delta Marsh, when samples from various periods and years were pooled, the zone with the greatest numbers of individuals per activity trap was the cat-tail zone, followed distantly by the hardstem bulrush and open water zones. When manipulated parts of the Delta Marsh were considered instead, the zone with the greatest numbers was the red goosefoot (*Chenopodium rubrum*) zone, followed by the whitetop, cat-tail, rayless aster, softstem bulrush, and open water zones (H. Murkin, personal communication, Institute for Wetland and Waterfowl Research, Oak Hammock Marsh, Manitoba). However, in both manipulated and unmanipulated wetlands, the relative rankings of zones based on their invertebrate densities varied by season and year.

Published data from emergence traps are more limited. From 0.5-m² emergence traps left in the Delta Marsh for 29 days in late summer, the mean density of the 10 commonest midges was 179 per trap, and the maximum was 640/m² (Wrubleski 1989). Emergence sampling of a permanent wetland 80 miles west of Delta Marsh found a maximum density of 977/m² (Bataille and Baldassarre 1993). On a total annual basis, emergence of midges in the part of Delta Marsh studied by Wrubleski varied from 2322 to 15,400 individuals/m², depending on vegetation type and cover ratio (Wrubleski 1989, Wrubleski and Rosenberg 1990).

Biomass estimates are influenced by mesh size, inclusion/exclusion of snail and clam shells, and sampling equipment. From part of the Delta Marsh, Kaminski and Prince (1981a,b) reported a mean biomass of invertebrates of 11,161 mg/m³ during 1 year and 2843 mg/m³ during another. Based on sweep net samples, the median invertebrate biomass of 18 semipermanent wetlands in North Dakota was about 1300 mg/m³ (N. Euliss, personal communication, NPSC, Jamestown, ND). In tube samples from the lakeside prairie marsh in South Dakota, Broschart and Linder (1986) reported means of 8524 and 6564 mg/m³ from ditched and unditched areas respectively.

On a per-area basis, mean biomass of core-sampled invertebrates in four South Dakota semipermanent wetlands ranged from 1543 to 5428 mg/m², and production ranged from 4604 to 21,800 mg/m² (Duffy and Birkelo 1993). In unmanipulated parts of the Delta Marsh, benthic biomass peaked at about 8000 mg/m² (Murkin et al. 1982, Murkin and Kadlec 1986b). Activity trap samples from four Minnesota semipermanent wetlands yielded a biomass per sample of from 0.38 g in one wetland to 3.23 g in another; seasonal peak biomass ranged from 0.89 g in

one wetland to 7.48 g in another (M. Hanson, personal communication, Minnesota Department of Natural Resources, Bemidji, MN). In a lakeside prairie marsh in South Dakota, Broschart and Linder (1986) reported biomass means of 1746 and 1314 mg/m² from ditched and unditched areas respectively. Data from submersed vegetation beds in 11 eastern Canadian lakes showed a range of invertebrate densities of 1000–2900 mg/m² (LaLonde and Downing 1992).

In unmanipulated parts of the Delta Marsh, when samples from various periods and years were pooled, the zone with the greatest biomass per activity trap was the cat-tail zone, followed by the softstem bulrush and open water zones (i.e., the same as when based on number of individuals). Samples from the open water zone weighed only one-quarter the weight of those from the cat-tail zone. When manipulated parts of the Delta Marsh were considered instead, the zone with the greatest biomass was the red goosefoot (*Chenopodium rubrum*) zone, followed by the softstem bulrush, whitetop, rayless aster, cat-tail, and hardstem bulrush zones; mean invertebrate biomass in the last of these was about half that in the first (H. Murkin, personal communication, Institute for Wetland and Waterfowl Research, Oak Hammock Marsh, Manitoba). However, as noted earlier, in both manipulated and unmanipulated wetlands the relative rankings of zones based on their invertebrate densities varied by season and year. Considering just the midge component of the invertebrate community, numbers and biomass of emerging individuals were greater in beds of pondweed (*Potamogeton pectinatus*) than in cat-tail or bulrush stands during a 2-year study in an unmanipulated part of the Delta Marsh (Wrubleski and Rosenberg 1990).

4.6.2 Temporal Variability

Species Composition

Within a season, species composition of invertebrates changes markedly. Water bugs (Hemiptera), water beetles (Coleoptera), and snails (Gastropoda) seem to be more evident later in the growing season in some prairie wetlands (Bartonek and Hickey 1969, Swanson et al. 1974). In unmanipulated parts of the Delta Marsh, data from activity traps and artificial substrates showed that seasonal peaks were attained mostly in late spring or early summer by mosquitoes, ostracods, and water mites; in mid-summer by lymnaeid snails and non-predacious midges; and in late summer by planorbid and physid snails, cladocerans, copepods, and amphipods (H. Murkin, personal communication, Institute for Wetland and Waterfowl Research, Oak Hammock Marsh, Manitoba). In three western Minnesota semipermanent wetlands, seasonal peaks were attained mostly in late spring or early summer by clam shrimps (Conchostraca), water beetles, dragonflies, and cladocerans; in mid-summer by mayflies, snails, leeches, and water mites; and in late summer by water bugs (Hemiptera), amphipods, and copepods. However, the same taxa did not necessarily show the same seasonal patterns in all wetlands, or even in the same wetland between years (M. Hanson, personal communication, Minnesota Dept. Natural Resources, Bemidji, MN).

Species Richness

In the Delta Marsh, Murkin et al. (1991) documented variation in taxonomic richness within a year. Richness in activity traps ranged from an average of about 11 families in late summer to near 0 during October sampling (the latest sampling of the year). Similarly, when samples from all years and zones within the unmanipulated wetlands were pooled, the data show relatively

constant richness until early to mid-September, at which time richness drops. Richness in the unmanipulated wetland ranged from a high of 33 families per seasonal period to a low of 11. None of the manipulated units had more than 31 families per seasonal period, and most had no more than 16. Some manipulated units had only 2 or 3 families per seasonal period (H. Murkin, personal communication, Institute for Wetland and Waterfowl Research, Oak Hammock Marsh, Manitoba).

Interannual variation also can be extreme. Of 54 families collected by activity traps in unmanipulated parts of Delta Marsh over a 5-year period, no more than 51 were collected in any single year, and in 1 year only 38 were found (H. Murkin, personal communication, Institute for Wetland and Waterfowl Research, Oak Hammock Marsh, Manitoba). In one of 18 semipermanent wetlands in North Dakota, the number of invertebrate families changed from 4 to 16 families between just 2 years, whereas in several other wetlands the number of families remained stable or decreased between years (N. Euliss, personal communication, NPSC, Jamestown, ND). In six prairie wetlands in southern Saskatchewan, the number of midge species over a 3-year period ranged from 3-9 in one wetland to 20-24 in another (Driver 1977). Annual species extinction rates varied from 0% to 44% (of all midge species present during the period), immigration rates varied from 0% to 58%, and turnover rates (the difference between immigration and extinction rates) varied from 11% to 100%. As expected, immigration and species replacement rates were greater in temporary wetlands than in semipermanent or seasonal wetlands.

Density and Biomass

The seasonal variation in availability of invertebrates in prairie wetlands is a critical factor affecting waterbird use. Although temporary wetlands (as noted above) harbor generally fewer species of invertebrates than do semipermanent wetlands, the species that are present typically occur in enormous quantities at a season when invertebrate biomass in more permanently flooded wetlands is relatively small or unavailable because of persisting ice cover. In sweep net samples from 3 undisturbed seasonal wetlands in North Dakota, invertebrates varied seasonally within the growing period from a maximum of about 18,964/m³ in one wetland in April to about 1000/m³ in another wetland in June (Swanson et al. 1974). In South Dakota, the density and biomass of benthic invertebrates in 3 seasonal wetlands was greatest in late June just before the wetlands dried up, whereas in a semipermanent wetland, density and biomass increased as the growing season progressed, reaching highest levels during the last sampling on September 21 (W. Duffy, personal communication, South Dakota State University, Brookings, SD). In 3 of 4 semipermanent wetlands in western Minnesota, peak biomass in activity trap samples occurred in late spring to early summer (M. Hanson, personal communication, Minnesota Department Natural Resources, Bemidji, MN).

In semipermanent wetlands of Delta Marsh, Murkin et al. (1991) and in an area 80 miles west, Bataille and Baldassarre (1993) documented considerable variation in density and biomass by season. Total numbers of individuals in activity traps ranged from about 2094 in early summer (Bataille and Baldassarre 1993) and 300 in late summer (Murkin et al. 1991) to near 0 during October sampling (the latest sampling of the year). Biomass ranged from about 150 mg in summer to near 0 mg in October. Peaks in invertebrate abundance and biomass generally occurred in spring, often coinciding with the period when waterfowl were laying eggs (Bataille and

Baldassarre 1993). A second peak, especially of zooplankton, sometimes occurs in late summer or early fall when inputs of plant litter to the water column peaked (Murkin et al. 1991). There is some interannual variability in the timing of seasonal peaks; a late summer peak was noticeable in some years and habitats but not in others, and the spring peak occurred earlier in some years than in others (Wrubleski and Rosenberg 1990; H. Murkin, personal communication, Institute for Wetland and Waterfowl Research, Oak Hammock Marsh, Manitoba). Cladocerans in particular experienced a midsummer depression in numbers, coincident with an increase in cover of submersed plants (Murkin et al. 1991).

Interannual variation can be tremendous. Between just 2 years, the number of individuals in each of several semipermanent wetlands in North Dakota varied by orders of magnitude, but in others it varied only slightly (N. Euliss, personal communication, NPSC, Jamestown, ND). The invertebrate density (mean number of individuals per sample per period) in two semipermanent wetlands of western Minnesota varied three- and five-fold between years, but density did not change significantly in a third (M. Hanson, personal communication, Minnesota Department of Natural Resources, Bemidji, MN). In unmanipulated parts of the Delta Marsh, numbers of invertebrates caught in activity traps varied sevenfold over a 5-year period, and biomass varied by a factor of 3. In emergence traps from the same general area, the mean abundance of midges varied from 21,499/m² one year to 29,627/m² another, while biomass changed little (Wrubleski and Rosenberg 1990). In manipulated parts of the Marsh, total invertebrate abundance varied tenfold over the 5 years, and biomass varied twofold (H. Murkin, personal communication, Institute for Wetland and Waterfowl Research, Oak Hammock Marsh, Manitoba). In one part of the Delta Marsh, interannual changes in abundance and biomass of invertebrates were least in areas that had been disturbed by mowing (Kaminski and Prince 1981b). Interannual fluctuations in the amphipod, *Hyaella*, appear to be particularly great (Voigts 1975), in one instance ranging between 0% and 71% of all invertebrates sampled in a wetland, depending on the year (Bartonek and Hickey 1969). Interannual changes are probably a reflection of changing vegetation and water conditions.

The part of the season during which the peak occurs in a particular wetland's invertebrate population is not necessarily consistent between years (Wrubleski and Rosenberg 1990). For example, in the same set of Minnesota wetlands, invertebrates in two of the wetlands appeared to peak in late summer one year but early summer the next, but in a third wetland, the peaks both years occurred in early summer.

Bioaccumulation

In an eastern Canadian lake, bioaccumulation of heavy metals in aquatic insects followed a seasonal pattern, and in no case varied more than sixfold over the course of a year (Hare and Campbell 1992). Bioaccumulation depends on characteristics of the particular contaminant, the abiotic environment, and characteristics of the invertebrate species, e.g., its feeding habits, body size, and microhabitat preferences (Krantzberg 1989, van Hattum et al. 1991, Hare et al. 1991).

4.6.3 Spatial vs. Temporal Variability

In a 4-year study of diving beetle communities in an Alberta lake, Aiken (1991) found that species composition varied less between years than between zones within the lake (e.g., sedge, cat-tail,

willow, willow-cat-tail, mixed, and open water). In a 2-year study of midges emerging from 3 habitats (pondweed, cat-tail, bulrush) of the Delta Marsh, Wrubleski and Rosenberg (1990) found that numbers and biomass varied less between years than among these habitats.

4.7 Collection of Ancillary Data

It is easier to separate the anthropogenic from the natural causes of impairment of community structure if data are collected or inferred simultaneously on the following variables of particular importance to wetland invertebrates:

- age of wetland and its successional status
- water or saturation depth
- conductivity and baseline chemistry of waters and sediments (especially pH, alkalinity or calcium, and organic carbon)
- sediment type
- presence of fish and salamanders
- density, type, and form of vegetation (particularly, total surface area)
- cover ratio
- duration, frequency, and seasonal timing of regular inundation
- time elapsed since the last severe inundation or drought.

All of these features vary to a large degree naturally as well as in response to human activities such as soil tillage, compaction, and erosion; fertilizer and pesticide application; and water-regime modification.

4.8 Sampling Design and Required Level of Sampling Effort

Locations within a wetland from which invertebrate samples are collected can be chosen according to many of the designs described for sampling wetland vegetation (Section 3.8). One EMAP sampling design is to collect invertebrate samples randomly from transects radiating in four compass directions from the center of each wetland.

4.8.1 General Considerations

The time required to collect an invertebrate sample varies somewhat among the sampling methods (sweep net, corers, etc.), and is about 1–3 minutes per sample, not including travel time to the sampling site. The largest collection-time differences among methods relate to the number of samples each method requires to achieve a prespecified level of precision and the sorting times required to separate invertebrates from debris in the collected samples. If sorting is done

at the wetland site, a screen such as designed by Swanson (1977b) and modified by Euliss and Swanson (1989) can expedite the process. More often, samples are sorted in a laboratory under high-intensity lights or in direct sunlight. Sorting time is about 15–30 minutes for samples from emergence and activity traps and artificial substrates, about 15–45 minutes for sweep net and tube samples, and at least 3 hours for some core and dredge samples (Murkin et al. 1983, Resh et al. 1985). These estimates depend, of course, on how numerous and cryptic the sampled organisms are, how large and "dirty" the sample is, and how completely one wishes to process the sample. Samples collected with a fine-mesh net (e.g., < 80 microns) will naturally contain many more individuals than samples collected with coarser nets.

Many investigators have used sugar flotation methods to separate invertebrates from debris; apparently only a few have used rose bengal stain to increase the detection of individuals in samples. Core samples are routinely sieved before sorting. Except for very small samples, live-sorting (sorting of samples in the field prior to preservation) is unlikely to succeed in removing more than a small proportion of individuals, which are usually the largest and most active forms and thus not necessarily representative of the invertebrate community. Because the time that would be required to find every individual in a dirty sample seems almost limitless, some investigators have used a single, standard sorting time for all samples, but most have simply exercised best professional judgement as to when they believe they have found nearly all specimens. If species richness is to be determined, then sorting only a fixed number of individuals is inadvisable because of a bias toward selecting larger and less cryptic individuals. More preferable is the complete counting of all individuals within randomly selected subsamples; subsamples may be delineated by drawing a grid on the bottom of the sorting pan. Once all individuals have been sorted, tallies of species richness are justifiably based on a fixed number of individuals (generally 100–1000) that are chosen randomly from within a sample.

If biomass determinations will be made, samples are typically dried in a drying oven to a constant weight. In some cases, snail and clam shells are first dissolved with acid and caddisflies are removed from their cases, which are not weighed.

An important consideration that affects sample costs is the desired level of taxonomic identification. Identifying aquatic invertebrates to the species level usually allows the investigator to make more refined statements about the condition of a wetland, but species-level identification can increase sample processing time at least fourfold and requires advanced training, experience, and the availability of region-specific taxonomic keys. There are no data to indicate whether, and under what conditions, identification of organisms only to the family level would be sufficient to define the ecological integrity of a prairie wetland. Determination of invertebrates to genus or species takes at least three times longer than when identification is made only to the family level (assuming the identifier is familiar with keys of all taxa). Processing samples (sorting, identification to family, data entry) from sweep nets and activity traps can be accomplished in 2–3 hours, but about 4 hours are required for processing samples collected using corers and tube samplers (N. Euliss, personal communication, NPSC, Jamestown, ND).

Sampling costs are determined not only by sample collection, sorting, and identification times, but also by the number of samples collected. This should depend on expected variability (coefficient of variation) and the desired precision. Although ecologists commonly consider acceptable a confidence level of 90% and standard errors less than 20% of the mean, Downing's

(1979) review of the literature on benthic sampling in lakes revealed that fewer than 3% of published studies attained this goal. For their work in a prairie wetland, Murkin and Kadlec (1986a) stated beforehand that they would accept estimates within $\pm 30\%$ of a mean, and as a result they were able to specify the number of samples needed to achieve this precision. Among prairie wetland researchers, they are apparently alone in using and reporting such relevant information.

Interpreting the collected data poses many other methodological challenges that cannot be addressed in this report. In particular, separating the component of variability that is due to natural causes (weather, vegetation, etc.) from the component that is due to anthropogenic causes is always challenging. In an analysis of stream invertebrate data from one California stream, Resh and McElravy (1993) found that the chance of detecting a significant interannual difference because of natural variability alone was at least 22% when species richness is used, 23%–35% when invertebrate density is used, at least 5% when a similarity index (Simpson's) is used, and 20%–35% when a purported "indicator species" was used. They also found, for that stream, the sampling regime in which five replicates were collected at a single site would result in an ability to detect a 56% interannual (2-year) change in species richness and a 73% change in the similarity index, whereas the same regime would only be sensitive enough to detect a > 200% change in invertebrate density and a > 300% change in the indicator species (with a 95% chance of being correct at the 5% level of significance).

From a review of 46 studies that monitored benthic invertebrates in lakes, Voshell et al. (1989) found that 3 was the usual number of replicates collected. Review of the prairie wetland literature indicates that previous studies (those intended to characterize the invertebrates or algae) usually collected, in each wetland and at each point in time, 2–4 samples per zone (and in a few cases, 2–3 replicates of each sample). For invertebrate biomass estimates in Minnesota lakes, Hanson and Butler (1990) reported that samples collected at 4- and 6-week intervals were very similar to those based on nine biweekly collections.

