Assessment of Floodplain Wetlands of the Lower Missouri River Using an EMAP Study Approach, Phase II: Verification of Rapid Assessment Tools

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by

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Table of Contents

Abbreviations....4
Background....5
Introduction....5
Methods....6

- Site selection....6
- Field methods....8
- Macroinvertebrates....9
- Disturbance Assessment....9
    - The Assessment....9
    - Wetland Attributes....10
    - Reference Indicators....11
    - Disturbance....12

Results....13

- Explanation of statistical analyses and graphical representations....13
- Floristic Quality Assessments....14
- *In situ* water quality....19
- Depth measures....21
- Nutrient Analyses....22
    - Nitrogen....22
    - Phosphorus....24
    - Carbon....25
- Herbicides....27

Comparisons between Phase I and II studies of the lower Missouri River floodplain wetlands....28

- Introduction and Background Information....28
- Floristic Quality Assessment Results....29
    - Plant Richness - All Species....30
    - Plant Richness - Native Species....31
- Spatial attributes....32
    - Wetland area....32
    - Depth to Flood....33
    - Mean Depth and Maximum Depth....34
- Water quality....36
    - Dissolved oxygen....36
    - Turbidity....36
    - Carbon....38
    - Nitrogen....39

Phosphorus 42
TN:TP ratio..43
Chlorophyll-a..44
Specific Conductance..46
Herbicides..48

Resampled sites..50
Disturbance Assessment 52
Macroinvertebrate MMI 54

Metrics..54
a priori Groups and Metric Selection..56
Metric Testing..57
The Macroinvertebrate Multiple Metric Index (MMI) 64
MMI in Relation to Other Measures..68
Differences in Wetland Types 69
Wetland Types and MMI Scores 70
MMI Result Conclusions 72

References 73
Appendix A. Goals and objectives of EPA Award R7W0812. 76
Appendix B. Study sites for Phase II. 78
Appendix C. Laboratory measurements and analyses. 81
Appendix D. Disturbance assessment scoring form. 82

Abbreviations

AB – Aquatic beds
ANOVA – Analysis of Variance
AP – Agricultural Pesticides
BU – Burrower
Chl-*a* – Chlorophyll-*a*
CPCB – Central Plains Center for BioAssessment
CN – Clinger
DA – Disturbance Assessment
DEA – Desethylatrazine
DIA – Desisopropylatrazine
DOC – Dissolved Organic Carbon
DTF – Depth to Flood
EM – Emergent macrophyte beds
EMAP – Environmental Monitoring and Assessment Program
EPA – Environmental Protection Agency
EPT – Ephemeroptera, Plecoptera, and Trichoptera
ETO – Ephemeroptera, Trichoptera, and Odonata
FQA – Floristic Quality Assessment
FQI – Floristic Quality Index
FC – Filterer-Collector
GC – Gatherer-Collector
GIS – Geographic Information System
GPS – Global Positioning System
HM – Heavy Metal
IQR – Interquartile Range
KBS – Kansas Biological Survey
MIX – Wetlands with equally dominant AB, EM, and UB
MMI – Multiple Metric Index
MS – Microsoft
NCSS – Number Cruncher Statistical System
NOD – Nutrient and Oxygen Demanding chemicals
NTU – Nephelometric Turbidity Units
PA – Parasite
Pheo-*a* – Pheophytin-*a*
PI – Piercer
PL – Planktonic
POC – Persistent Organic Carbons
PR – Predator
RTV – Regional Tolerance Value
STDDEV – Standard deviation
STDERR – Standard error
SP – Sprawler
SSS – Suspended Solids and Sediments
TOC – Total Organic Carbon
UB – Unconsolidated beds

Background

In 2007 the Central Plains Center for BioAssessment (CPCB) at the Kansas Biological Survey (KBS), University of Kansas, studied a set of 22 reference wetland sites located in the Missouri River floodplain (Kriz *et al.* 2007). During that Phase I study, wetland assessment tools were developed that could be useful for Level 1 (landscape assessment using a geographic information system (GIS) and remote sensing) studies and could be applicable to Level 2 and Level 3 studies (see Fennessy *et al.* 2004). This report describes a Phase II study in which we continued development of the assessment tools by sampling and analyzing a series of abiotic and biotic factors associated with 42 randomly selected wetlands in 2008 and 2009. The objectives of this Phase II study of these randomly selected lower Missouri River floodplain wetlands were to 1) obtain a "snapshot" of the ecological condition of the study population, 2) test the applicability and responses of the previously developed wetland assessment metrics, and 3) compare "reference" wetlands (from Phase I) with this random sample population of wetlands. Four groups of attributes were examined for each study wetland: water quality, floristic, macroinvertebrate community, and landscape. Analysis of relationships among buffer and landscape attributes, water chemistry, and biological attributes are described.

Assessment data gathered for this population of 42 randomly selected wetlands were compared against the reference sites studied in Phase I to identify baseline reference conditions for water quality and benchmarks for determining wetland health. Project objectives are linked to EPA's Strategic Plan, Goals 4.3.1.1, 4.3.1.3, and 4.3.2.1, by identifying and assessing critical wetlands, developing rapid assessment tools, and providing baseline data, thus enhancing our ability to track loss and degradation of wetland resources and identify opportunities for wetland protection or restoration to support the "no overall net loss" goal of EPA's Strategic Plan. Specific project goals are described in Appendix A.

Introduction

The floodplain ecosystems of the Missouri River basin have been severely impacted over the course of U.S. history; this has been especially true since the completion of the six main-stem dams built between 1930 and 1950 (Chipps *et al.* 2006). The transformation of natural prairies, riverine areas, and wetlands to agricultural land via clearing, draining, and filling has destroyed much of the wetland acreage once found there. The loss of wetland acreage is a continuous trend with an increasing amount of disturbance due to urbanization and extension of rural areas through the development of roads and other infrastructure (Dahl 2000). After 633,500 acres were lost between 1986 and 1997, an estimated 100 million acres of freshwater wetlands remained within the U.S. (Dahl 2000). Alterations to the Missouri River, including berms and levees, have disrupted the connectivity between the river and remaining floodplain wetlands. Wetland loss also is occurring due to natural succession caused by the changing course of the river, however these natural processes are now constrained by human control of flooding. Nevertheless, human disturbance has had great impacts on the Missouri River floodplain wetlands and their capacity to provide crucial ecosystem services such as wildlife habitat, nutrient cycling, carbon sequestration, and contaminant removal from upland and riverine systems.

The biological integrity of the aquatic ecosystem has become an important component for assessing wetland condition and quality. Aquatic macroinvertebrates respond to an assortment of abiotic and biotic factors. Many wetland assessments use multiple tier approaches to quantify wetland health and to identify perturbations that may cause degradation to a system. This study was designed to assess the quality of wetlands in the lower Missouri River floodplain using remote sensing technology, a rapid on-site landscape and hydrological assessment, a floristic quality assessment, *in situ* water quality and nutrient measures, and benthic macroinvertebrate collections. A multiple metric index (MMI) development approach was chosen to evaluate the aquatic invertebrate community as a quantifiable measure of how these organisms respond to other wetland parameters and assessment outcomes developed in this study. As an index of biological integrity (IBI), the macroinvertebrate MMI was developed by scrutinizing the stressor-response relationships between the chemical and physical measures, and components of the benthic macroinvertebrate community. Results of the macroinvertebrate MMI were consistent with other studies using invertebrate metrics for assessing the biological integrity of aquatic ecosystems when comparing the reference and random sample populations. The developed MMI was then tested for congruency with the other assessment results, relationships to hydrological connectivity, and internal wetland structural features that were evaluated. The macroinvertebrate MMI responded significantly to observed physical and chemical anomalies, and provided insight to dominant wetland features such as landscape, hydrology, water chemistry, and plant communities, that influence wetland conditions.

Methods

<u>Site selection</u>

During Phase I (e.g. reference wetland identification, characterization, development of assessment tools) of this two-part study, geospatial data from several sources were analyzed using ArcView 3.3 and ArcGIS. From this a map was developed of all wetlands in the lower Missouri River valley. We also developed a flooding model that identifies flood-prone areas within the valley. NWI maps were merged into a single seamless data theme for the entire study area, and a 500-year floodplain boundary was used to select wetlands within areas of interest. Two classes of wetlands (as defined by Cowardin *et al*. 1979) were studied: lacustrine and non-woody palustrine.