4.8.2 Asymptotic Richness: Results of Analysis

For this report, we analyzed invertebrate taxonomic richness from three data sets gathered from prairie wetlands. One data set consisted of replicates collected from four semipermanent wetlands during a single growing season (Duffy, unpublished data). Data from four corer replicates collected within a wetland were first combined into a single list containing all taxa for each wetland-date combination. Two of the wetlands were sampled four times before they dried up, and analysis of data from each showed that half the total number of species (from all four dates) could have been detected in collections from any two dates, but that to detect 95% of the species, sampling on all four dates was required (Appendix O). For a wetland that was sampled on six dates, analysis suggested that half the species could have been found in samples from any two dates, but that to detect 95% of the species, sampling on all six dates was required. Finally, for a more persistently flooded wetland that was sampled on nine dates, the analysis indicated that half the species could have been found (as before) in samples from any two dates, but that to detect 95% of the species, sampling on all nine dates was required.

A second data set (Euliss, unpublished data) consisted of 381 invertebrate sweep-net samples collected from multiple transects in 19 prairie wetlands during a 2-year period. Without pooling

any of the samples, our analysis indicated that half the 29 species that were present collectively could have been detected with only five samples, but to detect 90% of the taxa, at least 178 samples would be required (Appendix O). We then examined data just from the wetland that had the greatest richness (wetland 28-III), and determined that any two samples would produce half the 25 taxa found in all 26 samples, but 21 samples would be required to find 95% of the species.

Finally, we examined a very large data set gathered from invertebrate activity traps used in the MERP research effort on Delta Marsh, Manitoba (Murkin, unpublished data). Samples had been collected at various depths in various zones and during various weeks over a 5-year period. See Appendix L for a full description of the monitoring design and data structure. We conducted the following analyses.

Number of Seasonal Periods

Just the data for the 2-zone-year combinations that had the greatest richness in the unmanipulated "reference" unit of the Delta Marsh were compiled. These combinations were zone 4 (*Scirpus acutus* zone) in 1985 (31 taxa) and zone 5 (cat-tail zone) in the same year (30 taxa). In the first instance, half the taxa that were present in the samples from 10 periods could have been detected if only 2 periods (sampling weeks) had been covered, and 95% of the taxa could have been detected from 9 periods. In the second instance, half the taxa present in the samples from 20 periods could have been detected if only 3 periods had been sampled, and 95% taxa could have been detected from 15 periods.

Number of Years

Just the data for the two zone-year combinations that had the greatest richness in the unmanipulated "reference" unit of the Delta Marsh were compiled. These combinations were zone 4 during period 4 (last week of May) and the same zone during period 7 (late June). In both instances, half the 25 taxa that were present in the samples from all 5 years could have been detected if only 2 years had been sampled, but to detect 95% of the taxa, the full 5 years are required. Species accumulated at a slightly more rapid rate (i.e., interannual conditions were more similar) in late May than in late June.

Number of "Replicates"

Data from all 246 sampled combinations of year, period, and zone were examined, from the unmanipulated "reference" unit. Half the 53 taxa that were present collectively in the 246 samples could have been detected if only 13 samples had been collected, and 225 samples would be needed to detect 95% of the taxa.

4.8.3 Power of Detection: Results of Analysis

The Components of Variance approach, as described in Section 1.5, was applied to invertebrate data from four data sets. These data sets (Duffy, Euliss, Hanson, and MERP) are described in Appendix L.

Taxonomic Richness

Core sampling, as implemented by the Duffy study, was better able to detect interwetland differences in the total sampled number of individuals than in the total sampled number of taxa. The Hanson, MERP, and Euliss studies showed the converse. The apparent results of these comparisons might be due less to the type of sampler used than to the relative intensities of sampling. Analysis of the Duffy core data suggests that a sample size of 10 wetlands would allow detection of interwetland differences of seven taxa, whereas if activity traps or sweep nets were used, sampling the same number of wetlands would allow detection of interwetland differences of two and 3 taxa, respectively. These taxa would likely be different because different sampler types capture different species. For wetland types, sampler types, and experimental designs similar to those of these studies, sampling additional wetlands beyond 6–13 wetlands has little effect on increasing the precision of the richness estimates.

Total Number of Sampled Individuals

Analysis of the data suggests that a sample size of 10 wetlands would allow detection of interwetland differences of 9200 individual organisms per core sample. Sampling the same number of wetlands with activity traps would allow detection of interwetland differences of 1600 individuals (Hanson data) or 6200 individuals (MERP data), whereas sampling with sweep nets or sediment traps would allow detection of differences of 1300 or 120 individuals, respectively. The sediment trap data also show that 10 transects would allow detection of intertransect differences of 67 total individuals. For wetland types and experimental designs similar to those of these four studies, sampling additional wetlands beyond 5–10 wetlands has little effect on increasing the precision of the density estimates. For the sediment trap approach, sampling more than 14 transects brings diminishing returns with regard to precision of estimates of the number of individuals.

Biomass

The data suggest a sample size of 10 wetlands allows detection of differences between total biomass means of 3 g (using corer samples or activity traps), 2.1 g (using a sediment sampler), 0.7 g (sweep net sampling), or 0.180 g (more intensive activity trap sampling). Data also indicate that beyond a sample size of about 6–12 wetlands, adding additional wetlands has little effect on increasing the precision of estimates (i.e., the ability to distinguish between means of the total sample biomass of any two of the region's wetlands). For the sediment trap approach, sampling more than 10 wetlands or 12 transects brings diminishing returns with regard to precision of biomass estimates.

Numbers of Individuals: Specific Taxa

Core sampling, as implemented by the Duffy study, was best able to detect interwetland differences in the total sampled number of individuals of (in decreasing order of power of detection) Chironomidae, Anostraca, Conchostraca, Amphipoda, Ostracoda, and total. The data suggested that a corer sample size of 10 wetlands would allow detection of interwetland differences of between 11 (Chironomidae) and 6000 (Ostracoda) individuals. For wetland types

and experimental designs similar to those of the Duffy study, core-sampling additional wetlands beyond about 9 wetlands has little effect on increasing the precision of estimates of most taxa.

Activity trap sampling, as implemented by the MERP project, was best able to detect interwetland differences in the total sampled number of individuals of (in decreasing order of power of detection) Tanytarsini (a Chironomid group), Amphipoda, Ostracoda, Physidae, Cladocera, and total. In comparison, Hanson's activity trap data were best able to detect interwetland differences in the sampled number of individuals of (in decreasing order of power of detection): Hirudinea, Amphipoda, Conchostraca, Ostracoda, Copepoda, and Cladocera. The MERP and Hanson data suggest that sampling 10 wetlands with activity traps would allow detection of interwetland differences of between 2 (Tanytarsini) and 2100 (Cladocera) individuals. For wetland types and experimental designs similar to those of the MERP and Hanson studies, placing activity traps in additional wetlands beyond 5–12 wetlands has little effect on increasing the precision of estimates of total sampled numbers of most taxa.

Sweep net sampling, as represented by the Euliss data, was best able to detect interwetland differences in the total sampled number of individuals of (in decreasing order of power of detection): Ephemeroptera, Physidae, Conchostraca, Lymnaeidae, and Chironomidae. The data suggest that sampling 10 wetlands with sweep nets in the manner of Euliss' study would allow detection of interwetland differences of between 3 (Ephemeroptera) and 500 (Chironomidae) individuals. For wetland types and experimental designs similar to those of the Euliss study, conducting sweep net sampling of additional wetlands beyond about six wetlands has little effect on increasing the precision of estimates of total sampled numbers of individuals of a particular taxon.

The sediment trap approach, as used by Euliss, was best able to detect interwetland differences in the total sampled number of individuals of (in decreasing order of power of detection) Lymnaeidae, Cladocera, and Ostracoda. The data suggest that sampling 10 wetlands with sediment traps in the manner of Euliss' study would allow detection of interwetland differences of between 1.6 (Lymnaeidae) and 100 (Ostracoda) individuals.

Biomass: Specific Taxa

Activity trap sampling, as implemented by the MERP project, was best able to detect interwetland differences in the total sampled biomass of individuals of (in decreasing order of power of detection) Amphipoda, Ostracoda, and Cladocera. The data suggested that sampling 10 wetlands with activity traps would allow detection of interwetland biomass differences of, at best, between 0.011 g (Amphipoda) and 0.066 g (Cladocera). For wetland types and experimental designs similar to those of the MERP and Hanson studies, placing activity traps in more than 5–12 wetlands has little effect on increasing the precision of estimates of the sampled biomass of most taxa. Similarly, for designs similar to the Euliss study, placing sediment samplers in more than 8–13 wetlands or 11–14 transects per wetland does little to increase the precision of estimates of the sampled biomass of most taxa.

4.9 Summary

The species composition of invertebrate communities, and to a lesser degree their species richness, demonstrates diagnostic responses to changes in prairie wetland salinity, water regime, and sedimentation/turbidity (Table 8). Invertebrates also respond sensitively to changing vegetative cover, nutrient levels, and presence of some contaminants, but existing information is too limited and confounding effects are too prevalent to currently allow widespread use of invertebrates to diagnose impairment of prairie wetlands from these stressors. Even for the better-known responses, few thresholds have been documented consistently, and the ability to use invertebrates to distinguish natural from anthropogenic levels of stressors is currently limited.

Invertebrate communities are being monitored with increasing frequency in prairie wetlands partly because of their recognized importance as food for waterbirds. Invertebrates that appear to be sensitive to the widest variety of stressors include amphipods, mayflies, clam shrimp, and fairy shrimp. Because of their high dispersal abilities and reproductive capacity, prairie wetland invertebrate communities appear to recover quickly (within weeks or months) from the direct effects of acute nonpersistent stressors. Because of this, they are poor temporal integrators of prairie wetland condition, unless the expense of frequent sampling is acceptable, or a systematic analysis of decay-resistant remains found in sediments is implemented. Results of such an analysis of decay-resistant remains can help establish "reference conditions" for development of regional water quality standards, but further information is first required on the tolerance thresholds of the taxa most commonly found decay-resistently.

Individual prairie wetlands that are semipermanently flooded generally contain about 20–40 invertebrate families, at densities of 1–20,000 organisms/m². Estimates of species composition, richness, and density are strongly influenced by the type of sampling gear and by sampling design. Several studies have quantified the interwetland and interannual variability of invertebrate communities in prairie wetlands. Variability spanning several orders of magnitude is often strongly linked to long-term wet-dry cycles and associated vegetation changes in individual wetlands.

Additional research is needed to document invertebrate response thresholds to all stressors, but particularly to sedimentation and water level change. Before biocriteria can be fully developed, information is also needed on the potential loss or gain of data resulting from various levels of specimen identification and use of various sampling protocols.

Table 8. Summary evaluations of possible invertebrate indicators of stressors in prairie wetlands.

Evaluations are based on technical considerations, not cost or practicality. A rating of FAIR or POOR is assigned when too few data (FD) suggest potential as an indicator, or when confounding effects (CE) of other variables often overshadow the effects of the listed stressor on the indicator.

Stressors	Possible Indicators	Evaluation
Hydrologic stressors	Species composition Richness Density, biomass	GOOD GOOD FAIR (CE)
Changes in vegetative cover conditions	Species composition Richness Density, biomass	GOOD FAIR (CE) GOOD
Salinity	Species composition Richness Density, biomass	GOOD FAIR (CE) POOR
Sedimentation & turbidity	Species composition Richness Density, biomass	FAIR (FD) FAIR (FD) POOR
Excessive nutrients & anoxia	Species composition Richness Density, biomass	GOOD POOR (CE) FAIR (CE)
Herbicides	Species composition Richness Density, biomass	FAIR (FD) FAIR (FD) FAIR (FD)
Insecticides	Species composition Richness Density, biomass	GOOD FAIR (FD) GOOD
Heavy metals	Species composition Richness Density, biomass	FAIR (CE) POOR (FD) POOR (FD)

5. Amphibians as Indicators of Prairie Wetland Integrity

5.1 Ecological Significance

There is considerable concern among scientists about possible worldwide declines in amphibians (Barinaga 1990, Wyman 1990), yet relatively little is known about the relative sensitivities or ecological significance of amphibians (frogs, salamanders) in prairie wetlands. Larval stages of most species feed largely on algae, but adults are mostly insectivorous and are consumed by birds (e.g., bitterns, egrets, pelicans). Populations and biomass of amphibians can reach relatively high levels under some conditions, but species richness is lower than for other groups discussed in this report.

5.2 Potential Indicator Metrics

The following measurements and metrics deserve consideration when amphibian communities are used to characterize conditions in reference wetlands, identify the relative degree of past disturbance to a prairie wetland complex, or assess the current inhibition of key processes:

- number of individuals per unit area, by season
- reproductive success
- interannual variability in density
- bioaccumulation.

5.3 Previous and Ongoing Monitoring

Apparently only two broadscale attempts have been made to survey amphibians in prairie wetlands. One is a county-wide survey conducted in Iowa (Lannoo et al. 1993). The other was sponsored by EMAP, and it involved surveys in 11 North Dakota wetlands in summer 1993. Productivity of salamanders in some prairie wetlands is reported by Deutschmann and Peterka (1988).

5.4 Response to Stressors

Few studies examine amphibians in prairie wetlands. Species are too few to allow amphibian richness to be used meaningfully at the individual site level as an indicator of impaired wetland integrity. Nonetheless, at a regional level the distribution, abundance, and perhaps richness of amphibians might be a sensitive indicator of overall changes in wetland water regimes, sedimentation, eutrophication, and other stressors. Amphibians appear to be particularly sensitive to pesticides because they absorb many chemical substances directly through their skins (Harfenist et al. 1989). At a site level, various biomarkers might be used as indicators (e.g., Leboulenger et al. 1982, Licht et al. 1983, Moore and Miller 1984), as well as amphibian fecundity, incidence of deformities, and bioaccumulation.

5.5 Monitoring Techniques

Amphibians can be sampled using methods and equipment described by Scott (1982), Vogt and Hine (1982), Halvorson (1984), Jones (1986), Bury and Raphael (1988), Moser et al. (1993), Heyer et al. (1994), and others.

5.5.1 General Considerations

Sampling amphibians effectively normally requires several repeated visits to a wetland or to a series of wetlands along a survey route. Amphibians are best sampled during the mid- to late growing season when maximum numbers of developing juveniles (e.g., tadpoles) are present. However, many species are easily found only after the first few days of rain following a drought, during late-summer thunderstorms, during the first spring thaw in northern areas, during mid-day basking hours, or at night (Kaplan 1981). Occasionally, traditional winter hibernation areas can be located and used to count individuals representing a larger (but undefinable) area.

5.5.2 Equipment and Methods

Amphibians are sampled using pitfall and funnel traps (often with drift fences and bait), visual belt transects, direct capture methods, and vocalization recording. EMAP-sponsored efforts to collect amphibians in prairie wetlands have used a setup involving drift fences and funnel traps. Fence and funnel methods can provide relatively quantitative data, when arranged systematically and level-of-effort (e.g., "trap-hours") is standardized. Efficiency can be increased by channeling movements of amphibians in the direction of the fence or funnel. This is commonly done with "drift fences" (Gibbons and Bennett 1974). These are fences constructed of wire screen or polyethylene plastic, with lengths upwards of 15 m. Traps are placed at both ends of the drift fence, along the fence at various points, or at the junction of several intersecting fences. The bottom edge of the fence is implanted in the ground, or at least no space is provided for amphibians to crawl under the fence.

Pitfall and funnel traps are perhaps the methods most widely used (Jones 1986). Pitfall traps involve implanting a container in the soil, either on the periphery of the wetland or within it (if surface water is absent), with the lip of the container placed flush with the ground surface. Amphibians stumble in and cannot climb the steep sides to escape. Because some species can drown if the container fills with rainwater, Jones (1986) recommends placing floatable material (e.g., styrofoam) in the container to reduce mortality. Pitfall traps are impractical in all but the most temporary parts of wetlands because otherwise the water table is so close to the land surface that pits fill rapidly with water.

Pitfall and funnel traps often produce more species per sampling effort than direct capture methods (Jones 1986). With funnel traps, animals enter a screened area and cannot find the opening to escape. They are subsequently identified, counted, measured, and released. To reduce loss of trapped animals to predation, traps and funnels are checked regularly (at least every other day) and can be shaded, and/or filled with sufficient moist plant litter to minimize physiologic stress to animals. Funnel openings are usually oriented toward land for greatest effectiveness. The size of the trap, baits used, and trap placement can affect the species that are caught.

Drift fences and pit traps can be more effective and less biased in capturing amphibians than walking transects, electroshocking, or searching and digging through litter. However, drift fences are expensive; time and cost estimates for drift fence trapping are provided by Gibbons and Semlitsch (1982). Drift fence/pitfall trap methods are less effective for quantifying populations of frogs, toads, large snakes, terrestrial turtles, and salamanders than for quantifying populations of small snakes (Jones 1986). Sizes and shapes of containers and associated drift fences and their configurations vary greatly, depending partly on target species and wetland type. Various designs are described by Stockwell 1985, Vickers et al. 1985, and others. For sampling seasonal and semipermanent prairie wetlands, Euliss (personal communication, NPSC, Jamestown, ND) has designed and used a particularly effective drift-fence-and-trap array.

The above methods require many visits to a wetland, first to set up and later to check traps. Amphibians can also be monitored directly, that is, during a single visit, or without having to wait for traps to catch individuals. However, direct methods usually do not provide accurate quantitative data on abundance. Unless frequent visits are made and the correct microhabitats are searched at the proper times of year, direct methods are also unlikely to yield good estimates of species dominance or richness. However, they can provide a useful complement to trap methods, locating species that are not easily trapped.

The simplest type of direct search involves scanning a wetland with binoculars to observe the more obviously visible forms such as basking frogs. In some cases, floating egg masses of amphibians can also be detected visually and identified to species. Observational methods can be done formally along defined transects. Searches on foot, perhaps employing many people shoulder-to-shoulder (e.g., Marshall and Buell 1955) have been used, but these searches could be impractical and destroy habitat in many wetlands. To enhance opportunities for encountering amphibians during direct searches, electrofishing can be used, at least for retrieving larger salamanders and frogs. Frogs can sometimes be located more easily at night because their eyes reflect light in the beam of a flashlight. Vocalizations of many frogs and toads are easily identified (commercially available recordings are available to learn these) and can be used to augment observations along survey routes. Frogs and toads can sometimes be induced to vocalize by introducing sharp, loud sounds or by playing back tape recordings of vocalizations.