Wetlands were filtered by size (i.e. surface acres) to identify those that meet our minimum size criterion of 10 acres in area. Imposition of this wetland size criterion was done for four reasons. First, it ensures a high likelihood of open water during spring to early summer. Second, larger sites have a higher probability of being correctly classified in the NWI database. Third, larger sites generally support higher levels of native biodiversity, more wetland functions, and greater wildlife value. Fourth, bigger wetland area are more likely to be in public ownership and therefore more likely to have been studied in the past.

For this Phase II study, from the population of wetlands meeting the location, class, and size criteria, 400 were identified using EMAP selection protocols. From this population, 42 study sites were selected randomly within a spatially hierarchical sampling framework called Generalized Random Tessellation Stratified Designs (GRTS) (Figure 1). In GRTS, a hexagonal grid is imposed on the map of the target population. The grid scale is adjusted to appropriate levels of resolution. Grid elements (and sampling units) are then randomly selected using a robust, selection algorithm. GRTS simultaneously provides true randomness, ensures spatial balance across the landscape, and enables the user to control many parameters.

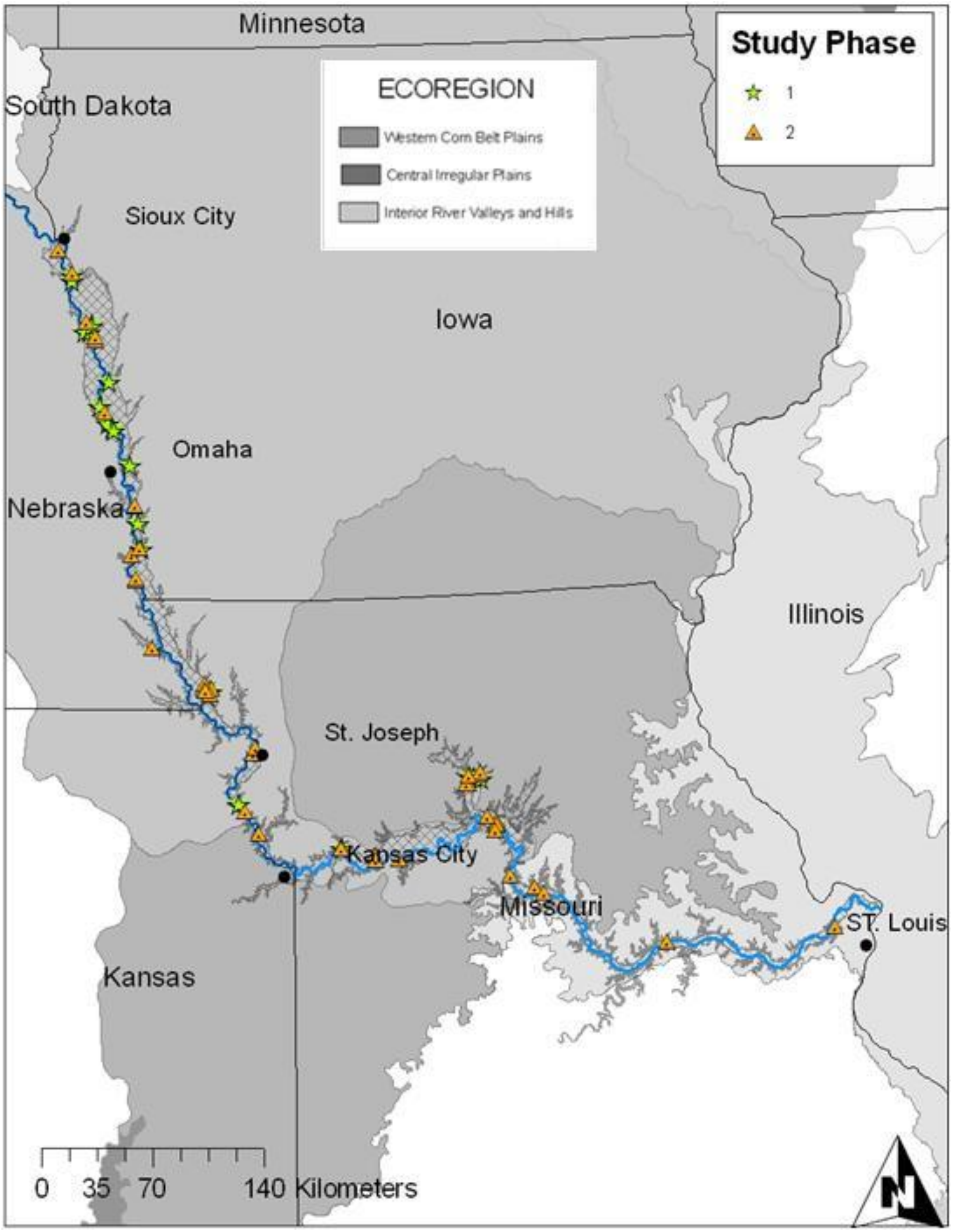

Figure 1. Map of the Lower Missouri River floodplain wetlands studied in Phase I and II. Phase I studies focused on 22 candidate reference wetlands and their characterization. Phase II studies focused on 42 randomly chosen wetlands that had open water and macroinvertebrate samples

Field methods

See the project Quality Assurance Project Plan (QAPP) for details of sampling methods (http://www.cpcb.ku.edu/research/assets/PhaseIIwetlands/QAPP_wetlandsII.14Aug.pdf). The disturbance assessment and the floristic quality assessment are composed of metrics (values that represent qualitative aspects). Metrics from each study wetland were combined to produce a score that estimated the wetland's condition with respect to the amount of disturbance or the quality of plant community, respectively. The floristic quality index is only one component for assessing the plant community in wetlands. Other factors, such as native wetland plant species richness, may also indicate the condition of the wetlands health or quality to maintain diverse communities of invertebrates and vertebrates, including amphibians, water fowl, and small mammals. *In situ* water quality measures in this study consisted of mean values for water depth, Secchi disk depth, water temperature, turbidity (NTU), conductivity (mS/cm), dissolved oxygen, and pH. Water depth was measured with a surveyor's telescoping leveling rod to the nearest centimeter. Water properties were measured with a Horiba U10 Water Quality Checker. One liter samples were collected along three imaginary transect lines at right angles to a line extending along the longest axis of the study wetland and combined in a 5-liter carboy as one composite sample (Figure 2). Chemical laboratory analysis was conducted on composite water samples for concentrations of chlorophyll-*a*, nitrates, nitrites, ammonia, total nitrogen, total phosphorus, total and dissolved organic carbon (TOC and DOC), and six agriculturally applied herbicides, including atrazine and its two major metabolites. Chlorophyll-*a* analysis was conducted using fluorometric methods, nitrogen and phosphorus concentrations were determined with inline digest flow injection analysis, TOC and DOC were measured with a Shimadzu TOC analyzer, and herbicide concentrations were determined using Gas Chromatography/Mass Spectrometry (see Appendix C for analytical and measurement methods). All water quality analyses were conducted in CPCB's chemistry laboratories except the herbicides analyses, which were performed at the University of Kansas's Chemistry Department laboratories.

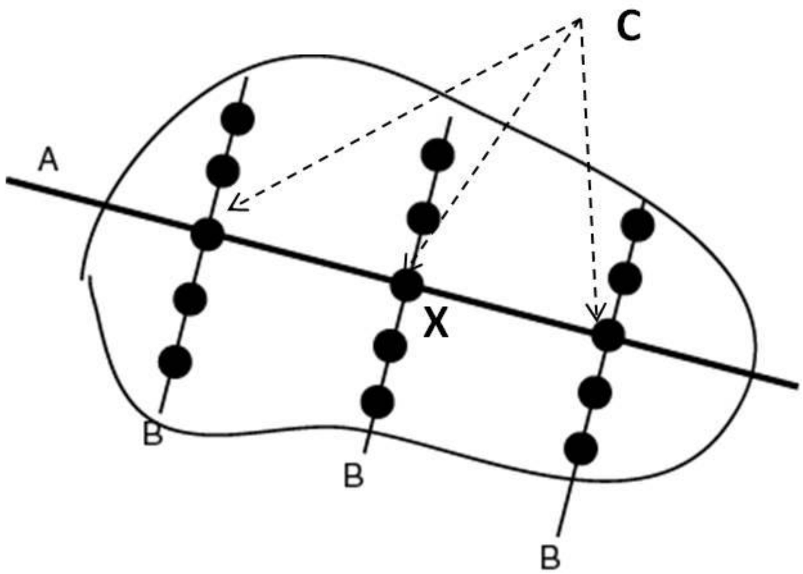

Figure 2. Illustration of wetland survey layout. X = wetland centroid where GPS location was recorded. A = long axis transect line. B = cross axis transect lines. C = composite water sample. ● = *in situ* water quality measurement locations.

Macroinvertebrates

Macroinvertebrate sampling was conducted at four sites in the littoral zone of the major vegetated habitat areas within each wetland. These zones were usually transitional areas between open water and emergent macrophyte beds, more commonly referred to as 'edge' habitat. At each zone, a kick and sweep method with a 500-micron D-frame aquatic net was used to capture invertebrates in the benthos substrate. The surface of the benthos was disturbed for 30 seconds with movement of the foot through the approximately top 10 centimeters of substrate, while sweeping the net through the water column directly above the turbulence. The contents of the aquatic net sample from each of the four zones were transferred from the net to a one-liter Nalgene collection bottle to create a composite sample. To ensure proper preservation of invertebrate collection, multiple bottles for each sample site were used with each sample bottle filled to one-third the volume with collected substrate. Bottles were labeled and samples were preserved in 10% buffered formalin with rose Bengal.

Macroinvertebrate samples were relinquished to the custody of the CPCB macroinvertebrate lab, rinsed of field fixative, and sorted to a 500 organism count according to the USEPA EMAP methods (USEPA 1995, USEPA 2004), explained in the Standard Operating Procedure (SOP) of the CPCB at the KBS (Blackwood 2007). Specimens were identified to the genus level for most taxonomic groups when possible (Blackwood 2007). Data were recorded on data sheets and then entered into a Microsoft Access relational database.

Macroinvertebrate data containing taxonomic names and specimen counts were linked to an integrated taxonomic information system (ITIS) (www.itis.gov/index.html) data table, and fields containing higher taxonomic groups were created (Phylum, Class, Order, etc.). Errors in nomenclature were identified and corrected before further field creation and classification commenced. In ECOMEAS software (Slater 1985), total taxon richness, Shannon's diversity index, and other diversity indices were computed for each sample. Feeding guilds, habitat behavior, tolerance, and sensitivity values were added to the macroinvertebrate database (Barbour *et al.* 1999, Huggins and Moffett 1988). Taxa without this information were updated from the aquatic insect identification and ecology literature (Smith 2001, Thorp and Covich 2001, Merritt and Cummings 2008). Additional metrics were calculated from this information and all macroinvertebrate metrics exported along with water quality, herbicide, floristic, and disturbance variables to the Number Cruncher Statistical System (NCSS) (Hintze 2004) for statistical analysis.

Disturbance Assessment

The Assessment

After considering several reviews of wetland rapid assessment methods (Fennessy *et al.* 2004, Fennessy *et al.* 2007, Innis *et al*. 2000), the Ohio Rapid Assessment Method (Mack 2001) and the California Rapid Assessment Method (Sutula *et al*. 2006) were used as models in designing the Missouri River Floodplain Wetland Disturbance Assessment. While the California and Ohio methods attempt to provide a more or less comprehensive evaluation of wetland rapid assessment parameters, the disturbance assessment developed for this study focused on *Wetland Attributes, Reference Indicators, and Disturbance* (Table 1). *Wetland Attributes* are used to score how able the wetland is to deal with disturbance (or how it is currently dealing with it).

Reference Indicators are those wetland characteristics and conditions most often associated with least impacted or minimally impacted wetlands. Other indicators might include public use restrictions, protective regulations associated with some wetland areas and other factors that might be protective of wetland structure and function. *Disturbance* is defined as evident physical perturbations or known observable impairments that may occur as a result of them, such as excessive sedimentation and/or altered hydrology. Some overlap between assessment metrics was inevitable, but care was taken to avoid redundancies in scoring. Metrics dealing directly with the classification scheme used in this study (*i.e.* depth and the temporal dimension of inundation) were also left out. Finally, metrics pertaining to the water and floristic quality response variables measured in the field that deal with known ecological impacts of disturbance were limited so as not to affect adversely a comparison with data from a Floristic Quality Assessment.

The resulting assessment method is advantageous in the sense that it is a subjective scoring process in which the user is evaluating human impacts without being asked to make specific judgments about the more technical aspects of wetland ecological integrity. Though the three sections in the disturbance assessment are meant to be used together to estimate an overall score for a wetland or specific area within a wetland complex. In addition attributes and scoring within each of the three sections can be examine individually to more specifically assess or describe certain wetland characteristics or trends in wetland condition.

Table 1. Assessment parameters used in quantifying disturbance. Wetland attributes are scored up to 3 points each, and reference and disturbance parameters ±1 point. See Appendix D for field sheet used in scoring.

Wetland Disturbance Assessment Parameters		
Wetland Attributes	**Reference**	**Disturbance**
Size (acres)	*Legal Protection*	*Sedimentation*
Buffer Width (m)	*Amphibian Habitat*	*Upland Soil Disturbance*
Surrounding Land Use	*Waterfowl Habitat*	*Presence of Cattle*
Hydrology (Water Source)	*Endangered/Threatened Species*	*Excessive Algae*
Vegetation Coverage	*Interspersion*	*>25% Invasive Plants*
	Connectivity	*Steep Shore Relief*
		Altered Hydrology
		Management

Wetland Attributes
Three wetland size classes (<25 acres, 25-50 acres, and >50 acres) were selected based on the range of surface areas for individual wetlands and wetland complexes in the lower Missouri River floodplain and the findings of other rapid assessment methods (*e.g.*, the Ohio Rapid Assessment Method) gauged as appropriately "large" wetlands.

Natural buffer width or buffer thickness was an important metric according to several published assessment methods. Natural buffers are thought to provide protection against local disturbances.

Surrounding land use is defined as intensive, recovering, undisturbed, or a mixture of intensive and undisturbed (scored the same as “recovering” landscape). Row crops, grazed pasture, residential areas, and/or industrial complexes that are adjacent to the study area were considered intensive uses. Natural buffer should be considered part of the surrounding land in the ‘undisturbed’ category.

Hydrology can be an indicator of wetland class and vary independently of human disturbance. However, in the context of assessing human disturbance and in some respect functionality in the landscape (in terms of connectivity), different hydrological variables were scored according to potential and actual water source(s) for individual wetlands. Historically, floodplain wetlands probably received water; 1) directly as a result of local precipitation events (e.g. rainfall and localized runoff), 2) as groundwater from the shallow water table of the floodplain, and 3) from flood waters as a result of the historical hydrologic regimes. Wetlands develop rapidly with a continual (or seasonal) inflow of river water (or overland flow), which maintains steady propagule/organism inflow and allows for mixing of basins during floods, a process known as ‘self-design’ (Mitsch *et al.* 1998). Since the most natural functional condition for floodplain wetlands would include their filling and flushing by floodwaters associated with natural hydrological events within the river basin, the assessment of the degree of hydrological disturbance must include an estimate of “disconnection” of the wetland from the river system. While assessment of all factors (e.g. number of dams, amount of channelization and levees) that may affect the hydrological connection between the river and wetland is difficult due to scale issue an attempt was made to estimate and score natural hydrological conditions highest, wherein less natural sources, such as storm water drains or channelized ditches receive an intermediate and low scores.

Vegetation coverage below 20% was thought to be indicative of a disturbed wetland or a wetland that is more venerable to perturbations. Coverage of over 70% often reduces the amount of open water to vegetation “edge” and the potential for habitat diversity, so receives an intermediate score. Finally, 40-70% coverage was thought to be ideal for floodplain wetlands because a moderate amount of vegetation coverage suggests a high occurrence of edge habitat between open water and vegetated areas, providing for a diversity of habitats.

Reference Indicators

Indicators of reference conditions refer to the absence of human disturbance within the wetland. Metrics that reflect undisturbed ecological condition can be combined for a condition score used to track the status of a site. Reference indicators are a combination of factors that impede and control human disturbance or indicate the presence of valuable wetland features or “value-added metrics” (Fennessy *et al.* 2007). The inclusion of reference indicators was necessary to facilitate the inclusion of factors that were not numerically quantifiable like those evaluated in the Wetland Attributes section, but were better evaluated by their presence or absence.