5.5.3 Bioaccumulation

Methods for collecting wetland amphibians and assessing bioaccumulation of contaminants in their tissues are described in Moser et al. (1993).

5.6 Variability and Reference Points

Species Richness

Although 40 species of amphibians and reptiles have been found in South Dakota and 25 in North Dakota, perhaps only 3—the tiger salamander, leopard frog, and chorus frog—appear to be widespread and intimately associated specifically with prairie wetlands (Hubbard et al. 1988). Biweekly sampling of 17 seasonal and semipermanent wetlands in North Dakota captured 6 species of reptiles and amphibians (N. Euliss, personal communication, NPSC, Jamestown, ND). A survey of prairie wetlands in Iowa found 7 amphibian species; 2 species present in the 1920's

(mudpuppy, *Necturus maculosus*; Blanchard's cricket frog, *Acris crepitans blanchardi*) apparently were no longer present (Lannoo et al. 1993). Minnesota prairie wetlands support at least 4 toad species (Oldfield and Moriarty 1994).

Density, Biomass, Production

A 2-year study of 3 prairie lakes in North Dakota revealed the maximum density of larval salamanders to be 5000/ha. Maximum biomass was 180 kg/ha and maximum annual production was 565 kg/ha (Deutschman and Peterka 1988).

5.7 Collection of Ancillary Data

It is easier to separate the anthropogenic from the natural causes of impairment of community structure if data are collected or inferred simultaneously on the following variables of particular importance to wetland amphibians:

- water depth
- temperature (site elevation, aspect)
- conductivity and baseline chemistry of waters and sediments (especially pH, DO, and suspended sediment)
- shade
- amount and distribution of cover (logs, muskrat houses, etc.)
- cover ratio
- extent of plant litter
- vegetation type
- duration, frequency, seasonal timing of regular inundation
- time elapsed since the last severe inundation or drought.

All of these features vary to a large degree naturally, as well as in response to human activities such as soil tillage, compaction, and erosion; fertilizer and pesticide application; and water regime modification.

5.8 Sampling Design and Required Level of Sampling Effort

No quantified estimates of interwetland or interannual variability were found in the published literature from the region, and no data sets were obtained for analysis, so requisite sample sizes cannot be estimated.

5.9 Summary

Although apparently constituting a large portion of the annual animal production of some semipermanent wetlands, amphibian communities have seldom been investigated in prairie wetlands. This is due in part to their relatively low species richness and spotty spatial and temporal distribution. Limited information suggests amphibians in prairie potholes may be highly sensitive to some chemical contaminants and to landscape-level fragmentation of wetland resources. Considerably more research is required before amphibian species composition, richness, and biomass can be used as unambiguous indicators of prairie wetland condition.

6. Birds as Indicators of Prairie Wetland Integrity

6.1 Ecological Significance

Birds are an obvious feature of prairie wetlands during the growing season. Birds inhabiting prairie potholes include waterfowl, shorebirds, large wading birds, and songbirds. The ecological significance of birds in prairie wetlands stems from at least two characteristics:

- They are highly mobile, moving frequently among as many as 20 prairie potholes during a growing season and between prairie potholes and wetlands in other regions during migrations. In the course of these movements, they often passively carry with them various invertebrates and seeds, which subsequently become established in new areas.
- Their movements and feeding within a wetland can alter vegetation structure (especially submersed plants), invertebrate densities, and the mixing of sediments (which in turn can affect wetland fertility).

The usefulness of birds as indicators of ecosystem integrity has been widely discussed (e.g., Reichholf 1976, Morrison 1986, Temple and Wiens 1989). Specific factors that make birds attractive as indicators of wetland integrity include:

- ease of monitoring (usually no samples to process); simple identification, and willingness of capable non-scientists to assist with surveys
- availability of established survey protocols
- tendency of some species (e.g., many raptors and wading birds) to accumulate toxic substances because of their position at the end of food chains
- longer life spans than other bioindicators (this may make them more sensitive to some cumulative impacts and more able than other groups to integrate the effects of episodic events)
- usefulness for *in situ* assessments (confined or behaviorally imprinted individuals)
- availability of the only relatively extensive nationwide databases on trends, habitat needs, and distribution
- availability of moderately extensive bioassay databases.

Certain characteristics usually considered disadvantages for using birds as indicators of wetland integrity include:

- absence from most prairie wetlands in winter
- mobility makes it difficult to locate site-specific causes of mortality (could be factors that operate thousands of miles away)

- mobility makes it difficult to assume that wetlands used by birds also support other organisms (birds may only be resting rather than feeding on these wetlands)
- no opportunities for routine analysis of decay-resistant remains (as with diatoms and some invertebrates), to establish historical reference conditions in a wetland
- nonbreeding individuals present in the breeding season (their presence in a wetland then denotes little about the condition of the species' population)
- survey protocols well-established, with nonsystematic biases (e.g., some species are much easier to detect than others)
- bird community structure highly controlled by physical habitat, predation, and perhaps mortality as a result of being hunted by humans rather than by contaminants.

In summary, birds are likely to be poor indicators of the integrity of a specific wetland, but their trends in species composition and relative abundance when measured throughout a region can integrate changes occurring in wetlands across the region. Given the current availability of data and tested protocols, birds are the only taxonomic group capable of serving this purpose.

6.2 Potential Indicator Metrics

The following measurements and metrics deserve consideration when bird communities are used for characterizing conditions in reference wetlands, identifying the relative degree of past disturbance to a prairie wetland complex, or assessing the current inhibition of key processes:

- richness of species and functional groups (per unit area, or per number of randomly chosen individuals)
- number of individuals per unit area by season
- relative dominance and richness of species that are characteristically associated with a particular habitat condition (e.g., grazing-sensitive species)
- reproductive success, including (see Sheehan et al. 1987 for definitions) numbers of breeding pairs, nest density, clutch size, nest success, hatch success, number of broods produced, brood size at fledging, broods per pair, and recruitment
- daily duration of specific activities (e.g., feeding, roosting) in the wetland complex, i.e., time budget analysis
- interannual variability in richness, density, and reproductive success
- bioaccumulation.

The specific ways some of these metrics have been or could be interpreted as an indication of stressed conditions are described in Section 6.4.1.

6.3 Previous and Ongoing Monitoring

Of the more than 80 publications describing field studies of birds in prairie wetlands, only 14 (18%) involved species other than waterfowl. The parameters most commonly measured in waterfowl studies are the frequency of nests and broods. An impressive 16 surveys covered more than 100 wetlands, but 15 studies were based on only a single year's data.

Few studies have systematically surveyed non-waterfowl species outside the breeding period. Collection of data on habitat use by migrant shorebirds in particular has been limited (Eldridge 1992, Eldridge and Krapu 1993), despite the fact that available evidence suggests that prairie wetlands are used extensively. In some instances, counts of shorebirds in the northern prairies exceed those known from any other location on the Central Flyway of North America (G. Krapu, personal communication, NPSC, Jamestown, ND). Compared to other mid-continent populations, populations of migratory shorebirds that occur in Dakota wetlands contain a higher proportion (55%) of long-distance migrants which depend most heavily on wetlands to replenish their energy supplies during migration (Skagen and Knopf 1993).

No State agencies are currently monitoring birds for the specific purpose of using the data to estimate the ecological integrity of prairie wetlands. At a regional level, USEPA's EMAP investigated the use of estimates of four breeding waterfowl species as indicators of landscape quality. Other ongoing regional efforts (Appendix K) include 1) the FWS's annual breeding waterfowl surveys (18-mile long aerial and ground transect surveys), 2) the FWS's annual Breeding Bird Survey (25-mile long roadside transects; an average of 69 routes have been run in US and Canadian parts of the prairie region and contain an average of 14 years of data); and 3) Breeding Bird Censuses (plot-based intensive surveys). At more localized levels, birds are being tested for possible use as indicators of the success of wetland restoration efforts in Iowa (Dinsmore et al. 1993, Zenner and LaGrange 1993) and cover management practices of the Conservation Reserve Program. Research on ecological relationships affecting waterfowl in particular continues to be conducted by NPSC and universities.

6.4 Response to Stressors

The following subsections describe responses of the bird communities to hydrologic stressors, vegetative cover conditions, salinity, sedimentation/turbidity, excessive nutrient loads/anoxia, and pesticide and heavy metal contamination.

6.4.1 Birds as Indicators of Hydrologic Stressors

Species Composition

Birds are affected both directly and indirectly by hydrologic changes. The assemblage of breeding birds that have established territories in a particular prairie wetland can generally indicate the present water depths of the wetland. For example, the regular presence of western grebes and certain diving ducks can indicate relatively deep water (> 2 m) and consequently, the likely seasonal persistence of water in an individual wetland. Much of the information on depth requirements is summarized by Fredrickson and Taylor (1982), Fredrickson and Reid (1986), and Short (1989). Species that are likely to be the most sensitive indicators of water levels might be

those that 1) nest along water edges, 2) feed on mudflats (e.g., shorebirds), 3) require a particular combination of wetland hydroperiod types in a region (e.g., Kantrud and Stewart 1984, Maxsson and Riggs 1996). In contrast, species (e.g., marsh wren, some diving ducks) that characteristically nest well above the water level might be less directly vulnerable, and thus are probably weaker indicators.

Only when data are combined at a regional level is it likely that trends in bird community composition will reflect trends in the hydrologic integrity of wetlands overall. Species composition of the bird community in a single prairie wetland is a poor indicator of past hydrologic stresses to that particular wetland because most birds can move freely among wetlands and among regions (although this can reduce reproductive success). As documented by radiotelemetric and modeling studies, many species appear to require wetland complexes, a particular combination of wetland hydrologic types at a particular density on the landscape or in close proximity to each other (Cowardin 1969, Weller 1975, Patterson 1976, Flake 1979, Talent et al. 1982, Kantrud and Stewart 1984, Rotella and Ratti 1992a,b). Years of regional drought temporarily reduce the number and perhaps the variety of wetland types, and subsequently cause drastic changes in species composition for an indefinite number of years thereafter (Hammond and Johnson 1984). Interannual fluctuations in bird numbers are likely to be smaller in landscapes containing intact wetland complexes because the complexes support a "shifting mosaic" of water depths that provides at least minimally suitable habitat regardless of regional drought or flood conditions (Skagen and Knopf 1994).

Single-species Indicators

Mallards and other waterfowl species are widely monitored throughout the prairie region. It is not apparent, however, that simple presence of a single species, its nest, and/or broods in a particular wetland is evidence of good hydrologic integrity. Mallards, for example, seem to inhabit a wide range of wetland types (as defined by hydrologic permanence). There are likely to be many situations where hydrologic conditions in wetland complexes are sufficient to support one or a few species such as mallard, but are too degraded (e.g., through drainage) to support many other species, including some plants, invertebrates, and other vertebrates that are crucial contributors to regional biodiversity because of their narrower habitat preferences.

Species Richness

At an individual wetland level, avian species richness is often greater in semipermanent and permanent wetlands than in temporary and perhaps seasonal wetlands (Faanes 1982, Weber et al. 1982). By reducing the number and perhaps the variety of wetland types, sustained regional drought diminishes species richness in many individual wetlands and wetland complexes. Among six restored prairie wetlands that were sampled in Iowa for 2 years, avian richness was greater during the wetter year in all but one wetland, where it did not change (Hemesath 1991). Richness in wetlands restored after being drained for > 30 years did not differ significantly from richness in wetlands drained more recently. Birds colonized formerly drained wetlands within 1 year of restoration; other prairie wetland studies report similar results (LaGrange and Dinsmore 1989 a,b, Sewell 1989, Zenner and LaGrange 1993).

Density and Biomass

Among waterfowl, pair density during the early summer is usually greater in temporary and seasonal wetlands that have ponded water, than in semipermanent and permanent wetlands (Krapu and Duebbert 1974, Kantrud and Stewart 1977, Ruwaldt et al. 1979). When presence/absence of ponded water is not considered, seasonal wetlands have the highest pair densities. However, later during the summer and perhaps during dry years, the more permanent wetland types support the greatest number of individuals per wetland area (Stewart and Kantrud 1971, 1973; Duebbert and Frank 1984; Talent et al. 1982). In contrast, for birds as a whole (all species combined), breeding densities are greatest in semipermanent wetlands (Faanes 1982). By reducing the number and perhaps the variety of wetland types, sustained regional drought diminishes density of birds in many individual wetlands and wetland complexes (Greenwood et al. 1995, Bethke and Nudds 1995).

Reproductive Success

Reproductive success of waterfowl and undoubtedly other prairie birds is diminished during drought years (Higgins et al. 1992, Greenwood et al. 1995, Bethke and Nudds 1995).

6.4.2 Birds as Indicators of Changes in Vegetative Cover

Species Composition

Birds in prairie wetlands respond strongly to changes in vegetation density and type, both within wetlands (Weller and Spatcher 1965, Lokemoen 1973) and in the surrounding landscape (Duebbert and Kantrud 1974, Huber and Steuter 1984). Many species, primarily waterfowl and shorebirds, benefit from (or tolerate) reduced ground cover and increased openings in dense stands of vegetation (Keith 1961; Weller and Spatcher 1965; Weller and Fredrickson 1974; Krapu et al. 1979; Kaminski and Prince 1981b, 1984; Blixt 1993; McMurl et al. 1993). For example, breeding waterfowl in four semipermanent wetlands responded positively to thinning of dense cat-tail stands for at least 4 years after the stands had been thinned by herbicides (Solberg and Higgins 1993a). The waterfowl also used the treated wetlands to a greater degree than they used untreated wetlands that had natural openings in the vegetation. However, effects of increased open water on waterfowl as a result of another experimental application of herbicides (Blixt 1993) were equivocal.

Several species, including sora (Fannucchi et al. 1986), other rails (Weller et al. 1991), northern harrier, short-eared owl, and ring-necked pheasant (USDA Soil Conservation Service 1985, Homan et al. 1993) do not necessarily benefit from reduced cover density. One North Dakota study that used herbicides to reduce vegetation cover found a reduction in densities of marsh wren, red-winged blackbird, yellow-headed blackbird, and common yellowthroat up to 2 years after application (Blixt 1993, Linz et al. 1993, 1995, 1996). A Minnesota study found no positive correlation between cover ratio and numbers of yellow-headed blackbird, song sparrow, or sora (Olson 1992). Limited surveys of restored wetlands in Iowa seldom found certain species that occurred only in natural (vs. restored) wetlands: least bittern, American bittern, sora, Virginia rail—or more abundantly—common yellowthroat, red-winged blackbird, swamp sparrow (Delpey and Dinsmore 1993, Dinsmore et al. 1993). Among waterfowl species, the northern pintail and

northern shoveler appear to tolerate or benefit from partial removal of cover in surrounding landscapes (e.g., from grazing) to a greater degree than do teal, gadwall, and American wigeon (Stewart and Kantrud 1973).

Impacts of cover removal might be most evident among 1) species that nest in uplands, 2) species with relatively large territories, and/or 3) species that nest early in the growing season, before there is appreciable new growth by crops or pasture grasses (Batt et al. 1989). Species in prairie wetlands that appear to benefit from light-intensity grazing (or mowing during the prior autumn) include Wilson's phalarope, common yellowthroat, and red-winged blackbird; species most sensitive to heavy grazing include LeConte's sparrow and sedge wren (Kantrud 1981). At a regional level, changes in the frequency or range sizes of the species cited above (and others) might indicate changes in the overall condition of vegetative cover. Literature on bird response to vegetation removal in wetlands is summarized by Skovlin (1984) and Kantrud (1986a). Since Kantrud's 1986 synopsis was published, an additional 15 research studies on the topic have been published (Appendix J). Based on the literature, Short (1989) categorized 88 species of birds that breed in the prairie region according to the vegetative cover types they prefer for nesting and foraging.

Single-species Indicators

It is likely that conditions of vegetative cover that are suitable for nesting mallards and other waterfowl are suitable for sustaining relative high levels of avian richness generally. Nonetheless, cover needs vary among waterfowl species, and there are some bird species (e.g., piping plover) that do not generally occur in wetlands that are optimal for waterfowl. Definitions and measurements of wetland integrity must be broad enough to account for needs of such species.

Species Richness

High species richness within prairie wetlands typically occurs where there is a mix of vegetation types, and/or a mixture of about 30%–50% open water with 50%–70% vegetation (Weller and Spatcher 1965, Weller and Fredrickson 1974, Kaminski and Prince 1981b, 1984, Hemesath 1991, Olson 1992). However, avian richness in prairie wetlands cannot always be predicted by structural diversity of vegetation (Olson 1992).

As dense stands of vegetation are thinned, the diversity of bird species using a wetland typically increases or remains stable (Blixt et al. 1993), especially if open water begins to occupy spaces cleared in the vegetation (Kaminski and Prince 1981b, 1984; Harris et al. 1983). Thus, "moderate" levels of grazing, herbicide application, mowing, and/or tillage, if occurring at a time of year that does not disturb nests, either have no effect (Kaminski and Prince 1981a,b, 1984) or increase wetland bird species richness (Kantrud 1981). However, severe grazing, mowing, fire, or herbicide application at inappropriate times is detrimental to waterfowl (Kantrud and Stewart 1984, Higgins 1977, Higgins et al. 1992). Also, wetlands that are tilled during the breeding season tend to support fewer non-waterfowl wetland bird species than do untilled wetlands (Weber et al. 1982). Ongoing studies of Conservation Reserve Program (CRP) lands by the

NPSC are intended to determine relationships of avian richness to patch sizes of unfarmed land. The sizes, types, and distribution of wetlands on the study plots are being recorded incidentally.

Density and Biomass

Field data show that density of waterbirds tends to be greatest in prairie wetlands with the most even balance between open water and vegetation (Stewart and Kantrud 1971, Weller and Spatcher 1965, Weller and Fredrickson 1974, Kaminski and Prince 1981a,b, 1984, McMurl et al. 1993). In an Iowa lakeside marsh, open water patches that were 0.01 ha in size (about 10 × 10 m) were little-used by waterbirds, but patches > 0.02 ha were used by several species, especially if they exceeded 100 m in their longest dimension (Weller 1975). Between years, changes in cover density may also mediate the response of breeding waterfowl to limnological factors (Lillie and Evrard 1994).