Protected wetlands deter certain types of human disturbance over time, thereby increasing the probability that the wetland experiences relatively little disturbance (except for management, which is discussed in the next section).

Evidence that *waterfowl* and/or *amphibians* are present or would be present during the migratory season, suggests the wetland is capable of providing wildlife habitat, including food and nesting cover.

Endangered or Threatened Species warrant further protection of the area under federal laws and would thus generally discourage disturbance.

Interspersion (Mack 2001) refers to natural non-uniformity in wetland habitat design. Some native wetland species require multiple habitat-types. If these habitat-types are not in close proximity to one another, or interspersed throughout the wetland area, then it may be difficult for such species to survive. The assumption is that between two wetlands of the same size and with the same proportions of open-water to vegetated habitat, the one exhibiting the greatest interspersion of habitats likely will support greater native wetland biodiversity and will be more similar to a ‘reference’ state.

Connectivity refers to a wetland’s functional and structural connection to other landscape and hydrologic features. Features that disrupt connectivity, such as river or stream impoundments, levees, berms, or other water structures, can be easily identified on a local level and indicate disruptions to historical hydrologic regimes. It is more difficult to assess broad-scale and cumulative hydrological impacts to floodplain wetlands since at some scale nearly all floodplains and riverine systems have become hydrologically altered to some degree. This assumes most floodplain wetlands were originally connected to the river or that water was able to cycle between these systems intermittently.

Disturbance

Metrics that indicate human disturbances known to degrade wetland health are listed in this section of the Disturbance Assessment. For each disturbance a point is subtracted. If the disturbance is unusually severe or at a high rate of occurrence, then more than one point can be subtracted.

Sedimentation is a natural process for wetlands in the Missouri River floodplain, however modern land use changes that affect the spatial and temporal extent of permanent ground cover can accelerate soil loss and increased sedimentation (observed as plumes or fresh deposits within wetlands) that dramatically affect the structure and function of wetlands. Scoring the extent of wetland sedimentation is not dependant on the identification of anthropogenic or natural causes.

Upland soil disturbance or tillage in the immediate area drained by the wetland is scored separately as a local disturbance that demonstrates the potential for excessive sedimentation, although it may not be observable at the time of evaluation.

The *presence of cattle* is not considered a natural occurrence, even in circumstances where the cattle graze the wetland periodically throughout the year.

Excessive algae usually suggest an imbalance within an aquatic ecosystem (*i.e.* excessive nutrients or eutrophication). Regardless of whether the cause is fertilizer run-off, sediment resuspension, or cattle, the presence of excessive algae can impede the growth of aquatic/emergent plant life and threatens the survival of some aquatic organisms.

Wetland surface area is comprised of over 25% invasive species. Invasive plant species are themselves a disturbance and an indicator of degraded wetland conditions (e.g. hydrological alterations, soil disturbance) that favored their growth over native species.

Steep shore relief is a common occurrence in created wetlands that were constructed during the last few decades of the 20th century. Examples would include "barrow" pits from road construction, farm ponds, or natural wetlands that were dredged to reduce the littoral zone. Some of these wetlands exhibit a uniform depth and, although they may cover areas of hundreds or thousands of acres, they may exhibit little shore relief. In nature, a high shore length to surface area ratio and gradual relief in littoral zones generally characterize floodplain wetlands in the Midwestern US. The structural uniformity of some created and altered wetland systems may favor invasive species and decrease biodiversity.

Hydrologic alterations that contribute to "disconnection" of the wetland from the historical flow regime of the river are differentiated from alterations that contribute to their historic connectivity with the riverine system.

Management for specific purposes, such as hunting, fishing, or wildlife preservation may result in systems that are broadly impaired and do not fully support other wetland uses or functions. Management practices can be observed at particular wetland sites and their objectives confirmed by conversations with the landowners or designated managers.

Results

<u>Explanation of statistical analyses and graphical representations</u>
Comparisons between study phases, ecoregions, major wetland classes, and vegetative types were performed on FQA, disturbance assessments, water quality parameters, and macroinvertebrate metrics with ANOVA means analysis and Tukey-Kramer multiple comparison t-tests when sample populations were found to be normally distributed or when normal distribution could be obtained via log transformation. When ANOVA assumptions of distribution could not be assumed either due to number ($n < 5$) or distribution (i.e. skew, log factor, kurtosis), Kruskal-Wallace non-parametric variance analysis and normal Z-tests were performed. All statistical significance was measured at 95% confidence ($\alpha = 0.05$) with Kruskal-Wallace p values corrected for ties. Relationships between parameters were investigated with Pearson auto-correlations matrix having significant p values (≤ 0.05). Correlation coefficients and p values are reported when significance is found. Relationships were further scrutinized with robust linear regression routines that accommodate discrepancies associated with outlier data. Adjusted R^2 values and significant p values associated with linear regression t–tests are reported when statistically significant values were obtained. When statistical significance is not obtained, no value of p, R^2, or Pearson correlation coefficient is reported, and it can be assumed the level of significance was not achieved ($p \geq 0.05$) and the relationship was not substantial.

Box plot representations are used extensively throughout the text because range, distribution, and identification of moderate and extreme outliers become readily apparent. Box area represents inner quartile range (IQR), while "whiskers" represent the upper observation that is less than or equal to the 75th percentile plus 1.5 times the IQR upper value and the lower observation that is greater than or equal to the 25th percentile minus 1.5 time the IQR lower value.

Floristic Quality Assessments

Floristic Quality Assessments were conducted for all 42 sites visited during the 2008 and 2009 seasons; mean and median values of plant community metrics and final Floristic Quality Indices (FQI) are reported in Table 2. Only mean values and variance in mean conservatism were found to be significantly different between sample populations of the Western Corn Belt Plains (n = 21) and Central Irregular Plains (n = 16) ecoregions based on ANOVA evaluation and Tukey-Kramer multiple comparison tests. The mean value for the Interior River Valleys and Hills sample population (n = 5) fell between the other two ecoregion sample populations, with mean value of mean conservatism of native plant species for the Central Irregular Plains, Interior River Valleys and Hills, and Western Corn Belt Plains regions being 4.58, 4.08, and 3.64, respectively. Mean conservatism for all plant species mean ecoregion values were slightly lower than that of native plant species but maintained the same hierarchy (Table 2, Figure 3).

Table 2. Descriptive statistics for Florist Quality Assessment metrics of the random population of wetlands in the floodplain of the Missouri River.

Metric	Count	Mean	STDDEV	Median	Min	Max
FQI All	42	17.18	4.40	16.57	9.43	26.11
FQI Natives	42	18.17	4.31	17.69	11.09	27.14
Richness All	42	27.12	15.14	25.00	5.00	66.00
Richness Native	42	23.76	12.99	22.00	5.00	55.00
Percent Adventive	42	11.20	8.13	10.00	0.00	30.43
Mean Conservatism All	42	3.64	1.07	3.63	1.70	6.00
Mean Conservatism Natives	42	4.05	0.97	4.10	2.16	6.00

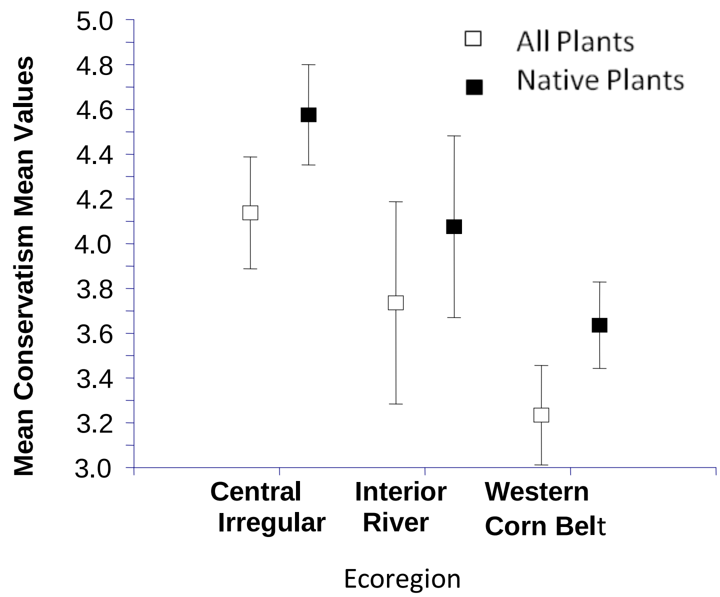

Figure 3. Ecoregional means of mean conservatism values for plant richness of all and native species.

Though the majority of wetland polygons surveyed were found to be palustrine systems based on Cowardin's 2-meter depth criterion, wetlands were assigned dummy variables that indicating their dominant hydrological influence. Lacustrine systems in this survey were of two types: polygons identified as lakes by the NWI dataset and having dominant aquatic plant establishment or polygons that were littoral zones of lakes with wetland features. Palustrine systems were either identified by NWI as freshwater emergent wetlands or lakes, yet had consistently shallow depths and were dominated by emergent macrophytes. Riverine systems were those wetlands identified by field observation and GIS mapping that were backwaters and sloughs having continuous connectivity or frequent connectivity with the Missouri River. Significant differences in FQI, plant richness, mean conservatism, and percent adventive species were identified in ANOVA evaluation based on this assigned wetland classes. The palustrine sample population maintained statistically significant higher mean FQI All and FQI Native scores over the riverine sample population. The palustrine sample population also had significantly higher plant richness for all and native species. The riverine sample population had higher mean percent adventives than the lacustrine sites with palustrine sites falling somewhere between and similar to both lacustrine and riverine types. Finally, lacustrine types had significantly higher mean values of mean conservatism for all and native species. All mean values and variance were significant for the relationships between FQA metrics and wetland hydrological classes mentioned above based on ANOVA and Tukey-Kramer multiple comparison tests ($p < 0.05$, Table 3-5, Figure 4-7).

Table 3. Descriptive statistics for Floristic Quality Index (FQI) scores for wetland classes: lacustrine, palustrine, and riverine.

		Lacustrine	Palustrine	Riverine
	Count	15	21	6
FQI ALL	Mean	16.62	18.85	12.74
FQI ALL	Median	16.36	18.57	10.73
FQI ALL	StdDev	3.6	4.2	4.01
FQI ALL	Min	11.4	11.67	9.43
FQI ALL	Max	22.39	26.11	19.45
FQI NATIVE	Mean	17.22	20.04	14.01
FQI NATIVE	Median	17.03	20.23	11.98
FQI NATIVE	StdDev	3.57	4	3.87
FQI NATIVE	Min	12.56	13.15	11.09
FQI NATIVE	Max	23.7	27.14	20.43

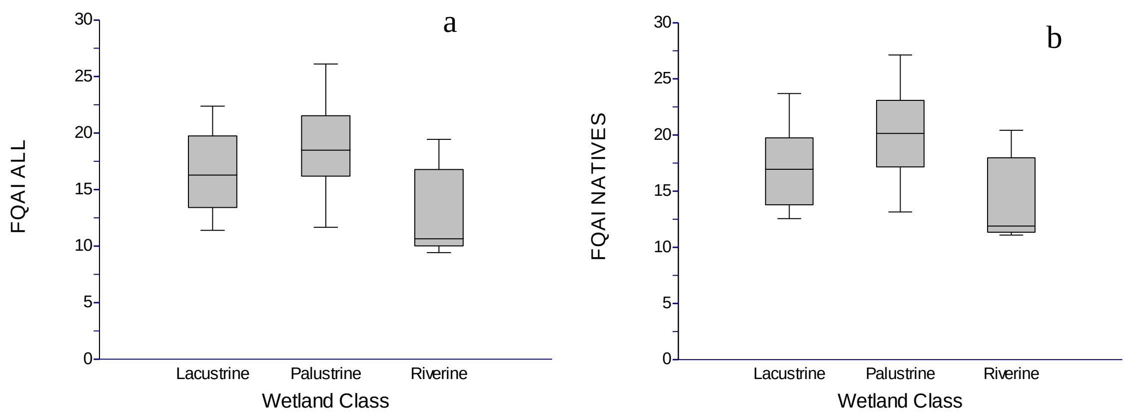

Figure 4. Box plots of floristic quality index scores (FQAI) for: (a) all plant species and (b) native plant species.

Table 4. Descriptive statistics of plant richness among lacustrine, palustrine, and riverine wetland classes for both native plants and the entire community of plants.

		Lacustrine	**Palustrine**	**Riverine**
	Count	15	21	6
RICHNESS ALL	Mean	14.4	37.43	22.83
	Median	13	36	22.5
	StdDev	6.05	13.44	10.07
	Min	5	16	7
	Max	27	66	35
RICHNESS NATIVES	Mean	13.4	32.57	18.83
	Median	12	30	17.5
	StdDev	5.85	11.44	9.33
	Min	5	16	6
	Max	27	55	30

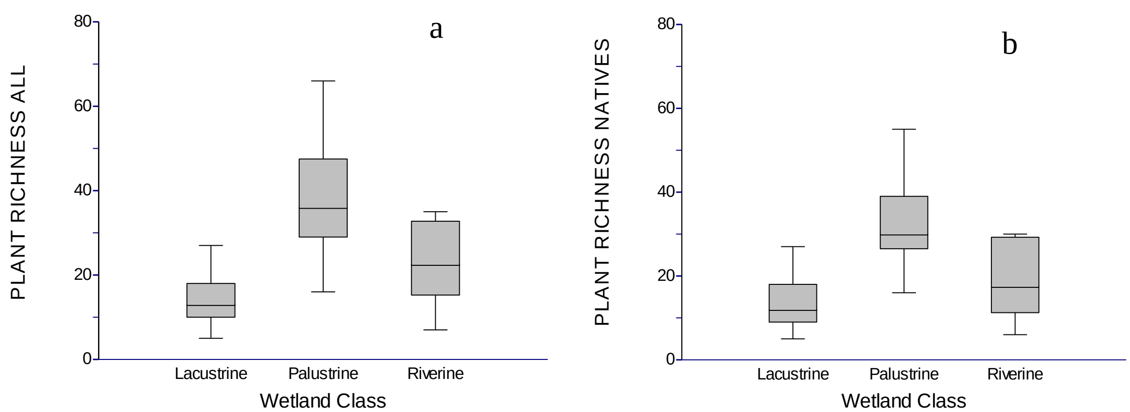

Figure 5. Box plots showing distribution of plant richness values for: (a) all and (b) native species among the lacustrine, palustrine, and riverine wetland classes.

Table 5. Descriptive statistics of selected FQI metrics (percent adventive species and mean conservatism for all and native species) among lacustrine, palustrine, and riverine wetland classes.

		Lacustrine	Palustrine	Riverine
	Count	15	21	6
PERCENT ADVENTIVE	Mean	7.04	12.14	18.3
	Median	7.69	10	14.29
	StdDev	6.64	7.56	8.61
	Min	0	0	9.38
	Max	18.18	27.08	30.43
MEAN CONSERVATISM ALL	Mean	4.54	3.23	2.8
	Median	4.54	3.45	2.5
	StdDev	0.77	0.9	0.76
	Min	2.76	1.7	2.13
	Max	6	4.88	4
MEAN CONSERVATISM NATIVES	Mean	4.87	3.64	3.4
	Median	4.94	3.56	3.11
	StdDev	0.68	0.83	0.72
	Min	3.36	2.16	2.68
	Max	6	4.88	4.67

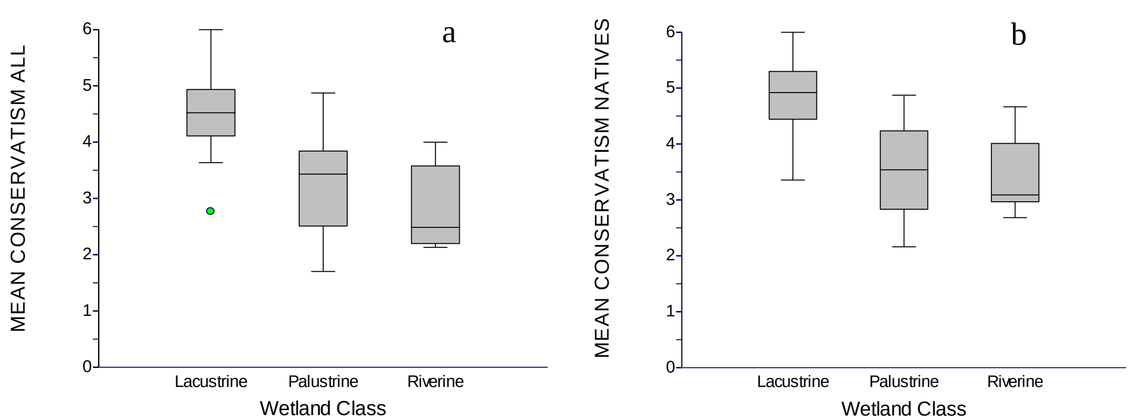

Figure 6. Box plots of mean conservatism values for: (a) all and (b) native species among lacustrine, palustrine, and riverine.