Waterfowl pair densities in tilled wetlands (especially wetlands with little crop debris) are only 20% of densities in untilled wetlands (Kantrud and Stewart 1977), and are lower than in grazed wetlands (Barker et al. 1990). However, Kantrud (1981) found the total number of individual birds (of all species) to increase with grazing intensity in North Dakota. In South Dakota, Bue et al. (1952) found virtually no duck nests in areas grazed by cattle for more than 15 days per acre per year.

Reproductive Success

Many studies have shown that reduced reproductive success in waterfowl can be a strong indicator of loss of cover in a wetland or surrounding landscape because of grazing, herbicides, cultivation, or other factors (Dwernychuk and Boag 1973, Higgins 1977, Duebbert and Frank 1984, Cowardin et al. 1995). Analysis of data from Canadian prairie wetlands indicates that waterfowl populations might decrease once cropland occupies more than 56% of a landscape (a rectangular 25.6-km² area), and average nest success might decrease four percentage points for every 10 percentage points increase in cropland (Greenwood et al. 1995). Refinement of research study designs is needed (Clark and Nudds 1991).

6.4.3 Birds as Indicators of Wetland Salinity

Species Composition

Many waterfowl avoid hypersaline or alkali prairie wetlands unless freshwater wetlands are located nearby (Kantrud and Stewart 1977, Lokemoen and Woodward 1992). However, a few other waterbird species occur regularly in alkali wetlands during the breeding season (e.g., American avocet, phalaropes, killdeer) or migration (e.g., tundra swan; white-rumped, semipalmated and Baird's sandpipers)(see Faanes 1982, Kantrud 1986b, Eldridge and Krapu 1993, and Earnst 1994). These relatively salt-tolerant species also occur in less saline wetlands, but their abundance often is greatest in hypersaline wetlands and is related to sharp seasonal peaks in the abundance of brine shrimp and other salt-tolerant invertebrates. Although changes in the frequency or range sizes of these species at a regional level might indicate changes in the occurrence of hypersaline wetlands, birds generally do not appear to be sensitive indicators of less extreme variations in salinity, especially at a site level.

Single-Species Indicators

It is likely that saline conditions that are suitable for nesting mallards and other waterfowl are suitable for sustaining relatively high levels of biodiversity in general. Nonetheless, there are some bird species (e.g., American avocet) and many plants that do not generally occur in wetlands whose salinity is optimal for waterfowl. Definitions and measurements of wetland integrity must be broad enough to account for needs of such species.

Species Richness

Avian richness is predicted reliably by salinity only among wetlands that are the most saline. Avian richness is generally low in hypersaline or alkali wetlands of the prairie region (Faanes 1982).

Density and Biomass

The density of birds nesting in saline wetlands is generally low (Faanes 1982); this is particularly true of waterfowl (Savard et al. 1994). Pair densities of breeding waterfowl in alkali (highly saline) prairie wetlands are only one-tenth the densities in fresher wetlands (Kantrud and Stewart 1977). However, densities of some migrating waterbirds can be high in saline wetlands, sometimes exceeding densities in many fresher wetlands (Kingsford and Porter 1994).

Reproductive Success

Although moderately saline wetlands can be highly productive, reproductive success of some waterfowl species is limited in highly saline wetlands if they cannot gain access to fresh water. For example, mallard ducklings are generally not present (or experience reduced growth) in wetlands with salt concentrations greater than 10–20 $\mu\text{S}/\text{cm}$ unless freshwater springs are present (Swanson et al. 1983, Swanson et al. 1988). In a survey of part of the Canadian prairie, 2% of the wetlands were found to be potentially too saline to support waterfowl reproduction (Leighton and Wobeser 1994).

6.4.4 Birds as Indicators of Sedimentation and Turbidity

Species Composition

Bird species (e.g., redhead) that feed on submersed plants and their associated invertebrates can be defined, and they are likely to be affected the most by turbid conditions in prairie wetlands. At a regional level, changes in the occurrence, frequency, or range sizes of such species might indicate overall trends in turbidity and sedimentation. Using statistical regression analysis, Flake et al. (1977) reported that turbidity negatively influenced numbers of mallard pairs in stock ponds in western North Dakota, whereas a regression analysis in British Columbia (Savard et al. 1994) found positive correlation between wetland turbidity and dabbling duck densities.

Species Richness

Changes in avian richness in response to increased turbidity and sedimentation are being investigated by ongoing work sponsored by the NPSC and USEPA.

Density and Biomass

Changes in avian density and biomass in response to increased turbidity and sedimentation are being investigated by ongoing work sponsored by the NPSC and USEPA.

Reproductive Success

Changes in avian reproductive condition in response to increased turbidity and sedimentation are being investigated by ongoing work sponsored by the NPSC and USEPA.

6.4.5 Birds as Indicators of Excessive Nutrient Loads and Anoxia

Species Composition

No documentation exists for birds being influenced directly or measurably by the nutrient status of prairie wetlands, and accordingly they are probably unsuitable indicators of this stressor. Effects of nutrient enrichment are likely to be expressed as increases in density of vegetation cover or turbidity (from algal blooms), to which birds respond mostly negatively (see Sections 6.4.2 and 6.4.4). In wetlands in other regions, the abundance and/or on-site diversity of songbirds (Brightman 1976, Hanowski and Niemi 1987) and sometimes waterfowl (Piest and Sows 1985, Belanger and Couture 1988) have tended to increase with increased abundance of aquatic invertebrates as in the case of wetland enrichment. However, some anecdotal observations in prairie wetlands suggest that wetlands experiencing prolonged anaerobic conditions following major runoff-induced algal blooms tend to support lower densities of birds (G. Krapu, personal communication, NPSC, Jamestown, ND).

Single-species Indicators

Simple presence of a single species, its nest, and/or broods in a particular wetland is insufficient evidence that the wetland is relatively enriched.

Species Richness

Migrant shorebirds and gulls often appear to concentrate at nutrient-enriched sites, e.g., wastewater lagoons, both in other regions (Fuller and Glue 1980, Campbell 1984) and in the prairies (Swanson 1977a, Maxson 1981). Thus, overall avian diversity might be greater in moderately enriched prairie wetlands than in unenriched ones. However, definitive data are lacking.

Density and Biomass

Large numbers of waterbirds congregate at all seasons in wastewater wetlands where the cover of emergent vegetation is at least partly controlled (e.g., Swanson 1977a, Maxson 1981, Brady and Giron-Pendleton 1983). This high level of use is attributable largely to the ready availability of high densities of invertebrate foods in these areas. Nonetheless, little is known about waterbird responses in prairie wetlands subject to eutrophication from fertilizer runoff.

Reproductive Success

Data are lacking to describe the effects of enrichment of prairie wetlands on waterbird reproductive success.

6.4.6 Birds as Indicators of Pesticide and Heavy Metal Contamination

Species Composition

Few if any pesticides appear to be acutely toxic to waterbirds in prairie wetlands when applied as prescribed, many indirect effects, i.e., mortality of foods upon which waterbirds depend and loss of nesting cover, can be significant (Grue et al. 1986, 1988, 1989, Mineau 1987, Sheehan et al. 1987, Tome et al. 1990, 1991). Some studies from other regions (Hunter et al. 1986) and more recently in prairie wetlands (Martin and Solomon 1990, McCarthy and Henry 1993) have demonstrated indirect impacts to individual birds as a result of pesticide-induced loss of foods. Whether loss of plant and/or invertebrate foods reduces bird populations depends partly on the degree to which birds in a particular situation can avoid contaminated foods or shift (without physiologic damage) their preferences from impacted foods to non-impacted foods.

Foods chosen by birds that inhabit prairie wetlands are relatively species-specific. Thus, the exposure of wetlands to a pesticide or other contaminant that kills only a particular insect or plant species or group might be reflected by the absence or widespread decline of just those bird species that are associated with the target organism/group. For example, a local decline in populations or breeding success of American wigeon and gadwall—ducks that rely most heavily on plant foods—could signify impacts from herbicides. A local decline in northern shoveler could indicate impacts to nektonic invertebrates that are its primary food. Invertebrate food choices of various other prairie waterbirds are summarized from the literature in Appendix C. As noted above, however, the effects on any species of loss of a particular food will depend on the likelihood of unimpacted foods being selected and meeting the physiologic needs of birds. Seasonal timing is also important.

Direct toxicity levels and descriptions of the effects of several heavy metals, selenium, and synthetic organics are given in Hudson et al. (1984), USEPA's "TERRETOX" database, and in the FWS's "Contaminant Hazard Reviews" series that summarizes data on arsenic, cadmium, chromium, lead, mercury, selenium, mirex, carbofuran, toxaphene, PCBs, and chlorpyrifos.

Single-species Indicators

Contaminant levels or population declines of a single species is seldom sufficient to indicate that a particular wetland has been exposed to the contaminant.

Species Richness

Declines in avian richness would be expected at wetland complexes or regions heavily contaminated by pesticides or heavy metals. However, data from prairie wetlands are lacking.

Density and Biomass

It is likely that declines in avian density and biomass should be expected at wetland complexes or regions heavily contaminated by pesticides or heavy metals. However, data from prairie wetlands are lacking.

Reproductive Success, Fledgling Growth, Population Demographics

Many studies have documented birds failing to reproduce or grow successfully in wetlands severely contaminated with heavy metals (e.g., Scheuhammer 1987, Kraus 1989) and particular pesticides, e.g., phorate (Dieter et al. 1995).

Bioaccumulation

Selenium levels of > 0.050 mg/L, or > 0.030 mg/g of body weight, pose a potential risk to many waterbird species because selenium is rapidly accumulated in food chains and body tissues (Welsh et al. 1993). Analysis of waterbirds collected from > 24 wetlands in the prairie region, mostly on National Wildlife Refuges, 1986–1992, revealed problems with selenium accumulation in only a few localities (Ludden 1990, Welsh and Olson 1991). Selenium was not detected in water samples from any of 238 wetlands in a part of the Canadian prairie with selenium-rich soils (Leighton and Wobeser 1994). Incidences of organochlorines, PCB's, and mercury accumulating in prairie birds, especially raptorial and fish-eating species, have been reported (Jackson 1986, FWS 1989, DeSmet and Shoesmith 1990, Larson 1990, Welsh and Olson 1991).

Physical Condition, Deformities, Behavior

Eggshell thinning, physical deformities of embryos and hatching birds, and feather loss in adult birds, are symptoms of severe contamination of wetland food chains with certain chemicals, such as selenium (Scheuhammer 1987, Ohlendorf et al. 1990). Drooping wings and abnormal neck posture can indicate poisoning by carbamate or organophosphate insecticides (Facemire 1991).

Biomarkers

The FWS's Biomonitoring of Environmental Status and Trends (BEST) program has proposed use of several biomarkers, including the following relatively well-established ones:

delta-aminolevulinic acid dehydratase (ALAD). Elevated concentrations of this enzyme in birds and perhaps amphibians can indicate sublethal exposure to lead from highway runoff or birdshot within the previous month.

acetylcholinesterase (AChE). Depressed concentrations of this enzyme in birds, amphibians, and invertebrates can indicate exposure generally within a few hours or days to organophosphorus and carbamate insecticides (Ludke et al. 1975), and perhaps to some heavy metals.

cytochrome P450 mono-oxygenase system (MO). Elevated concentrations of this enzyme in birds can indicate exposure, within the previous few days or weeks, to various organic hydrocarbons.

hexacarboxylic acid porphyrin (HCP). Elevated concentrations of this enzyme in birds can indicate ongoing exposure to various organic hydrocarbons.

retinol (vitamin A). Depressed concentrations of this enzyme can indicate reduced viability of individual birds (Wobeser and Kost 1992).

thyroid hormones. Depressed concentrations of various thyroid hormones in birds can indicate ongoing exposure to various organic hydrocarbons.

Laboratory costs for analysis of any of the above biomarkers generally range from \$15 to \$75 per sample, processed at a rate of about 20 to 30 samples per day. Other potential biomarkers for use with terrestrial vertebrates are described in Harder and Kirkpatrick (1994).

6.5 Monitoring Techniques

Methods for censusing of waterfowl in prairie wetlands are summarized by Hammond (1969), Klett and Johnson (1981), Klett et al. (1986), Ball et al. (1988), and Higgins et al. (1992). Although not specific to prairie wetlands, Kirby (1980) and Eng (1986) also discuss waterfowl censusing. Methods for censusing marsh and shorebirds are discussed by Connors (1986), Weller (1986), and Clark and Murkin (1989). Methods for surveying entire bird communities within individual habitats are described by Burnham et al. (1980), Ralph and Scott (1981), Halvorson (1984), Verner (1985), Verner and Ritter (1985), and others.

6.5.1 General Surveys

Observations that are part of a survey covering several wetlands should occur simultaneously or be made within consecutive days unless severe weather conditions intervene. If the objective is to compare between-year trends in a species, total species, or species richness, then simple count methods (e.g., transects) are probably appropriate. However, if the objective is to rank

wetland types or relative abundance of species, more time-consuming censusing techniques are required to develop estimates of density (Steele et al. 1984). Determination of indices of relative annual abundance, rather than an exhaustive census of the population, is suitable for most purposes (Emlen 1981).

Although most common songbirds will not be disturbed by frequent visits by monitoring personnel, raptors, waterfowl, other large or colonial species, and ground-nesting species can be susceptible. Wetland songbird surveys are commonly conducted during May through July, when breeding birds are most detectable by song. Species detection (especially of most songbirds) is greatest during early morning hours. However, in winter some species are active at mid-day. Night-time coverage is sometimes warranted, not only for typically nocturnal species such as owls, but also for waterfowl and wading birds which sometimes use different prairie wetland types for roosting and for feeding (e.g., Swanson and Sargeant 1972). Secretive species (e.g., rails, some passerines) have sometimes been surveyed more effectively by playing back tape recorded calls, using predator decoys, using dogs, and dragging ropes or chains through wetlands (e.g., Glahn 1974, Ralph and Scott 1981, Gibbs and Melvin 1993).

Surveys can be conducted from ground level, from elevated observation posts, or aerially. Ground-level, visual techniques cannot be used effectively in wetlands with tall vegetation. Boats are typically used for surveys of wetlands wider than about 100 m, as visibility from shore, even using a spotting scope, becomes restricted.

6.5.2 Reproductive Success

Where birds that colonize bird boxes are present, boxes provide a convenient means of monitoring reproductive success with minimal disturbance and without the labor of having to find nests. In other regions, boxes have been used successfully to monitor impacts from heavy metals (Kraus 1989, Peterson and McEwan 1990) and acid precipitation (St. Louis and Barlow 1993).

6.5.3 Time Budget Analysis

Studies on prairie wetlands (Murkin and Kadlec 1986a, Eldridge and Krapu 1993) have demonstrated that estimates of bird density are not necessarily sufficient to indicate a degraded wetland condition (i.e., a wetland with diminished invertebrate densities), yet documenting the hours of use of the wetland by various species can successfully indicate such a condition. Such a time-budget approach usually requires purchase and installation of video equipment that automatically photographs portions of the wetland at specified intervals. From viewing the tapes, the duration of each activity (e.g. feeding) of visible birds in each photographed zone can be determined. This method would be costly to implement for studies intended to survey more than a few wetlands.

6.5.4 Bioassay Methods

A review of laboratory, outdoor mesocosm, or *in situ* bioassay methods involving birds or other wildlife is beyond the scope of this report. Use of bioassays to explore direct contaminant toxicity to birds has been relatively limited in prairie wetlands.

6.5.5 Bioaccumulation

Methods for assessing bioaccumulation of contaminants in bird tissues are described in Moser and Rope (1993a).

6.6 Variability and Reference Points

The following subsections describe spatial and temporal variability in avian community composition of prairie wetlands.

6.6.1 Spatial Variability

Species Richness

As a point of reference, of the 302 bird species that occur regularly at some season in the prairie region (Faanes and Stewart 1982), about 104 occur regularly in wetlands, 35 solely as migrants, and 69 additionally as nesters (interpretation based on published literature and personal experience, see Appendix C). During the nesting season, this wetland component of the avifauna represents 78% of all 88 nesting species that Short (1989) notes are found regularly in any habitat in the prairies.

In Iowa, a breeding-season survey of 17 restored prairie wetlands found 2–18 species per wetland (Hemesath 1991). No species occurred in all wetlands and four species occurred only in one. A survey of another 11 Iowa wetlands, both natural and restored, reported a cumulative total of 22 species (Delphay and Dinsmore 1993). In yet another survey in Iowa, covering 30 natural wetlands ranging in size from 0.2 to 182 ha, Brown and Dinsmore (1988, 1991) found a range of 2–17 species per wetland, similar to the 7.2 species per wetland found by Dinsmore et al. (1993).

In North Dakota, a 2-year survey of 452 North Dakota prairie wetlands, in which each wetland was visited 1–2 times annually to include the breeding period, found an average of three species per wetland (range 0–45 species) (Igl and Johnson, unpublished data, NPSC, Jamestown, ND). Richness varied among six subregions of the prairie region, from 1.38 species per wetland in one subregion to 3.74 species per wetland in another. Cumulatively (among all the wetlands), 101 species were found the first year and 113 the second. In a single-visit survey of 95 randomly selected plots in North Dakota wetland subregions, Kantrud (1981) found an average of about seven breeding species per 31.5-ha plot. Avian richness was less variable among landforms than was avian density. About 750 acres of one North Dakota wetland (Kraft Slough) supported 29 breeding species (Krapu and Duebbert 1974). In a 1-year survey of breeding species in the Cheyenne Lake area of North Dakota, Faanes (1982) found 36 species in a cumulative area of 76 ha of permanent wetland, 25 species in 24 ha of seasonal wetland, 22 species in 20 ha of semipermanent wetland, and 17 species in 44 ha of alkali (saline) wetlands.

A major source of information on the species composition, distribution, and relative abundance of prairie birds is the FWS's Breeding Bird Survey (BBS). This is a database containing data collected, in some instances, as far back as 1966. Birds seen or heard at each of 50, 3-minute stops along 25-mile roadside routes are recorded. Both waterfowl and nonwaterfowl are

surveyed, as well as both wetland and nonwetland habitat (which are not identified specifically by the survey). At a regional level, the FWS has calculated trends in abundance of each species; trends of wetland species are shown in Appendix C ("Priority" column). For Appendix C of this report, data from 160 routes in BBS strata 37, 38, and 40 were tabulated for the years 1966–1993. Each of these strata is located predominantly within the region commonly considered to be the prairie pothole wetland region. Along these 160 BBS routes, a cumulative total of 74 wetland species (71% of all wetland species that occur regularly at any season in the region) have been recorded at least once during 27 years. During any year, the median number of wetland species per route is 15 (14% of the region's wetland avifauna) and ranges from 46 species (44%) on the richest route during its highest-count year to three (3%) on the poorest route during its lowest year. Among years, the richest route averages 37 wetland species, and the poorest route averages five wetland species.