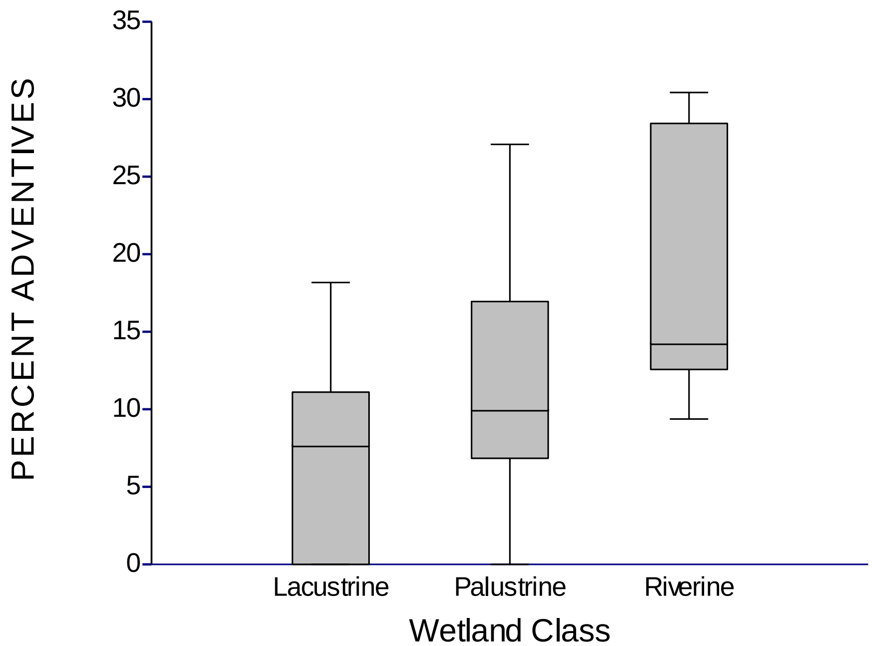

Figure 7. Box plots of percent adventive values among lacustrine, palustrine, and riverine wetland classes.

In addition to class, wetlands were identified as having three dominant plant community structures and were classified according to the type of vegetated conditions observed. Aquatic beds (AB) were wetlands with open waters zones commonly inhabited by obligate aquatic submergent and emergent hydrophytes. Unconsolidated beds (UB) were wetlands that had open water zones, but were more frequently observed having little to no hydrophytes or fringe flora such as geophytes (i.e. cattail, bulrush, etc). Emergent macrophyte beds (EM) were commonly very shallow palustrine sites with dense stands of cattail, bulrush, reed canary grass (*Phragmites sp.*), and other facultative wetland plants. Wetlands that were found to have all three types equally dominant were classified as a mixed type (MIX). This is further discussed in the comparison of Phase I and Phase II results.

In situ water quality

In situ water quality measures were collected at 38 of the 42 sites visited. Data collected included depth measurements and water chemistry readings from the Horiba U10 water quality checker including: water temperature, dissolved oxygen, mean pH, and mean turbidity (Table 6). Significant differences among wetland classes were not observed for any of the water chemistry metrics for the wetland population. However, log-transformed mean conductivity mean values were significantly ($p < 0.000$) lower for the wetland population of the Central Irregular Plains ecoregion than both the Western Corn Belt Plains and the Interior River Valleys and Hills ecoregions (Figure 8**Error! Reference source not found.**).

Table 6. Descriptive statistics of random population in situ water chemistry measures.

Parameter	Count	Mean	Standard dev	Standard error	Min	Max	Median	25th percentile	75th percentile
Mean depth m	38	0.63	0.43	0.07	0.11	2.08	0.51	0.35	0.81
Maximum depth m	38	1.06	0.82	0.13	0.2	4.2	0.82	0.49	1.29
Mean Secchi depth m	38	0.43	0.46	0.08	0.08	2.82	0.31	0.18	0.6
Mean temperature C	38	27.06	2.78	0.45	20.4	33.5	26.95	25.48	28.63
Mean dissolved oxygen	38	6.19	3.15	0.51	0.38	12	5.93	3.48	9.02
Mean pH	38	7.77	0.78	0.13	5.59	9.53	7.61	7.33	8.26
Mean turbidity NTU	38	67.68	60.32	9.79	3	242	55	17.75	110
Mean conductivity mS/cm	38	0.31	0.17	0.03	0.07	0.86	0.28	0.22	0.35

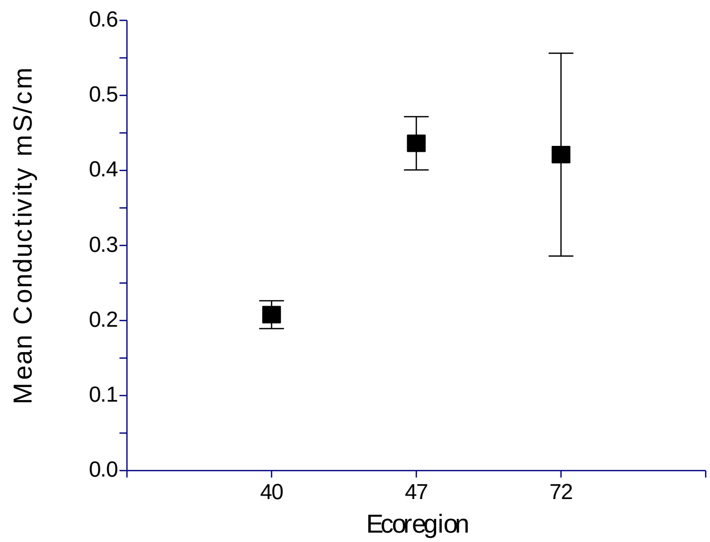

Figure 8. Error bar chart of ecoregional means of mean conductivity values: 40 - Central Irregular Plains, 47 - Western Corn Belt Plains, and 72 - Interior River Valleys and Hills. Error bars are measures of standard error.

Mean value for the mean conductivity measures in the Central Irregular Plains ecoregion was 0.205 mS/cm, well below the values found in the Western Corn Belt Plain (0.342 mS/cm) and the Interior River Valleys and Hills (0.307 mS/cm). Median values for the ecoregions were similarly significantly different when Kruskal-Wallace non-parametric medians test was performed. Many significant relationships between the mean conductivity and other assessment metrics were observed and will be discussed later.

Depth measures

Mean and maximum depth measures for the Phase II samples (n = 38) were not normally distributed thus log transformation of the depth values was necessary to perform the ANOVA's to examine regional and class differences (Figure 9**Error! Reference source not found.**). Significant differences in mean and maximum depths were not observed among ecoregions. When major hydrological system classes were analyzed, log means and variance for the lacustrine sample population (n = 15) were significantly higher in mean and maximum depths than the palustrine sites (n = 18) for both measures and higher in mean depth than the riverine sample population (n = 5). Secchi depths were also observed as being statistically higher in means among the lacustrine than both palustrine and riverine samples.

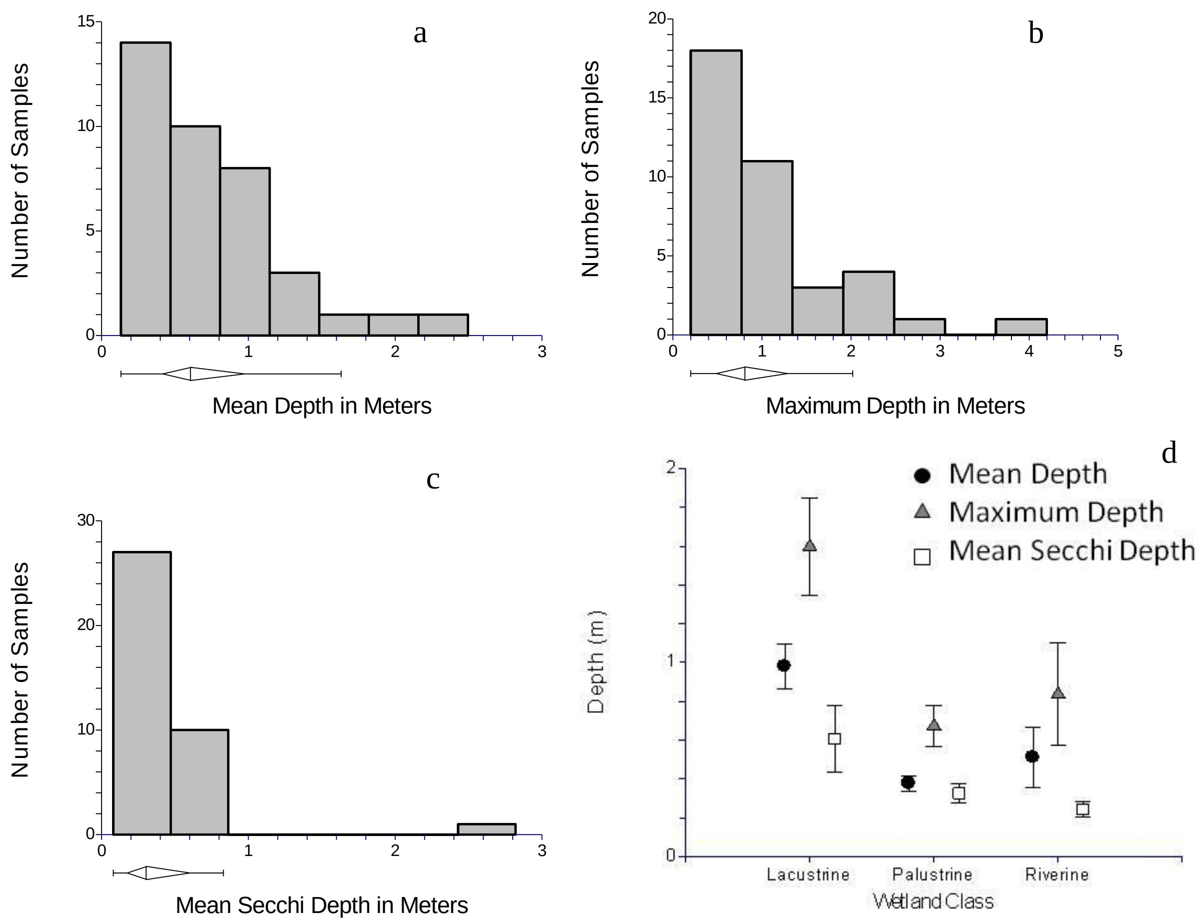

Figure 9. Random (Phase II) sample population distribution represented by frequency histograms of: (a) mean depth, (b) maximum depth, (c) Secchi depth, and (d) error bar chart of all in situ depth measures. Error bars are measures of one standard error.

Nutrient Analyses

Many of the analyzed nutrients exhibited broad ranges and extreme values (Table 7). The wide range of values reflects the large amount of variability observed across the lower Missouri River floodplain wetlands. Evaluation of the various nutrient fractions and totals can give us an idea of the primary productivity and nutrient cycling in the sample population.

Table 7. Descriptive statistics of nutrient measures for Phase II wetland water samples.

Nutrient Measure	Count	Mean	Median	STDEV	Min	Max
NO3 + NO2 mg N/L	38	0.03	0.01	0.03	0.01	0.13
NO2 mg N/L	38	0.01	0.01	0.00	0.01	0.02
NH3 µg N/L	38	85.01	48.95	115.95	18.90	555.00
Total N mg N/L	38	1.14	1.07	0.48	0.39	2.91
Dissolved N mg N/L	38	0.11	0.07	0.12	0.02	0.56
PO4 µg P/L	38	171.41	53.55	427.28	6.90	2630.00
Total P µg P/L	38	414.37	264.50	606.83	16.30	3710.00
Avail N:Avail P	38	3.04	1.21	5.44	0.05	29.63
TN:TP	38	5.34	4.19	4.79	0.48	23.93
Chlorophyll-*a* µg/L	38	30.68	24.47	30.99	0.70	171.83
Pheophytin a µg/L	38	12.38	10.17	10.96	0.71	65.41
TOC mg/L	38	10.54	9.80	3.81	5.60	20.74
DOC mg/L	38	9.23	8.75	2.98	5.40	17.93

Nitrogen

Measures of ammonia, nitrates, and nitrites were similar for all three major classes of wetlands and all three ecoregions when ANOVA and Kruskal-Wallace non-parametric means analysis were performed. Total nitrogen was almost significantly different between palustrine (1.34 mg/L) and both lacustrine (0.97 mg/L) and riverine (0.91 mg/L) classes (Figure 10-11). No significant difference was found with Kruskal-Wallace non-parametric medians analysis and the Tukey-Kramer multiple comparison tests. It was assumed that organic nitrogen component could theoretically be obtained by subtracting all dissolved available nitrogen fractions from the total nitrogen concentration value. Total nitrogen appeared to be comprised mostly of the organic nitrogen fraction with little available dissolved nitrogen compounds. This may reflect the overall high productivity that is generally associated with wetland ability to assimilate external and internal nitrogen sources into biomass. This concept is reinforced by the observed elevated concentrations in the palustrine wetlands which generally had higher plant richness and greater densities of standing emergent macrophytes. However, some effects of concentration over dilution may account for the variability in nitrogen concentrations.

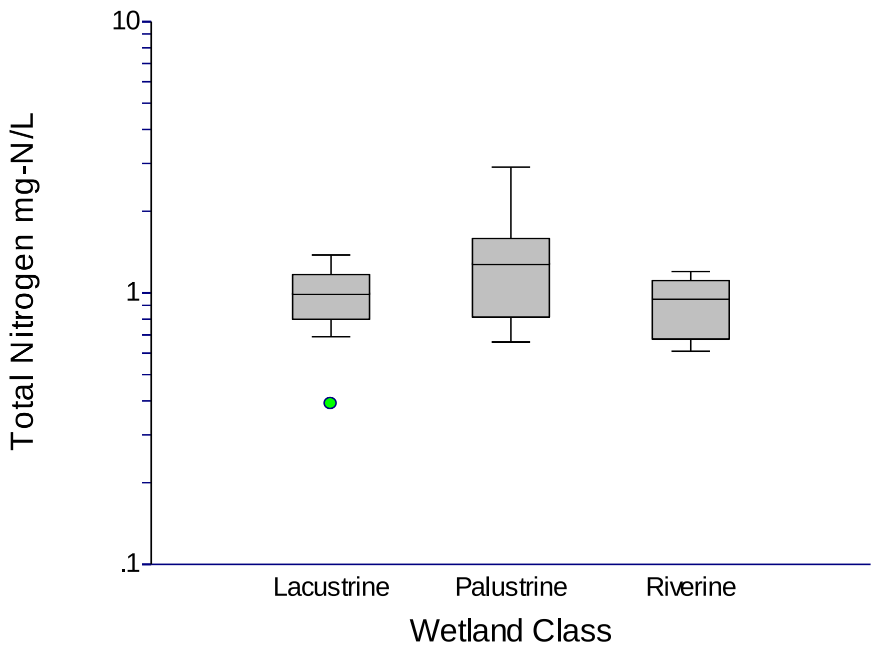

Figure 10. Box plots of total nitrogen concentrations among lacustrine, palustrine, and riverine classes in log scale.