Fewer data are available to describe bird richness during migration periods. In a 28-visit springtime survey of 13 varied wetlands in Brookings County, South Dakota, the cumulative species total ranged from 0 to 21 species per wetland (Brady and Giron-Pendleton 1983). A cumulative species total of 137 species (60 of them breeding) was found during a year-round, 2-year study of a 176-ha restored prairie wetland in Minnesota (Svedarsky et al. 1993).

Density

Many investigators have documented waterfowl pair densities, both in wetlands and in adjoining habitat, during the breeding season. Density estimates of waterfowl are difficult to compare, and habitat relationships are difficult to define because unit of area can be measured in numerous ways (Savard et al. 1994). Densities or regional population estimates based on probabilistic sampling of hundreds of wetlands of all types are reported by Stewart and Kantrud (1974), Brewster et al. (1976), Kantrud and Stewart (1977), Higgins (1977), Ruwaldt et al. (1979), Krapu et al. (1983), and Duebbert and Frank (1984). Densities from smaller numbers of wetlands are reported by Munding (1976), Krapu and Green (1978), Higgins et al. (1992), and many others.

Pair densities and/or regional abundance estimates of non-waterfowl species (as well) are reported from multiple wetlands by Stewart and Kantrud (1972b), Kantrud (1981), Faanes (1982), Weber et al. (1982), Kantrud and Stewart (1984), Brown and Dinsmore (1986), and Igl and Johnson (unpublished data, NPSC, Jamestown, ND). The Igl and Johnson study (described in Appendix L) found an average of 5.43 pairs of all species per wetland in the prairie region of North Dakota (median = 0, range = 0–744 pairs). The number of pairs believed to actually be breeding averaged 3.12 per wetland (median = 0, range = 0–397 breeding pairs). Mean density of breeding pairs varied spatially (among six subregions of the prairie region) from 2.04 to 13.92 pairs per wetland. The maximum pairs per wetland also varied among subregions, from 27 pairs in a Agassiz Lake Plain wetland to 397 pairs in a Northwestern Drift Plain wetland. About 750 acres of one North Dakota wetland (Kraft Slough) supported 2934 breeding pairs (Krapu and Duebbert 1974). Similar data from other areas of the prairie region (including some non-wetland habitat) are reported as part of the Breeding Bird Censuses published in the journals *American Birds* and *Audubon Field Notes*.

Considering just the 1967–1987 period, there were only 76 instances in which any of the 7255 ten-mile route segments (that constitute BBS routes in the prairie region) failed to contain a

single wetland species, and no segments have been devoid of wetland species during every year that they have been visited. During any year, wetland species are typically found at greater than 9 (18%) of the 50 stops along each prairie BBS route (range = 1–50), and the median number of individuals (all wetland species combined) per route is 159. The number of individuals of wetland species varies spatially from 1133 on the richest route to 30 on the poorest during years when numbers vary the least among routes. During years when numbers vary the greatest among routes, the number of individuals varies from 1247 on the richest route to 6 on the poorest.

Species Composition

Of the 67 wetland species ever recorded from prairie BBS routes, a majority have been recorded on at least 62% of the routes. However, of the 128 species found in prairie wetlands by Igl and Johnson (unpublished data, NPSC, Jamestown, ND), none were found in more than 41% of the individual wetlands, and a majority were present only in less than 1% of the wetlands surveyed.

Bioaccumulation

No published data pertaining to spatial variability of bioaccumulation in prairie wetlands were found.

Reproductive Success

Dozens of studies in prairie wetlands and adjoining grasslands have documented reproductive success rates of waterfowl (e.g., Sargeant et al. 1995). Nest success rates of about 50% are typical for many species (Solberg and Higgins 1993b), but spatial and annual variability is great.

6.6.2 Temporal Variability

Species Richness

Between years, the variety of breeding birds on a single prairie wetland can range from near 0 species to over 20, depending largely on water conditions. Among 6 restored prairie wetlands that were sampled in Iowa for 2 years, richness changed dramatically between years in 2 of the wetlands (from 1 to 5–6 species, as the wetland matured during its first post-restoration year), moderately in 3, and not at all in 1 (Hemesath 1991). In another survey in Iowa, the species richness in each of 30 natural wetlands did not change between two consecutive years in 14 (47%) of the wetlands, and there was no statistically significant difference in species richness between the years (Brown and Dinsmore 1988).

Igl and Johnson's 2 years of data from North Dakota prairie wetlands show that the number of species per wetland changed from 2.71 in 1992 (a dry year) to 3.22 in 1993 (a wet year). The greatest interannual variation was in the Agassiz Lake Plain subregion, where richness per wetland changed from 0.88 in 1992 to 1.77 in 1993.

Along most BBS routes in the prairie region, the number of wetland species has varied by a factor of less than 2.6 between years, but in one extreme instance changed from 1 to 11 species between years. This was calculated as the number of species on the route during the year

having the most species, minus the number of species on the route during the year having the fewest. Bird richness in prairie wetlands is lowest in winter, but the few birds present at that season are highly dependent on the vegetative cover of the wetlands.

Density

In a wetland complex near Woodworth, North Dakota, that was surveyed annually for 17 years, the density of waterfowl pairs varied annually from 19 to 56/km² and averaged 40/km² (Higgins et al. 1992). Brood densities ranged from 10 to 63/km² and averaged 12/km². Mallard densities over a 20-year period in another part of eastern North Dakota varied at least fourfold over a multiyear period (Krapu et al. 1983). Among waterfowl species, the northern pintail, green-winged teal, northern shoveler, and American wigeon appear to have the greatest interannual variability (Stewart and Kantrud 1974).

Igl and Johnson's 2 years of data from North Dakota prairie wetlands show that the mean number of pairs changed from 5.62 in 1992 (a dry year) to 5.64 in 1993 (a wet year). As was true of species richness, the greatest interannual variation was in the Agassiz Lake Plain subregion, where mean number of pairs per wetland changed from 1.48 in 1992 to 2.48 in 1993, indicating a rapid response to improved water conditions. Among wetlands that contained birds both years, the largest changes in numbers of pairs occurred in a wetland that experienced an interannual increase of 22 pairs (30% increase) and the largest decrease occurred in a wetland that experienced an interannual decrease of 49 pairs (12% decrease). Among wetlands that contained birds both years, the species that showed the greatest interannual change (averaged among all wetlands) were ruddy duck, eared grebe, bank swallow, black tern, American wigeon, gadwall, redhead (declined between years), and Forster's tern, green-winged teal, American coot, double-crested cormorant, and Franklin's gull (increased between years). Such species might be good candidates as indicators of environmental change. However, in some situations the annual fluctuations in waterfowl densities are caused by different environmental factors in different wetlands (Lillie and Evrard 1994).

Data for prairie BBS routes during 1967–1987 indicate that the number of individuals of wetland species can vary interannually by a factor of 49 (the most temporally dynamic route), but on most routes varied by a factor of less than 3.58. This was based on 15–21 years of data from the 12 BBS routes having the most years of coverage. Along the routes, interannual trends in nonwaterfowl wetland birds weakly mimic trends in waterfowl. Specifically, the number of nonwaterfowl individuals (summed across routes) is correlated ($r = 0.38$, $p < 0.09$, $n = 21$) with waterfowl individuals summed across routes, and the frequency of BBS stops at which waterfowl were present is correlated ($r = 0.51$, $p < 0.02$, $n = 21$) with frequency of stops at which nonwaterfowl were present. The mean richness per route of all wetland species hit lows in 1971 and 1981, despite coverage of a normal number of routes during those years.

Among 60 wetland species for which there are sufficient BBS data to calculate long-term trends by subregion within the prairie region (see Appendix C), 49 species (82%) have declined in one or more of the three subregions (the number is 44 species if only the trends that are statistically significant are included). By subregion, the eastern and central subregions appear to have a larger percentage (67%) of decreasing species than the western subregion (44%).

Reproductive Success

Based on analysis of data from over 3000 nests during an 18-year period in the prairie region, Klett et al. (1988) concluded that average nest success has changed little for most waterfowl species in most subregions. Duck production in wetlands of the Woodworth complex ranged from 15 to 61 broods per 100 pairs over a 17-year period and averaged 30 broods per 100 pairs (Higgins et al. 1992).

Bioaccumulation

Based on 7 years of data from North Dakota wetlands, Welsh et al. (1993) speculated that bioaccumulation of selenium might be greater during drought years.

6.7 Collection of Ancillary Data

It is easier to separate the anthropogenic from the natural causes of impairment of community structure if data are collected or inferred simultaneously on the following variables of particular importance to wetland birds:

- distribution of water depth classes
- vegetation (type, and vertical and horizontal diversity and arrangement)
- conductivity and baseline chemistry of waters and sediments (especially conductivity)
- distance and connectedness to other wetlands of similar or different type
- surrounding land cover (particularly within 500 feet of wetland perimeter)
- shoreline slope
- wetland size
- cover ratio
- spatial interspersion among vegetation classes
- duration, frequency, and seasonal timing of regular inundation
- time elapsed since the last severe inundation or drought.

All of these features vary to a large degree naturally, as well as in response to human activities such as soil tillage, compaction, and erosion; fertilizer and pesticide application; and water regime modification. In addition, disturbance from the presence of humans visiting wetlands can directly alter the bird-community composition of the wetlands.

6.8 Sampling Design and Required Level of Sampling Effort

"Scale" is an important issue in monitoring the birds of prairie wetlands. Methods used to determine bird density and richness within a wetland become impractical and even inaccurate when the objective is to make comparisons among many wetlands or wetland complexes, especially at regional scales. Likewise, regional-scale methods are often too coarse for application to individual wetlands.

When monitoring birds within prairie wetlands, point count methods have been used most often. In Iowa, Brown and Dinsmore (1986) and Delphey and Dinsmore (1993) used fixed-radius (18 m) circular plots; the first plot was placed randomly in a wetland and the rest were placed equidistantly around the wetland until the investigators could not locate a plot at least 60 m from another (or when a total of five were established). Alternatively, when surveying wetlands with particularly tall, dense vegetation and limited access, the survey points might be placed such that the largest portion of the wetland is visible from the fewest points. A third option would be to allocate points in proportion to the sizes of various vegetation zones and water depths, if these strata can be delimited beforehand. Where quantitative estimates of populations are not needed, less formal survey methods can be used. For example, in bird surveys of quarter-sections (e.g., Kantrud and Stewart 1984), observers have simply walked in as straight a line as possible through all habitats they recognize within a fixed area.

Costs of surveying birds depend on the number of visits that need to be made per wetland, the number of points to be visited, and the duration of observations at each point. As with other taxa, if numbers of individuals are to be estimated, the intensity of effort should reflect the expected variability (coefficient of variation) and the desired precision. If the objective is to assess species composition and biodiversity, species accumulation curves should also be plotted, as described below (Section 6.8.1) and in Section 1.5.

Some biologists in other regions suggest that, for reasonably accurate estimates of breeding bird richness in a wetland, three visits spread over the breeding season is usually desirable (Brooks et al. 1989, Weller 1986). This is advisable because some waterfowl species breed in May, most songbirds breed in June, and the remaining songbirds breed in July and August. In studies of breeding birds in Iowa wetlands, the number of visits per wetland ranged from two (LaGrange and Dinsmore 1989b) to five (Delphey and Dinsmore 1993). The time spent per observation point ranged from six to eight minutes. Only a single visit was made to most of the 128 areas covered by a survey of nongame birds conducted by the NPSC.

The foregoing discussion has described sampling of individual wetlands or quarter-sections. If the objective is to estimate species composition, richness, and numbers at only a regional level, then a different design can be used. For example, the Breeding Bird Survey bases estimates of avian distribution, relative abundance, and trends on just a single 3-minute visit annually to hundreds of points in a region. To conduct a regional survey of prairie avifauna, Stewart and Kantrud (1972a, 1973) and Kantrud and Stewart (1984) selected quarter-sections (64.7 ha) as plots. The plots were randomly selected by a cluster sampling (without replacement) process, in which 120–130 quarter sections were grouped as 30 clusters, with clusters reflecting the major landforms of the prairie region. A stratified random and cluster sampling design was also implemented, in two stages, in regional avian surveys by Brewster et al. (1976) and Ruwaldt et

al. (1979). They found that cluster sampling reduced the number of zero observations and travel time, and thus increased the number of wetlands that could be visited. In just one field season, they were able to do an avian survey involving two visits to 500 quarter-sections (64.7 ha each). Four of these quarter-sections were selected, one in each of the four compass directions, from a corner of each of 125 townships which had been selected randomly, for a total of 500.

Two hours were spent surveying birds in each quarter-section (about two minutes per ha). A two-person survey of 128 quarter-sections was able to cover about 160 acres in 1–2 hours (about three minutes per observer per ha) (L. Igl, personal communication, NPSC, Jamestown, ND). Because he was surveying a much smaller region and had somewhat different objectives, Faanes (1982) used smaller (16.2 ha) plots which he was able to visit for longer duration.

If not only richness, but density, must be determined, then at least eight visits are probably needed (Ralph and Scott 1981).

6.8.1 Asymptotic Richness: Results of Analysis

For this report, we used two data sets to estimate species accumulation and asymptotic richness. The monitoring design and data structure of the BBS data are detailed in Appendix L. The Igl and Johnson data are detailed in Appendix O. The BBS data, 1967–1993, were from all routes in the prairie region, and the Igl and Johnson study covered 452 individual wetlands during 1992–1993. For the BBS data set, we examined only the assemblage of species that are most characteristic of wetlands (see introduction to Appendix C), whereas for the Igl and Johnson data, we examined all species.

Analysis of the Breeding Bird Survey (BBS) Data

Several issues were considered in the analysis of the BBS data:

Number of years. We analyzed data from the two routes having the greatest species richness in each of the three subregions of the prairie (strata 37, 38, and 40). On these routes, half the wetland species found during the entire interannual period of the route could generally be found during any 2 years. To find 90% of the wetland species collectively present during the entire period of coverage required a number of years equivalent to 37%–83% of the route's total years of coverage. Of the six species-rich routes, the one with the longest coverage required 15 years (range, 9–22 years) to detect 90% of the 49 species that were found collectively during the entire 27 years of that count.

Number of routes. In each of the three subregions, we analyzed data from the 2 years in which the most species were detected. Half the wetland species found collectively among all 29–40 routes run per subregion during these years could generally be found on any 2–3 routes. To find 90% of the wetland species collectively present on all these routes during a given year required between 15 and 19 routes (or 44%–59% of the total routes run during those years in the subregion). During the year (1993) in which the most BBS routes were run, 16 routes (range, 5–32) were needed to detect 90% of the species in each of two of the subregions.

Another subset of the data also was examined to estimate the requisite number of routes. This subset included just 11 routes that had been run during the same 26 or 27 years. This analysis indicated that half the wetland species present collectively among all these routes during the entire 26–27 years could be detected on just two routes at least sometime during that period. Finding 90% of the species would require four routes (range, 2–7).

Number of route segments. The objective of the analyses described above was to estimate the requisite number of routes. Each route contains 50 point counts whose totals are aggregated into five subtotals, each representing 10 point counts conducted over a 5-mile segment. To analyze these segment subtotals, we considered just three routes, selected on the basis of their being the richest routes run during any year in their subregion. This analysis indicated that two segments were sufficient to detect half the wetland species found collectively with all five segments, and four segments (range, 2–5) were needed to detect 90% of the species total.

Analysis of the Igl and Johnson data

Species accumulation among wetlands was determined separately for 1992 and 1993 as well as for breeding individuals vs. total individuals (presumed non-breeding as well as breeding). Species accumulation among wetlands rose and began to level off sooner for breeding individuals than for total individuals, and accumulation was slightly faster in 1993 than in 1992 (Appendix O). If only half the number of wetlands had been sampled, the cumulative species list would have been about 75%–80% as large.

6.8.2 Power of Detection: Results of Analysis

Analysis of the BBS data

The BBS monitoring program was better able to detect inter-route differences in the total sampled number of wetland species than in the total number of stops containing wetland species or in the total number of individuals of wetland species. The data suggested that, for the prairie pothole region as a whole, a sample size of 10 BBS routes would allow detection of inter-route differences of six wetland species, 29 stops containing wetland species, or 140 individuals of wetland species. When variability is reduced by analyzing data from just the routes that have had the most consistent coverage over the years, the respective detection limits are two species, 25 stops, and 120 individuals. The data suggest that conducting additional BBS routes in the prairie region, beyond about six routes, has diminishing effects on increasing the precision of the richness-per-route estimates. Increasing beyond 10 routes has diminishing effects on the increase in precision of the other two variables (stops with wetland species, total individuals of wetland species).

Analysis of the Igl and Johnson data

If wetlands similar to those examined by Igl and Johnson are visited twice annually for 2 years, approximately 10 wetlands would need to be visited to detect a difference of two species

between any two wetlands. To detect a difference of 10 breeding pairs, about 35 wetlands would need to be visited.

6.9 Summary

The species composition of bird communities, and to a lesser degree their species richness, demonstrates diagnostic responses to changes in water levels and duration, and to vegetative cover conditions, within a prairie wetland complex (Table 9). Birds also may respond over the long term to changing wetland nutrient levels, sedimentation, and contaminant levels, but existing information is too limited and confounding effects are too prevalent to currently allow widespread use of birds to diagnose impairment of prairie wetlands from these stressors. Even for the responses to water regime and vegetation change, the ability to use birds to distinguish natural from anthropogenic levels of wetland disturbance is currently limited.

Bird communities are practical to monitor because sampling is nondestructive, and identification is relatively simple. Although their high mobility confounds attempts to use birds as indicators of the condition of individual wetlands, changes in species composition within a wetland complex or subregion can demonstrate impacts to wetlands that are occurring at such broad scales. Birds are the only group suitable and practical for indicating such impacts.

Individual prairie wetlands that are semipermanently flooded generally contain about three pairs of breeding birds, representing three species. Some wetland complexes and larger individual wetlands can support at least 400 breeding pairs representing 40 species. Between years, the variety of breeding birds on a single prairie wetland can range from near 0 species to over 20 species, depending mainly on water conditions.

Additional data collected and applied primarily at a landscape or regional scale are needed to support hydrologic and water quality criteria for nesting waterfowl and migratory shorebirds. Related information is needed on the degree to which surrounding condition of upland habitats influences the hydrologic and water quality requirements of wetland birds. Data are clearly needed on factors that influence use of prairie wetlands by migratory shorebirds and on the impacts of sedimentation and nutrient enrichment on the sustainability of wetlands as habitat for waterbirds.

Table 9. Summary evaluations of possible invertebrate indicators of stressors in prairie wetlands. Evaluations are based on technical considerations, not cost or practicality. A rating of FAIR or POOR is assigned when too few data (FD) suggest potential as an indicator, or when confounding effects (CE) of other variables often overshadow the effects of the listed stressor on the indicator

Stressors	Possible Indicators (when measured at a regional or wetland-complex scale)	Evaluation
Hydrologic stressors	Species composition Single-species indicators Richness Density, biomass Reproductive success	FAIR (CE) POOR FAIR (CE) GOOD GOOD
Changes in vegetative cover	Species composition Single-species indicators Richness Density, biomass Reproductive success	GOOD GOOD FAIR (CE) FAIR (CE) GOOD
Salinity	Species composition Single-species indicators Richness Density, biomass Reproductive success	FAIR (CE) FAIR (CE) POOR POOR FAIR (CE)
Sedimentation & turbidity	Species composition Richness Density, biomass Reproductive success	FAIR (FD) FAIR (FD) POOR (FD) POOR (FD)
Excessive nutrients & anoxia	Species composition Single-species indicators Richness Density, biomass Reproductive success	POOR POOR (CE) FAIR (CE) FAIR (CE) POOR (FD)
Herbicides	Species composition Single-species indicators Richness Density, biomass Reproductive success	FAIR (FD) POOR (FD) POOR (FD) POOR (FD) POOR (CE)
Insecticides	Species composition Single-species indicators Richness Density, biomass Reproductive success Bioaccumulation Physical condition, behavior Biomarkers	FAIR (FD) POOR (FD) POOR (FD) POOR (FD) GOOD FAIR (CE) FAIR (CE) GOOD
Heavy Metals	Species composition Single-species indicators Richness Density, biomass Reproductive success Bioaccumulation Physical condition, behavior Biomarkers	POOR POOR (FD) POOR (FD) POOR (FD) GOOD GOOD FAIR (CE) GOOD

7. Synthesis and Recommendations for Indicators

Determining the ecological integrity of a prairie wetland and diagnosing possible causes of impairment should involve monitoring multiple indicators. In most prairie wetlands the possibility of ongoing or recent past exposure to excessive sedimentation is probably best indicated by species composition of algae and invertebrates, with emphasis on the epibenthic forms (taxa that live on the top surfaces of the sediment). Epibenthic and epiphytic algae and invertebrates are also useful indicators of excessive enrichment, removal of vegetative cover, and turbidity that is occurring either currently or during past years as determined by analysis of decay-resistant remains. Ongoing or recent past changes of water regime and salinity, as well as overgrazing, in individual wetlands are perhaps best indicated by vascular plant species composition. Longer-term changes in these factors can be inferred by examining seed banks and decay-resistant remains of invertebrates. Exposure to pesticides and heavy metal contaminants can sometimes be inferred from species composition of invertebrates and from various biomarkers in amphibians and birds. For bioaccumulative contaminants, tissues of individual plants and birds can be examined. Birds are also uniquely valuable for spatially integrating information on the hydrologic stresses to wetlands across entire regions.

Although the choice of indicators and sampling methods is vital to establishing monitoring programs, equally important are questions of how to interpret the collected data. Information provided throughout this document is intended to support data interpretation, and potentially diagnostic symptoms of the ecological integrity of prairie wetlands are summarized in Table 10. Responses of wetland communities to environmental change are extremely variable and difficult to interpret or predict. Following are some examples of symptoms that sometimes are associated with particular causes.

Table 10. Representative, sample symptoms of changes in the ecological integrity of prairie wetlands and examples of possible causes (Because of a generally poor understanding of prairie wetland variability, these examples are anecdotal, not definitive.)

Symptom	Possible Cause(s)
Plant species richness or number of functional groups (Boutin and Keddy 1993) is declining or is low relative to reference areas.	Wetland is being exposed (or recently has been exposed) to contaminants, increasing or decreasing water levels, excessive nutrient or sediment inputs, or intense grazing.
Plant species richness or number of functional groups is increasing or is high relative to reference areas.	Wetland is being exposed (or recently has been exposed) to increasing or decreasing water levels, moderate nutrient inputs, or moderate levels of grazing or other vegetation-thinning activities.
Blooms of algae occur more often, for longer periods, and/or at atypical times of year.	Nutrient loading of the wetland from external sources has increased recently; and/or enriched sediment or decaying vegetation is being reflooded following a drier period; and/or grazing, mowing, herbicides, or other factors have removed vegetation that formerly shaded the water column; and/or contaminants or other factors have reduced populations of zooplankton and other organisms that otherwise control algae by grazing.
Percent cover and stem density of emergents and associated epiphytic algae is relatively small or declining, coinciding with larger or increasing cover of submersed and floating-leaved species and (perhaps) phytoplankton and benthic (epipellic) algae.	Recent years have been wetter than normal and/or wetland has recently been burned, mowed, tilled, treated with herbicides, or intensely grazed
Percent cover and stem density of emergents is extensive or increasing, coinciding with small or declining cover of submersed and floating-leaved species and/or phytoplankton and benthic (epipellic) algae.	Recent years have been drier than normal; and/or wetland has not been disturbed by fire, tillage, or similar disturbance for many years; and/or water is very turbid because of sediment runoff, wind resuspension of bottom sediments, or previous algal blooms triggered by excessive nutrient inputs.
The number of species and dominance of algae, vascular plants, and/or invertebrates known to be characteristically tolerant of turbidity and sediment deposition (Appendices A, B) is increasing relative to species that are not or is high relative to reference areas.	Wetland is being exposed (or recently has been exposed) to increasing runoff of sediment, shoreline erosion, resuspension of bottom sediments by wind or livestock.
The number of species and dominance of algae, vascular plants, and/or invertebrates known to be characteristically tolerant of high salinity (Appendices A, B, D) is increasing relative to freshwater species or is high relative to reference areas.	Wetland is being exposed (or recently has been exposed) to increasing salt concentrations as a result of increased evapotranspiration, discharge of groundwater into the wetland, or other factors.

Table 10 (continued.)

Symptom	Possible Cause(s)
The number of species and dominance of vascular plants known to be characteristically tolerant of tillage is increasing or is high relative to reference areas.	Wetland soils have recently been tilled.
Emergent plants are concentrated in middle of wetland, surrounded by a ring of open water.	Recent years have been wetter than normal and/or margins of the wetland have been intensely grazed.
Tall robust plant species are dominant or increasing in dominance.	Wetland is exposed to relatively large or increasing loadings of nutrients and/or sediment and/or wetland has not been disturbed by fire, tillage, or similar disturbance for many years.
Robust plants that are unpalatable to cattle are dominant or increasing in dominance.	Wetland has been intensely grazed, perhaps because prior years have been dry, allowing livestock better access to wetland vegetation.
There is poor germination of emergent plants after drawdown.	Drawdown occurred too late in the season (after July) and/or recently deposited sediment or plant litter (especially from submersed plants and filamentous algae) is reducing light and/or oxygen needed by seeds.
Few annual plant species are dominant, or annuals are declining in dominance.	Wetland is intensely grazed or mowed early in the growing season.
Seed bank is relatively devoid of wetland species, and soil samples wetted and incubated in the laboratory produce few macroinvertebrates.	Wetland was mostly dry during previous decades.

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APPENDICES

The content and abbreviations used in electronic appendices (Appendix A-O) are described on the following pages. The appendices themselves are contained on the computer diskette accompanying this report. To read the appendices, copy all the files on the diskette to a hard drive directory on an IBM compatible computer. At the DOS prompt type browse and you will see a list of the appendices, from which you can select the one you want to view. Press the esc key to exit the appendix at any time and return to the browse menu. If you have a commercial database program such as dBase or Paradox, you can import files of the appendices and use the program to sort, link, or tally information.

Appendix A. Taxonomic Index: Plant Species Tolerances and Responses to Water Regime, Drainage, Land Use, Salinity, and Turbidity; Food Value to Waterfowl; Toxicity Data Availability

This database primarily includes vascular plant species that the literature reports to be dominant or abundant in at least one prairie wetland; it does not include all prairie wetland plant species. Three aquatic mosses/liverworts and one alga are also included because of their large size. Blanks in this database indicate a lack of information for the species. Codes in the database mean:

Form [from Reed (1988)]

A	=	annual,
B	=	biennial,
P	=	perennial
N	=	native,
I	=	introduced
E	=	emergent
F	=	forb
G	=	grass
GL	=	grasslike
H	=	partly woody
H2	=	horsetail
J	=	algae
M	=	aquatic moss
P3	=	pepperwort
S	=	shrub
Z	=	submersed
\$	=	succulent
/	=	floating

Dependence [from Hubbard et al. (1988), Reed (1988)]

Reed (1988) notes that the dependence category should not be equated strictly to degrees of wetness because many obligate species occur in temporary or seasonal wetlands (although most occur in permanent or semipermanent wetlands).

OBL	=	Obligate wetland species that under natural conditions occur almost always (> 99% probability) in wetlands.
FACW	=	Facultative wetland species that usually occur in wetlands (67%–99% probability) but occasionally are found in nonwetland upland (terrestrial) habitats.
FAC	=	Facultative species equally likely to occur in wetlands or nonwetland upland habitats.
FACU	=	Facultative upland species that usually occur in nonwetlands (67%–99% probability) but occasionally are found in wetlands.

+ , - = Slightly wetter (+) or drier (-) than indicated by one of the four acronyms above.

WaterType [from Kantrud et al. (1989)]

SS = seasonally flooded wetlands
SP = semipermanently flooded wetlands
P = permanently flooded wetlands
T = temporarily flooded wetlands
CAPs = dominant in this habitat
lower case = less dominant but occurs frequently

Drainage

l = Seeds are relatively intolerant of sustained drainage; these are the species that Galatowitsch (1993a, b) found in natural wetlands but not in restored (reflooded) wetlands that had been drained for long periods.
t = Seeds are relatively tolerant of sustained drainage; these are the species whose seeds Wienhold and van der (1989) found to be viable even after 30 years of drainage.

Land Use [from Kantrud et al. (1989)]

g = Species typically invades grazed wetlands, or it is disproportionately unaffected by grazing.
h = Species typically invades hayed wetlands, or it is disproportionately unaffected by haying activities.
p = Species typically invades plowed wetlands, or it is disproportionately unaffected by soil tillage.
r = Species typically occurs mainly in relatively undisturbed (reference) wetlands.

Salinity [mainly from Kantrud et al. (1989)]

f = fresh (< 800 $\mu\text{S/cm}$ conductivity, or < 3 g/L salt).
o = oligohaline (800–8,000 $\mu\text{S/cm}$ conductivity, or 4–20 g/L salt).
m = mesohaline (8,000–30,000 $\mu\text{S/cm}$ conductivity).

- p = polyhaline (30,000–45,000 $\mu\text{S}/\text{cm}$ conductivity).
- e = euhaline or hyperhaline (> 45,000 $\mu\text{S}/\text{cm}$ conductivity, or > 70 g/L salt).
- * = documented specifically by literature [other entries were based on judgement of Kantrud et al. (1989).
- CAPS = salinities where it is dominant (not merely occurring) according to Kantrud et al. (1989).

Turbidity [mainly from Kadlec and Wentz (1974), Davis and Brinson (1980), Nichols (1984), Chambers and Kalff (1985)]

- t = Species typically invades wetlands with highly turbid water (minimal light penetration) or is disproportionately unaffected by turbidity increases.
- x = Species typically occurs in wetlands with the least turbid water (great light penetration) or is highly sensitive to turbidity increases.

Duck Food [mainly from Kadlec and Wentz (1974)]

- s = Seeds are frequently consumed by waterfowl.
- f = Foliage and/or tubers are frequently consumed by waterfowl.
- * = Highly preferred by some species of waterfowl.

RecsPhyto

The number of records for this species in USEPA's PHYTOTOX database as of March 1994. Each record represents the response of the species to one substance during one investigator's experiment. G = number of records for the genus (not necessarily this species). The database is presently in the process of being joined with USEPA's AQUIRE database.

RecsAquire

The number of records for this species in USEPA's AQUIRE database as of October 1993. Each record represents the response of the species to one substance during one investigator's experiment. The database can be publicly accessed, and it provides a quantitative report of each experiment and the citation to the source literature.

Appendix B. Taxonomic Index: Invertebrate Tolerances and Responses to Water Regime, Oxygen, Salinity, and Sediment; Food Value to Waterfowl

For each of > 160 invertebrate taxa, this database describes what is known about preference or tolerance with regard to water regime, oxygen, salinity, and turbidity. The next-to-last column indicates taxa known to be consumed frequently by waterfowl. This database does not include all invertebrate taxa found in prairie wetlands. Taxa are listed in phylogenetic order (as indicated by the Sequence field), and were included in this database if 1) by numbers or weight they constituted a large portion of samples collected for a published study or for an unpublished database that was made available and/or 2) literature indicated a strong association with the particular environmental variable. As is evidenced by the many blanks in the database, much information is unavailable for many of the included taxa; however, certain abbreviations and sources of information were used:

Reproduction (Repro) [mainly from Wiggins et al. (1980)]

- 1 = **Group 1—Overwintering Residents.** Capable of passive dispersal only. Aestivate and overwinter in the dry basin either as drought-resistant cysts/eggs or as juveniles and adults.
- 2 = **Group 2—Overwintering Spring Recruits.** Reproduce in springtime surface water before it disappears because egg-laying depends on water. Aestivate and overwinter in the dry basin mainly as eggs or larvae (or for a few beetles, as adults).
- 3 = **Group 3—Overwintering Summer Recruits.** Can reproduce even when basin is dry because egg-laying does not require presence of surface water. Overwinter as eggs or larvae within the egg matrix.
- 4 = **Group 4—Nonwintering Spring Migrants.** Reproduce in springtime surface water before it disappears because egg-laying depends on water. Adults of the subsequent generation(s) leave the wetland as it dries and overwinter in permanent wetlands.

Water Regime [mainly from Swanson et al. (1974), Driver (1977), LaBaugh and Swanson (1988), Neckles et al. (1990), Bataille and Baldassarre (1993)]

- t = Occurs regularly in temporary and seasonal wetlands, as well as (usually) in semipermanent and permanent wetlands.
- sp = Occurs predominantly in semipermanent and permanent wetlands, but may also be present in temporary and seasonal wetlands.

Oxygen [mainly from Beck (1977), Hilsenhoff (1982), Rosenberg and Resh (1993)]

- 1 = Most tolerant of oxygen deficits, as commonly occurs with severe eutrophication.

- 4 = Least tolerant of oxygen deficits.
- a = Numeric rating to which this code is appended refers to a broader or narrower taxonomic level to which this taxon belongs, and its applicability to this particular taxon is unknown.
- b = Numeric rating to which this code is appended refers to taxa that appear to be closely related, and its applicability to this particular taxon is unknown.

Salinity [mainly from Timms and Hammer (1986), Timms et al. (1986), Lancaster and Scudder (1987), Walker et al. (1995)]

- f = fresh (< 800 $\mu\text{S}/\text{cm}$ conductivity, or < 3 g/L salt)
- o = oligohaline (800–8,000 $\mu\text{S}/\text{cm}$ conductivity, or 4–20 g/L salt)
- m = mesohaline (8,000–30,000 $\mu\text{S}/\text{cm}$ conductivity)
- p = polyhaline (30,000–45,000 $\mu\text{S}/\text{cm}$ conductivity)
- e = euhaline or hyperhaline (> 45,000 $\mu\text{S}/\text{cm}$ conductivity, or > 70 g/L salt)

Sediment

- t = Species typically invades wetlands with highly turbid water (minimal light penetration) or is disproportionately unaffected by turbidity increases.
- x = Species typically occurs in wetlands with the least turbid water (great light penetration) or is highly sensitive to turbidity increases.

Duck Food

Literature from prairie wetlands, cited in Sheehan et al. (1987), indicates major use by the following waterfowl species (H = hens, Y = young): American wigeon (AmWi), blue-winged teal (BwTe), canvasback (Canv), gadwall (Gadw), lesser scaup (LeSc), mallard (Mall), northern pintail (Pint), northern shoveler (Shov), redhead (Redh), ruddy duck (RuDu)

Sequence

This code is used to place the taxa in their approximate phylogenetic sequence. The first digit to the left of the decimal distinguishes among taxa in different phyla or orders, whereas the first digit to right distinguishes among orders or families, the second one to the right among families or genera, and so on.

Appendix C. Taxonomic Index: Bird Wetland Type Associations, Relative Abundance, and Trends

This database includes bird species that occur regularly—generally annually—at some season in multiple prairie wetlands and seem to use wetlands to a greater degree than nonwetland upland or deepwater habitats. This judgement was the author's, and it is based on reviewed literature [e.g., Duebbert (1981), Faanes (1982), Kantrud and Stewart (1984), Short (1989)] and knowledge of the life history of species.¹

The database contains these columns:

AOU

A numeric code assigned by the American Ornithological Union, used to sort species into their approximate phylogenetic sequence.

Status (Migration, Breeding) (Status_mig, Status_br)

Information in this column on relative abundance is from Faanes and Stewart (1982) and applies to North Dakota; it is generally but not exactly applicable to other areas of the prairie region. Abundance of some species varies between spring vs. fall migration; in this database, only the larger abundance term was used. Terms are as defined by Faanes and Stewart (1982):

abundant	=	very large numbers and easily observed
common	=	large numbers
fairly common	=	fair to moderate numbers
uncommon	=	low numbers
rare	=	very low numbers, but occurs somewhere at least annually
*	=	locally more numerous than indicated by the term

Type of Wetland (Wet_type)

The type of wetland (as defined by water regime) in which the species occurs most often. Information in this database was interpreted from Provost (1947), Faanes (1982), Weber et al. (1982), Kantrud and Stewart (1984), Hop et al. (1989), Colwell and Oring (1990), and Short (1989). Although nearly all of the listed species occur in nearly all wetland types, only the wetland types used most often are noted. Seasonal/interannual variation, geographic variation, differences

¹Note that although the following species (which were not included) are usually believed to depend more on uplands than on wetlands, they were found in more than 5 (10%) of the North Dakota prairie areas classified as wetlands by Igl and Johnson (unpublished data, NPSC, Jamestown, ND): brown-headed cowbird, western meadowlark, eastern kingbird, morning dove, common grackle, horned lark, western kingbird, American robin, clay-colored sparrow, upland sandpiper, gray partridge, vesper sparrow, lark bunting.

between foraging and nesting preferences, and needs for multiple types are not accounted for. All species listed as occurring in temporary wetlands are also found along the margins of seasonal, semipermanent, and permanent wetlands. Where information allows, wetland types used to a greater degree are denoted by upper case.

- a = alkali basins
- p = permanently flooded basins
- sp = semipermanently flooded basins
- ss = seasonally flooded basins
- t = temporarily flooded basins

Layers

The general layers of habitat in which the species generally is found. Information in this database was interpreted from Provost (1947), Faanes (1982), Weber et al. (1982), Colwell and Oring (1990), and Short (1989). Although it is recognized that nearly all of the listed species can occur in nearly all layers, only the layers used most often are noted. Seasonal/interannual variation, geographic variation, and differences between foraging and nesting preferences, are not accounted for.

- es = inhabits emergent vegetation growing out of hydric soil (surface water generally is absent)
- ew = inhabits emergent vegetation whose stems are immersed in standing water
- ow = inhabits patches of open water that are unvegetated except for presence of submersed aquatic plants
- m = inhabits generally unvegetated mud, sand, bare soil, and gravel
- t = inhabits trees or shrubs surrounding wetlands

Phenology

Actual peak breeding times vary somewhat depending on weather during a particular year. A numeric code indicates the usual peak breeding period for the species in North Dakota:

- 1 = 24 April to 7 June
- 2 = 14 May to 10 July
- 3 = 22 May to 19 July

Total Pairs (Pairs_tot)

The sum of breeding pairs found in 1993 in 416 North Dakota wetlands visited by Igl and Johnson (unpublished data, NPSC, Jamestown, ND). All wetlands included in this tally are in the Prairie Pothole Region.

Number of Wetlands (Num_Wets)

The number of wetlands where the species was present, based on visits by Igl and Johnson (unpublished data, NPSC, Jamestown, ND). Numbers are just for 1992–1993 in North Dakota wetlands within the Prairie Pothole Region. Although data were collected primarily during the breeding season, these frequencies were calculated for migrant and breeding individuals combined.

Frequency in Wetlands (Freqwets)

The percentage of the wetlands where the species was present, based on visits by Igl and Johnson (unpublished data, NPSC, Jamestown, ND). Frequencies are just for 1992–1993 in North Dakota wetlands within the Prairie Pothole Region. Although data were collected primarily during the breeding season, these frequencies were calculated for migrant and breeding individuals combined.

Maximum per Wetland (Max_per_w)

The largest number of breeding pairs counted in any single one of the 452 North Dakota prairie wetlands visited by Igl and Johnson (unpublished data, NPSC, Jamestown, ND).

Region of Widest Distribution (Reg_frma)

The North Dakota subregion in whose wetlands in 1993 the species was most widely distributed, based on Igl and Johnson (unpublished data, NPSC, Jamestown, ND). Frequencies were calculated for migrant and breeding individuals combined. See Stewart and Kantrud (1972a) for a map of subregions.

Region of Greatest Mean Abundance (Reg_abumax)

The North Dakota subregion in whose wetlands the species had the greatest mean abundance (on a per wetland basis) during 1993, and based on Igl and Johnson (unpublished data, NPSC, Jamestown, ND). The mean abundances were calculated for migrant and breeding individuals combined.

Region (Bbs_region)

This is a marker for data in the columns that follow to the right.

- E = Data for the eastern part of the prairie region, including the Red River Valley in eastern North Dakota and extending north slightly into Manitoba, all of western Minnesota and nearly all of southern Minnesota (including some nonprairie areas), and north-central Iowa [corresponds to Breeding Bird Survey (BBS) stratum #40].

- C = Data for the central part of the prairie region, including much of eastern South Dakota, eastern and central North Dakota, a small part of southern Manitoba, and southcentral Saskatchewan and Alberta (corresponds to BBS stratum #37).
- W = Data for the western part of the prairie region, including central South Dakota, central and northwestern North Dakota, northern Montana, and southern Saskatchewan and Alberta (corresponds to BBS Stratum #38).

of Routes (Bbs_numrts)

The number of BBS routes in the region on which the species has ever been found. Approximately 135 routes were run in the region at least once during the period 1966–1991, which is the period from which the data in this database came. Each route consists of 50, 3-minute stops along a 25-mile roadside route. The routes encompass all habitats, not just wetlands.

Pct of Routes (Bbs_rts)

The percentage of BBS routes on which the species has ever been found in the specified region.

per Route (Bbs_avg_rt)

For the specified region, this is the number of individuals of the species found on the average along a BBS route.

Maximum Frequency (Bbs_maxrt)

For the specified region, this is the greatest frequency of occurrence (% of 50 stops) found along any BBS route.

Priority (Prioritybb)

For the specified region, this is the trend in frequency of occurrence amongst BBS routes during the period 1966–1991. Because of uneven coverage among routes and years during the period, a bootstrapping technique (Sauer and Droege 1990) was used by the FWS in calculating the trends. The author identified categories with these priorities:

- 1 = The frequency with which the species was encountered declined on more routes than it increased during the period, and the difference was statistically significant.
- 2 = The frequency with which the species was encountered **declined** on more routes than it increased during the period, but the difference was not statistically significant.
- 3 = The frequency with which the species was encountered **increased** on more routes than it decreased during the period, but the difference was not statistically significant.
- 4 = The frequency with which the species was encountered **increased** on more routes than it decreased during the period, and the difference was statistically significant.
- ? = The species may breed in the subregion but has not been detected along BBS routes.

Appendix D. Taxonomic Index: Published Field Studies of Plant-Salinity Relationships in Prairie Wetlands

This database indexes studies that have reported on plant associations in prairie wetlands with salinity. It primarily includes species that the literature reports to be dominant or abundant in at least one prairie wetland; it does not include all prairie wetland plant species. A few of the included species are normally "upland" species (Reed 1988) even though they were found in a wetland.

Appendix E. Taxonomic Index: Published Field Studies of Plant-Water Regime Relationships in Prairie Wetlands

This database indexes studies that have reported on plant associations in prairie wetlands with various water regimes. It primarily includes species that the literature reports to be dominant or abundant in at least one prairie wetland; it does not include all prairie wetland plant species. A few of the included species are normally "upland" species (Reed 1988) even though they were found in a wetland.

Appendix F. Taxonomic Index: Published Field Studies of Invertebrate-Vegetation Cover Relationships in Prairie Wetlands

This database indexes studies that have reported on invertebrate associations with various vegetation types, densities, and patterns in prairie wetlands. It primarily includes invertebrates that the literature reports to be dominant or abundant in at least one prairie wetland; it does not include all prairie wetland invertebrate species.

Appendix G. Taxonomic Index: Published Field Studies of Invertebrate-Water Regime Relationships in Prairie Wetlands

This database indexes studies that have reported on invertebrate associations with various water regimes in prairie wetlands. It primarily includes invertebrates that the literature reports to be dominant or abundant in at least one prairie wetland; it does not include all prairie wetland invertebrate species.

Appendix H. Dominant Algae in Prairie Wetlands

This database includes algae (and a few Protista) that the literature reports to be dominant or abundant in at least one prairie wetland; it does not include all prairie wetland algal species.

Appendix I. Rare Wetland Plants Reported From Prairie Counties of North Dakota

The list of rare plants with county distributions was provided by Douglas Eiken, North Dakota Natural Heritage Inventory in April 1991; it has been edited to include only species officially considered to be wetland-dependent according to Reed (1988). These codes are assigned to the data:

Form [from Reed (1988)]

A	=	annual
B	=	biennial
P	=	perennial
N	=	native
I	=	introduced
E	=	emergent
F	=	forb
G	=	grass
GL	=	grasslike
H2	=	horsetail
Z	=	submersed
\$	=	succulent
/	=	floating

Dependence [from Reed (1988)]

Reed (1988) notes that the dependence category should not be equated strictly to degrees of wetness because many obligate species occur in temporary or seasonal wetlands (although most occur in permanent or semipermanent wetlands). These abbreviations have been used:

OBL	=	Obligate wetland species that under natural conditions occur almost always (> 99% probability) in wetlands.
FACW	=	Facultative wetland species that usually occur in wetlands (67%–99% probability) but occasionally are found in nonwetland upland (terrestrial) habitats.
FAC	=	Facultative species equally likely to occur in wetlands or nonwetland upland habitats.

- FACU = Facultative upland species that usually occur in nonwetlands (67%–99% probability) but occasionally are found in wetlands.**
- +, - = Slightly wetter (+) or drier (-) than indicated by the acronym.**

Appendix J. Catalog of Published Biological Studies of Prairie Wetlands: Locations, Sampling Regimes, Key Variables, and Related Descriptors

This database lists virtually all published studies since about 1970 that reported collecting multispecies biological data from at least one prairie wetland. Abbreviations are interpreted as follows:

IndepVar

A list of the major independent variables that were measured by the study. Some variables (e.g., temperature, season) that are not primarily anthropogenic were not included in this table. The abbreviations are as follows:

h	=	hydrologic gradient or fragmentation (among sites or within a site)
H	=	hydrologic manipulation (depth, duration, etc.)
n	=	nutrient gradient (N, P)
N	=	nutrient manipulation/dosing
p	=	pesticide or heavy metal gradient
P	=	pesticide or heavy metal dosing
s	=	salinity gradient (or alkalinity, Ca, Mg, SAR)
S	=	salinity manipulated
t	=	turbidity, light penetration, or sedimentation—gradient
T	=	turbidity, light penetration, or sedimentation—manipulated
c	=	cover density or cover ratio—gradient
C	=	cover density or cover ratio—manipulated

#Yrs

The maximum number of years covered by the study; some treatments or collections that are a part of the study may have covered fewer years.

LocState1...3

This column indexes the State(s) or Province(s) where data were collected.

LocCo1...10

This column indexes the counties where data were collected; a few studies covered more than 10 counties.

LocName

This column provides more specific information describing the location. The succeeding columns first provide information on bird studies, then invertebrate studies, vascular plant studies, and amphibian studies, as follows:

BirdGrp

The group of birds surveyed:

B	=	all birds
f	=	waterfowl (ducks, geese, swans)
F	=	one species of waterfowl
G	=	one species of gamebird
s	=	shorebirds (sandpipers, plovers, etc.)
w	=	waterbirds (mostly nonpasserine aquatic species)

BirdVars

The measurements reported:

b	=	biomass, weight, or standing crop
f	=	frequency, numbers, or density (of individuals, by species)
j	=	duration of use
k	=	home range size
m	=	mortality
p	=	production or growth
r	=	richness
t	=	trace or heavy metal content
v	=	behavior

BirdWets

The maximum number of major sample units (e.g., wetlands, fields) surveyed.

BirdSeas

Season(s) covered by the survey:

p	=	spring
s	=	summer
f	=	fall
w	=	winter

BirdFreq

Average frequency of surveys:

d	=	daily
w	=	weekly (< w means more often than weekly but not daily)
bw	=	biweekly
t	=	triweekly
m	=	monthly
2	=	(the number of visits per year)

InvVars

The measurements reported:

b	=	biomass or weight or standing crop (by taxon)
B	=	biomass or weight or standing crop (all taxa combined)
f	=	frequency or numbers or density (of individuals, by taxon)
m	=	mortality
n	=	nutrient or caloric content
r	=	richness

InvWets

The maximum number of major sample units (e.g., wetlands) surveyed.

InvReps/Wet

The maximum number of samples or transects per wetland.

InvSeas

Season(s) covered by the survey:

p	=	spring
s	=	summer
f	=	fall
w	=	winter

InvFreq

Maximum frequency of sample collections:

w	=	weekly (< w means more often than weekly but not daily)
bw	=	biweekly
m	=	monthly
2	=	(the number of visits per year)

InvIDlevel

Most specific level to which most organisms were identified:

f	=	family (fc = only Coleoptera, fm = only midges)
o	=	order
s	=	species

VPlantVars

Same codes as for InvVars, plus:

a	=	alkaline (mono)phosphatase activity
b	=	biomass or weight or standing crop
c	=	percent cover by species (C= not broken out by species)
d	=	decomposition rate

e	=	electronic transfer system (ETS) flux
f	=	frequency, numbers, or stem density (of individuals, by taxon)
F	=	total number or density (not broken out by taxon)
g	=	germination rate by species (G= all species combined)
h	=	height or length
m	=	mortality
n	=	nutrient or calorific content
p	=	production or growth
r	=	richness
s	=	seed production
t	=	trace or heavy metal content
u	=	glucose mineralization or metabolism
x	=	oxygen consumption or respiration

VPlantWets

See InvWets above.

VPlantReps/Wet

See InvReps/Wet above.

VplantSeas

See InvSeas above.

Algae

Similar to VPlant columns.

Amph

Similar to VPlant columns.

Appendix K. Catalog of Ongoing Biological Studies of Prairie Wetlands: Locations, Sampling Regimes, Key Variables, and Related Descriptors

This database lists many, but surely not all, studies that are currently underway in the prairie region and involve collection of multispecies biological data from at least one prairie wetland. Included are funded studies whose field work has not been completed, recent studies whose field work has been completed but whose data have not been analyzed completely for publication, and recent studies whose results have been reported so far only as abstracts at symposia. Information fields include the investigator's name, phone number, bibliographic reference if any, starting year (YRSTART), projected completion year (YREND), number of wetlands studied (WETS), major variables examined (see Appendix J abbreviations), and location.

Appendix L. Descriptions of Data Sets Analyzed For This Report

Below are described the unpublished data sets that were analyzed and used to derive estimates of variance reported in Appendices M and N as well as the species accumulation reported in Appendix O.

I. Invertebrates

A. Hanson Activity Traps

Records are indexed by year, period (month), wetland, sample, and vegetation. Each record represents a unique combination of year-period-wetland-sample-vegetation for certain metrics: number of species, number of individuals (all species combined), biomass (all species combined). Only the data from wetlands that lacked high densities of fathead minnows were included. Data are from activity traps and cover 2 years. In year one, there were 5 sample periods, 3 wetlands sampled per period, and 8 random samples per wetland. In year two, there were 4 sample periods (3 identical to year 1), 4 sampled wetlands (the same 3 wetlands in year one plus one other), and 10 random samples collected per wetland. Vegetation condition (present/absent) of each sample was recorded incidentally and was not part of the sampling design. In both years, data were collected using the same equipment and mostly the same protocol. The total number of unique records is 320. The data are from wetlands in east Polk County in west-central Minnesota; they were provided by Dr. Mark Hanson (Minnesota Department of Natural Resources, Bemidji, MN).

B. Euliss Sweep Nets

Records are indexed by year, wetland, transect, and sample. Each record represents a unique combination of year-wetland-transect-sample for certain metrics: number of species and number of individuals (all species combined). Samples were collected monthly with a sweep net, but data are not broken out by month; only season totals were available. Data are from 2 years. In year one, 16 wetlands were sampled; there were 2–8 transects per wetland with 1–2 samples per transect for a total of 118 unique records. In year two, 18 wetlands were sampled (the same 16 wetlands plus two new ones), using 5 transects per wetland with 2–3 samples per transect for a total of 265 unique records. The data were provided by Dr. Ned Euliss (NPSC, Jamestown, ND).

C. Euliss Sediment Traps

Records are indexed by region, plot², wetland³, transect, wetland class, and health class. Data are from 36 wetlands, all sampled once in 1993. Each record represents a unique combination of region-plot-wetland-transect-wetlandclass-healthclass, for certain metrics: number of individuals (all species combined) and biomass (all species combined). There are 2 regions, 35 wetlands, 3 wetland classes (semipermanent, seasonal, temporary), 10 plots, 5 transects, and 2 health classes. There are 18 wetlands in each of the two regions, 8–10 plots per wetland class, and 5 transects per plot. For each of the two health classes ("healthy" and "unhealthy"), there

²A "plot" is a set of 1 to 6 wetlands, generally of diverse water regime types and all located within a 4-mi² area.

³Called "polygon" in the Euliss data set.

are approximately equal numbers of plots, wetlands, transects, wetland classes, and regions. Total number of unique records is 180. Data represent an entire growing season's collection of settled, decay-resistant remains of snails, cladocerans, ostracods, and clam shrimp, as measured using a standard protocol. The data were provided by Dr. Ned Euliss (NPSC, Jamestown, ND).

D. MERP Substrate Samplers

Records are indexed by year, period (month), zone (cover type), and depth class. Data are from 2–6 wetlands, but unfortunately the wetland identifier was lost and all the data from individual wetlands were combined. Each record represents a unique combination of year-period-zone-depth class, for certain metrics: number of species, number of individuals (all species combined), biomass (all species combined). Data cover 5 years. There are 21–24 periods per year, 6–7 zones per period, and 2 depth classes per zone. There are 7 zones per year and 18–25 periods per zone. Total unique records is 790. In all years, data were collected using the same equipment and a standardized protocol. The data are from experimentally manipulated wetland cells (mesocosms) of the Marsh Ecology Research Program (MERP) located in the Delta Marsh, Manitoba. The data were provided by Dr. Henry Murkin (Institute for Wetland and Waterfowl Research, Oak Hammock Marsh, Manitoba).

E. MERP Activity Traps

Records are indexed by year, period (month), wetland ("cell"), treatment, and zone. Each record represents a unique combination of year-period-wetland-treatment-zone, for the following metrics: number of species, number of individuals (all species combined), biomass (all species combined). Data cover 5 years (except biomass data, which exclude 1988). There are 23–24 periods per year, 1–6 wetlands per period, 1 treatment per wetland, and 2–6 zones per treatment. There are 20–25 periods per zone and 17–23 periods per wetland. There are 2–6 wetlands per zone and the same number per treatment. There are 1–4 zones per wetland and 6–7 zones per treatment. Total unique records is 1200. In all years, data were collected using the same equipment and a standardized protocol. Data are highly variable because conditions among wetlands were intentionally and dramatically manipulated as part of experiments. The data are from experimentally manipulated wetland cells (mesocosms) of the Marsh Ecology Research Program (MERP) located in the Delta Marsh, Manitoba. The data were provided by Dr. Henry Murkin (Institute for Wetland and Waterfowl Research, Oak Hammock Marsh, Manitoba).

F. Duffy Data Set

Records are indexed by wetland and period (sampling date). Each record is the mean of four samples per wetland per sample period, and it represents a unique combination of wetland-period for certain metrics: number of species, number of individuals (all species combined), biomass (all species combined). Data are all from a single year, representing four wetlands: 2 wetlands were sampled for four periods, 1 wetland for six periods (including the same four periods), and one for nine periods. Total unique records is 23. In all periods and wetlands, data were collected using the same equipment (a 3.5-inch PVC corer) and a standardized protocol. The data are from Deuel County, South Dakota, and they were provided by Dr. Walter Duffy (South Dakota Cooperative Fish and Wildlife Research Unit, Brookings, SD).

II. Breeding Birds

A. FWS Breeding Bird Survey

Records are indexed by year, State, and route. Each record represents a unique combination of year-State-route, for these metrics: number of species, number of individuals (all species combined), number of sample points containing wetland species). Routes are surveyed repetitively (but often noncontinuously) among years, so that a partly different set of routes constitutes each year's data. All data were collected using a standardized protocol. There are 21 years, 5 States (and provinces), and 51 routes. When routes are combined, there are 95 unique year-State combinations. Other characteristics are:

years per route:	1–21
routes per year:	19–30
routes per State:	2–16
routes per State per year:	1–14
years per State:	14–21
States per year:	5

The total number of unique records is 543; the data were provided by Sam Droege (National Biological Service, Washington, DC).

B. Igl & Johnson Data Set

Records are indexed by year, wetland, and visit. Each record represents one wetland, described in term of these metrics: number of breeding species, number of breeding + nonbreeding species, number of breeding pairs, number of breeding plus nonbreeding individuals. The maximum value from both visits and both years was used. Visits were made to 480 wetlands, 330 in 1992, and 416 in 1993. Each was visited 1–2 times per year, and visits were timed to cover both early and late breeders. The sampling design is as described by Stewart and Kantrud (1972 a & b). The study covered nearly all of North Dakota, but only the data from sites in the Prairie Pothole Region were used in this analysis (i.e., Missouri Slope and Little Missouri Slope subregional data were excluded).

Appendix M. Results of Power of Detection Analyses of Existing Prairie Data Sets

This table describes the results of the components of variance approach that was applied to estimate sample power of detection. Methods of calculation are described on Section 1.5. Results are reported under these column headings:

Taxon

The major group for which variability was determined.

Metric

A measured characteristic of the biological community that was reported by the original investigator.

Units

The units of measurement (e.g., grams) in which the metric is expressed.

Equipment

Sampling equipment used by the collector of the original data.

RandomVar

The variable for which the number of requisite samples and the associated level of precision were calculated.

Data set

The source of the data; the experimental design associated with the analyzed data is detailed in Appendix L.

Pr_forS10

The specified metric's precision that would be obtainable if 10 samples of the type described were collected, e.g., a sample size of 10 would allow the user to distinguish a difference between two means of (specified) grams, given the assumptions of the analysis. This figure is based on the "optimistic" equation, but in most instances the value from the "conservative" equation differed only slightly (see Section 1.5 for discussion of the equations). "Ten samples" was an arbitrary number selected so that relative levels of resultant precision could be roughly compared among taxa, sampling methods, and sampling designs.

BreakPt

The number of samples beyond which increasing the number of samples results in relatively little increase in the level of precision. This number was estimated visually and subjectively from plotted curves, so the true value could be plus or minus about two samples.

DetectDif1

An example of the detectable difference in two means. This example is for the smallest difference that can be predicted from the data we used (i.e., the "optimistic" estimate, as described in Section 1.5).

SSizeMin1

The smallest number of samples that would be able to detect the specified difference, based on the "optimistic" calculation method defined in Section 1.5.

SSizeMax1

The smallest number of samples that would be able to detect the specified difference, based on the "conservative" calculation method defined in Section 1.5.

DetectDif2

A second example of the detectable difference in two means. This example is for the largest difference that can be predicted from the data we used (i.e., the "conservative" estimated as described in Section 1.5).

SSizeMin2, Max2

As above, but for the second data set.

PooledVars

Variables that were subsumed in defining the samples (records) that were used for the analysis.

Appendix N. Library of Coefficients of Variation from Prairie Wetlands

This database quantifies the biological variability of prairie wetlands at multiple spatial and temporal scales using a standard statistic—the coefficient of variation—which is defined as:

$$CV = (100 * SD) / \bar{x},$$

where SD is the standard deviation of the data, \bar{x} is the mean, and CV is the coefficient of variation.

The 412 coefficients were calculated from 15 prairie wetland data sets, encompassing both published literature and raw data provided to the author by investigators in the region. The data's quality and representativeness of prairie wetlands in general are unknown. Data were obtained on a purely opportunistic basis, and it is not known how much of the variability described by any CV can be attributed to ecological variation as opposed to human-related effects.

This database was developed to illustrate the relative degree of spatial and temporal variability that can be expected in a given situation, recognizing that each situation is unique; therefore, the CVs must be interpreted with caution. Comparisons among CVs are confounded by the fact that the sampling designs that resulted in each CV were not necessarily well-balanced (e.g., for a given study, not all habitats were sampled equally, at the same times of year, etc.). More significantly, and especially when values were extracted from the literature, it was seldom clear what data might have been previously combined (e.g., replicates composited) in arriving at a particular mean, standard deviation, or CV. When it was apparent to what a particular mean was referring (i.e., which variables had been combined and/or averaged), data then were referenced accordingly in the database. CVs were always reported at the finest level of detail (least amount of pooling) possible as well as for pooled samples. The least-pooled sample CVs are indicated by presence of an "x" in the AllSamp column, coinciding with absence of an "x" or "a" from all remaining columns.

These abbreviations are used in the database:

Group

The broad taxonomic group that was the primary focus of the study, namely,

- b = birds
- i = invertebrates
- p = plants

SampType

The type of equipment or protocol used:

- emtrap = emergence trap
- actrap = activity trap

quad = quadrat depth
0–5 cm = seeds collected from sediment depths of 0–5 cm

Metric

biomass = collective weight of all individuals
numindiv = the number of individuals
numtaxa = the number of taxa (richness)
seed den = density of seedlings
shoots/m² = density of plant shoots/m²

Regions

Samples from different subregions of the prairie region:

x = The CV was calculated after pooling data from multiple regions.
a, A = The CV represents the among-region variability (the number following this letter indicates the number of pooled samples).
37 = The CV is only for the central part of the prairie region.
38 = The CV is only for the western part of the prairie region
40 = The CV is only for the eastern part of the prairie region

HealthCI

Samples from the two "wetland health" classes defined by the cited study:

x = The CV was calculated after pooling data from both health classes.
a, A = The CV represents the variability between the two health classes.

States

Prairie States or provinces covered by the BBS data:

x = The CV was calculated after pooling data from multiple States.
a, A = The CV represents the among-States variability after all routes and years within a State were pooled.

Routes

The BBS route data (each route is 25 mi long and contains 50 stops where data are collected):

- x = The CV was calculated after pooling data from multiple routes.
- a, A = The CV represents the among-route variability after each route's years were pooled.

WetTypes

Data by wetland types:

- x = The CV was calculated after pooling data from multiple wetland types.
- a, A = The CV represents the among-type variability.

When the CV is just for the specified wetland type:

- p = permanently flooded
- ss = seasonal
- sp = semipermanent
- t = temporary

Wets

Data by discrete wetland:

- x = The CV was calculated after pooling data from multiple wetlands.
- a, A = The CV represents the among-wetland variability.

When the CV represents variability just within a single wetland, a number has been arbitrarily assigned a reference number (1,2, etc. are arbitrarily assigned reference numbers).

Treat

Data by wetland treatment regime:

- x = The CV was calculated after pooling data from multiple wetlands, each having been hydrologically manipulated in a different manner.
- a, A = The CV represents the among-treatment variability.

When the CV represents variability just within a single treatment regime:

- d = Wetlands were subjected to drawdown.
- f = Wetlands were subjected to flooding.
- h = Wetlands in which emergent plants were harvested.
- t1, t2 = Two different treatment regimes.

Trans

Data by transects within wetlands:

- x = The CV was calculated after pooling data from multiple transects within the wetlands.
- a, A = The CV represents the among-transect variability.

Zones

Data by vegetation zones within wetlands:

- x = The CV was calculated after pooling data from multiple zones within a wetland.
- a, A = The CV represents the among-zone variability.

When the CV represents variability just within the specified zone, a zonal term has been included: wet meadow, shallow, etc. (zonal terms are mostly those of the originator of the data).

Depths

Data by depth class within wetlands:

- x = The CV was calculated after pooling data from multiple depth zones within a wetland.
- a, A = The CV represents the among-depth zone variability; the two zones were < 30 cm and > 30 cm.

Veg

Data by vegetated condition within wetlands:

- x = The CV was calculated after pooling data from both vegetated and unvegetated (open water) areas of the wetland.

a, A = The CV represents the between-area variability.

AllSamps

x = The CV was calculated after pooling data from multiple wetlands, transects, zones, months, years, etc., unless other columns are blank (see explanation in second example query below).

Yrs

Data grouped by year:

x = The CV was calculated after pooling data from multiple years.

a, A = The CV represents the among-year variability.

When the CV represents variability just within a single year, a number has been to distinguish discrete years (1, 2, etc. are arbitrarily assigned numbers).

Months

Data grouped by month or biweekly sampling period:

x = The CV was calculated after pooling data from multiple sampling periods.

a, A = The CV represents the among-sampling period variability,

When the CV represents variability just within a single sampling period, numbers or a season abbreviation were assigned arbitrarily to distinguish discrete periods (1,2, etc.):

es = early summer

ls = late summer

p = spring

CV

The coefficient of variation calculated for samples grouped in the manner defined by the preceding columns.

CVmin

The smallest of several coefficients of variation calculated for samples grouped in the manner defined by the preceding columns.

Cvmax

The largest of several coefficients of variation calculated for samples grouped in the manner defined by the preceding columns.

The organization and structure of the database are best illustrated by two example queries, as follows:

Query #1

How much does species richness (number of taxa) vary among wetlands?

Approach:

Find (sort⁴) all records that have an entry "numtaxa" (number of taxa) in the column "Metric" AND have an entry preceded by a lower-case "a" in the column "Wets" (among wetlands). In this example, there are 12 records meeting these criteria. Inter-wetland variability is quantified by the corresponding values in the column "CV." Different CV values for different records reflect other influencing conditions. In this example, one CV was calculated for each of six sampling periods indexed in the Months column. Four wetlands (indicated by "a4" in the Wets column) were sampled on May 9 and 31, June 14, and June 29 (indicated in the Months column) and the among-wetland CVs calculated for each date. An "x" in the AllSamps column indicates that individual samples from within each wetland had been pooled prior to calculating the CV. In addition, another "a4" record lacks any date in the Months column, but rather has an "x." This indicates a situation where, for each wetland in the data set, the data from all the months were pooled before calculating the CV. The CVmin column then reports the smallest among-month CV from the four wetlands and the CVmax column reports the maximum (i.e., duplicating information already in the CV column under the four separate dates).

These CVs have all been calculated from one study's data (the Duffy data set, as denoted in column 1). Among-wetland estimates of variation are available from three other data sets. The "Hanson activity traps" data set has a single entry meeting the query criteria. The "a4" in the Wets column indicates that the CV estimate is from a comparison of four wetlands, and an "x" in the Yrs, Months, Veg, and AllSamps columns indicates that samples from an unspecified number of years, months, vegetation types, and replicates were pooled to represent each of the four wetlands, prior to calculating the CV. The CVmin column reports the smallest CV that any of the four wetlands had from the pooling of these other variables; CVmax reports the largest.

The "Euliss sweep net" data set has two entries that meet the selection criteria. One is a comparison of richness among 18 wetlands (a18 in the Wets column) and the other, a similar comparison among 16 wetlands (a16 in the Wets column). The difference is that each inter-wetland CV is from a different year. As indicated in the Years column, the 18-wetland comparison is from 1993 and the 16-wetland comparison is from 1992. The "x" in the Trans and AllSamps columns indicates that for each among-wetland comparison, data from an unspecified number of transects and replicates were pooled prior to calculating the CV.

⁴Software for sorting the values is not provided. Users must either sort manually or import the file into a program such as dBase or Paradox.

Finally, the "Driver 1977" data set has two entries that meet the criteria. Both involve a comparison of 11 wetlands (a11 in the Wets column), in which data from an unspecified number of months and years had been previously aggregated (indicated by an "x" in the Months and Yrs columns). The difference between the two entries is apparent from viewing the WetTypes column: one CV estimate is from a comparison of 11 semipermanent (sp) wetlands; the other is from 13 temporary (t) wetlands. Also note, from the "Specific Taxon" column, that these richness estimates pertain only to the Chironomidae (midges). Taken as a whole, these CV estimates tentatively suggest that variability in midge species richness, as sampled by emergence traps, is greater among temporary wetlands (CV = 39.00) than among semipermanent wetlands (CV = 31.46).

Conceptually, the same approach as described in the above paragraphs can be used to scan the database for estimates of other types of variability. For example:

- To review estimates of interannual variability, retrieve records that have a lower-case "a" in the Yrs column and proceed as described above.
- To review estimates of variation among zones within a wetland, retrieve all "a" records in the Zones column and do likewise.
- To compare two metrics (e.g., numindiv and biomass) with regard to their variability, retrieve all records for these as indicated in the Metrics column, and then match records according to other characteristics to make the comparison of CVs as fair as possible.
- To compare two major groups (e.g., invertebrates and plants) with regard to their variability, retrieve all records for these as indicated in the Group column, and then match records according to other characteristics to make the comparison as fair as possible.

Query #2

Does precision appear to increase the most when samples from a particular study are grouped by year, by season, by wetland, by zone, or some combination of these?

Approach:

First, go to the AllSamps column and for the particular study/data set and metric of interest, find all entries consisting of a number preceded by an "x". For example, for the data set on the Hanson activity traps and the metric "numiindiv," locate the entry "x320." This indicates that the associated CV estimate is based on 320 samples. The simultaneous presence of an "x" in the columns for Wets, Veg, Yrs, and Months indicates that the 320 samples constitute all the samples from varied wetlands, vegetation conditions, years, and months.

This CV (281) can then be used as a baseline against which to compare the CVs based on various subsets of the 320 samples. For example, when data are grouped by month (a5 in the Months column), the CV drops from 281 to 92. The value in the CVmin column of the same row indicates that under one vegetation condition in one wetland during 1 year, the variability among

months was as low as 78. Likewise, the value in the CVmax column indicates that under one vegetation condition in one wetland during 1 year, the variability among months was as high as 223.

If instead of month, the data are grouped by year (a2 in the Yrs column), the CV drops more significantly from 281 to 80. The value in the CVmin column of the same row indicates that under one vegetation condition in one wetland during one month, the variability between years was as low as 119. Likewise, the value in the CVmax column indicates that under one vegetation condition in one wetland during 1 year, the variability between years was as high as 254.

A third option is to group the data by both year and month. This is indicated by a number (10, in this case) preceded by an upper-case "A" in both the Yrs and Months columns. The value in the CVmin column of the same row indicates that under one vegetation condition in one wetland, the variability among the ten possible combinations of year and month was as low as 61. Likewise, the value in the CVmax column indicates that under one vegetation condition in one (probably another) wetland, the variability among the 10 year-month combinations was as high as 206.

Finally, note the few records where the AllSamps column has an entry consisting of a number preceded by an "x" (e.g., x320) and none of the other columns have an entry preceded by an "x." The CVs for these records represent the variability among what are apparently true replicate samples, i.e., samples collected at the exact same location at the same time with the same equipment.

Appendix O. Results of Asymptotic Richness Calculations Using Existing Prairie Data Sets

This table documents the results of the asymptotic richness calculations. Methods were described in Section 1.5. Column headings are as follows:

Data Set

The source of the data; the experimental design associated with the analyzed data is detailed in Appendix L.

Equipment

Sampling equipment used by the collector of the original data.

Taxa

The category of organisms for which species accumulation rates were determined.

Samples

The type of samples (e.g., wetlands, quadrats, years) among which the taxa were accumulated.

Location

General location of the sampling; for BBS data:

stratum 37 = central prairie region

stratum 38 = western prairie

stratum 40 = eastern prairie

Total Taxa

Cumulative number of taxa in all samples of the analyzed data set.

Tot_NumSa

Total number of samples (wetlands, quadrats, years, etc.) among which species lists were combined and accumulated.

Num_for50...etc.

Number of samples (of the type specified) that are required to detect 50, 75, 90, 95, and 99% of the total taxa cumulatively present in the data set. The values given are the median for that threshold, based on the 100 random runs.

CurveTurn

A value of 0 indicates a curve terminating in a plateau (i.e., no slope). The larger the value, the farther from an asymptotic condition is the near-final part of the curve and the more likely that the sample was insufficient to estimate richness. Values were calculated as:

$$\text{CurveTurn Value} = 100 * (b-a) / (c-b) ,$$

where a = median for 90th percentile (Num_for 90),

b = median for 95th percentile,

c = median for 99th percentile.

CurveEnd

A value of 100 indicates that less than the full number of samples would have been required to sufficiently estimate richness; the smaller the value, the likelier is it that the sample size was more than sufficient, i.e., oversampling occurred. Values were calculated by dividing Num_for 99 by Tot_NumSam and multiplying by 100.

Qualifiers

Supporting information that describes the exact type of data used in the analysis.

PooledVars

Variables that were subsumed in defining the samples (records) that were used for the analysis.