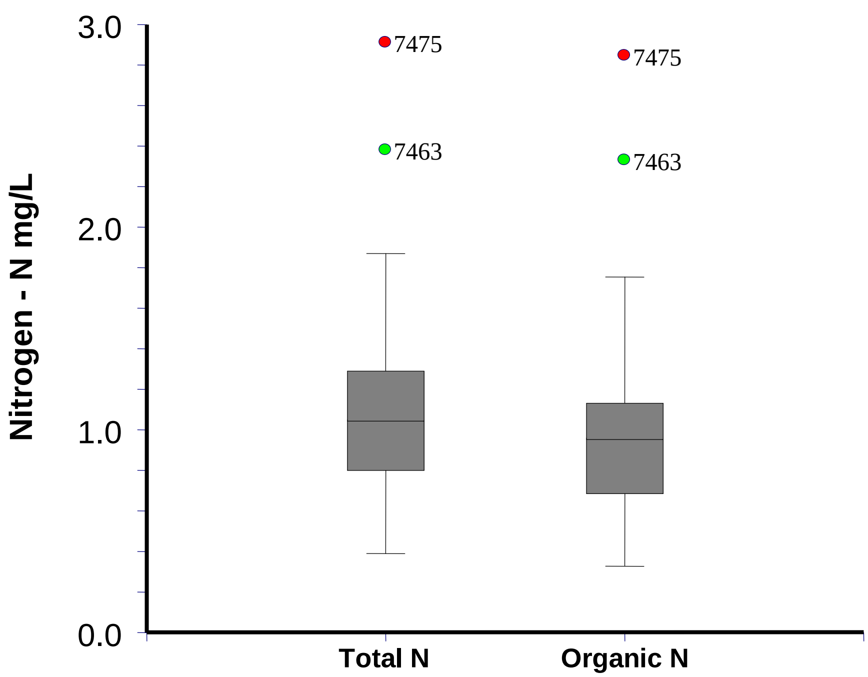

Figure 11. Box plots of measure total and calculated organic nitrogen for random population of Phase II study. Sites 7475 and 7463 were both emergent palustrine sites with very shallow mean and maximum depths. Site 7475 (French Bottoms) was densely covered with reed canary grass

with small intermittent pools having large amounts of detritus. 7463, located in the Swan Lake complex also had significant detrital matter, but was dominated by cattail and bulrush. In the Swan Lake site was edged with by a deeper pool allowing the establishment of some aquatic plants.

Phosphorus
Most of the total phosphorus in these wetlands appeared to be organic phosphorus (Figure 12). Median values for all forms of phosphorus were around 0.1 to 0.2 mg/L of P. However, total and organic phosphorus levels in some wetlands were well above 1000 ug/L.

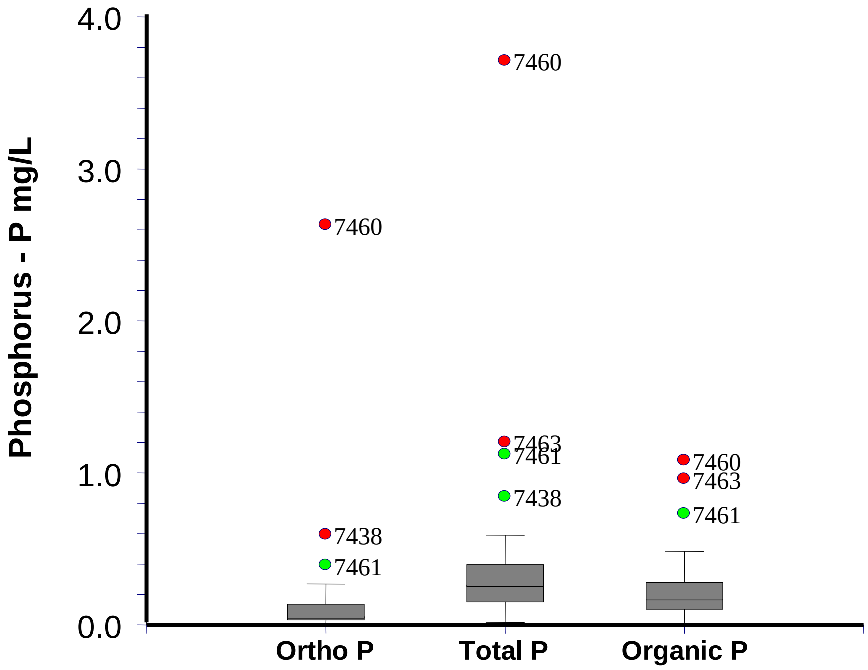

Figure 12. Box plots showing range of phosphorus values and moderate and extreme outliers among the random population. Ortho P is orthophosphate.

Wetland groups created by aggregating wetlands into ecoregion and hydrological classes shared similar log mean and median values among the nutrient measures of nitrogen and phosphorus. Nitrogen to phosphorus ratios were also similar among these groups, though more outliers were observed in the N:P groupings (Figure 13).

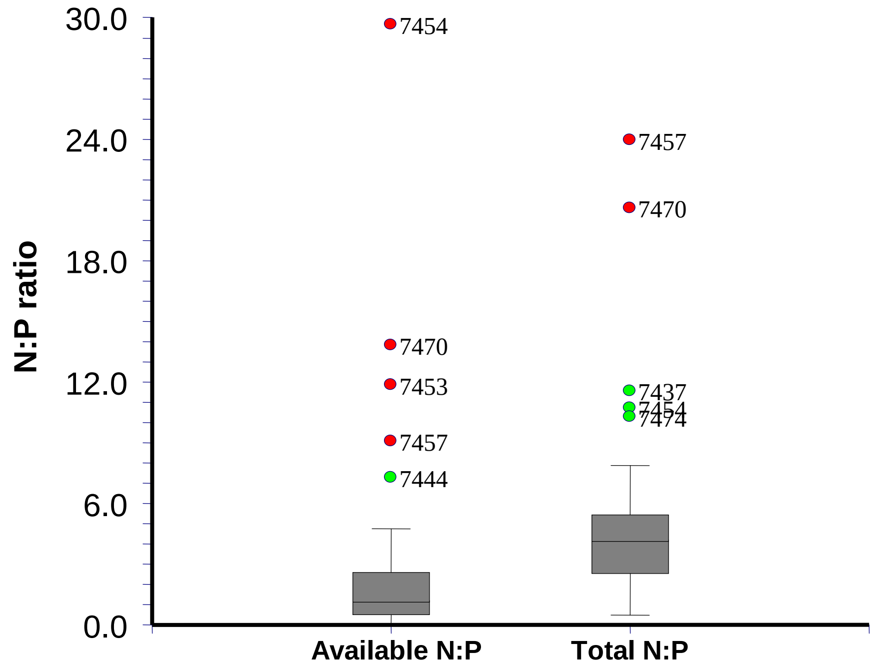

Figure 13. Box plots of nitrogen to phosphorus ratios, moderate, and extreme outliers among the random population.

Carbon

Unlike nitrogen and phosphorus, measures of variance of total organic carbon (TOC) and dissolved organic carbon (DOC) were found to be significantly higher in palustrine sites which had a wider range of values than lacustrine or riverine sites (Figure 14). Tukey-Kramer multiple comparison of mean values among classes revealed no significant differences. Medians test of data did not identify significant variance or differences in median values for TOC or DOC. The dissolved organic carbon makes up a considerable amount of the total water column carbon measure, approximately 88%, indicating that carbon was not incorporated in sestonic organisms and is in considerable surplus concentrations in these wetlands.

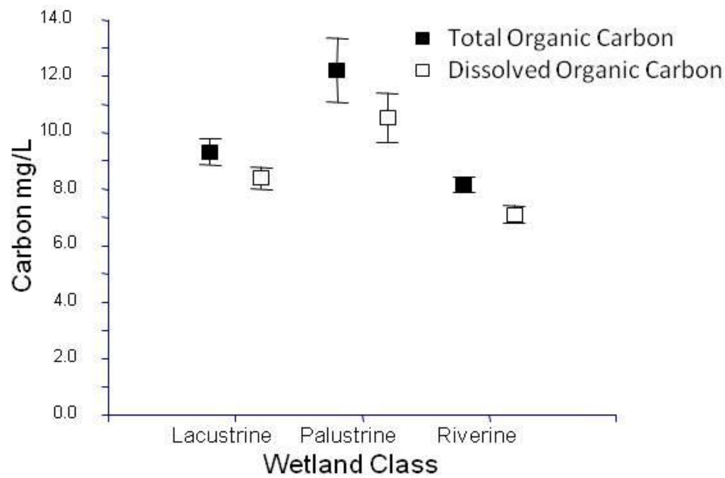

Figure 14. Error bar plots of total and dissolved organic carbon concentration means among the major wetland classes. Error bars are one standard error.

Normality tests of the chlorophyll-*a* and pheophytin-*a* data revealed that some samples were significantly different than the rest of the population (Figure 15). Attempts to achieve normal distribution via log transformation failed, thus data were analyzed for differences among ecoregions and classes using the Kruskal-Wallace non-parametric medians test instead of the ANOVA. The lacustrine sample population was determined to be significantly higher in median chlorophyll-*a* concentrations than the palustrine population (Figure 16). The riverine population shared ranges in variance with the other classes and the median value was similar to the others.

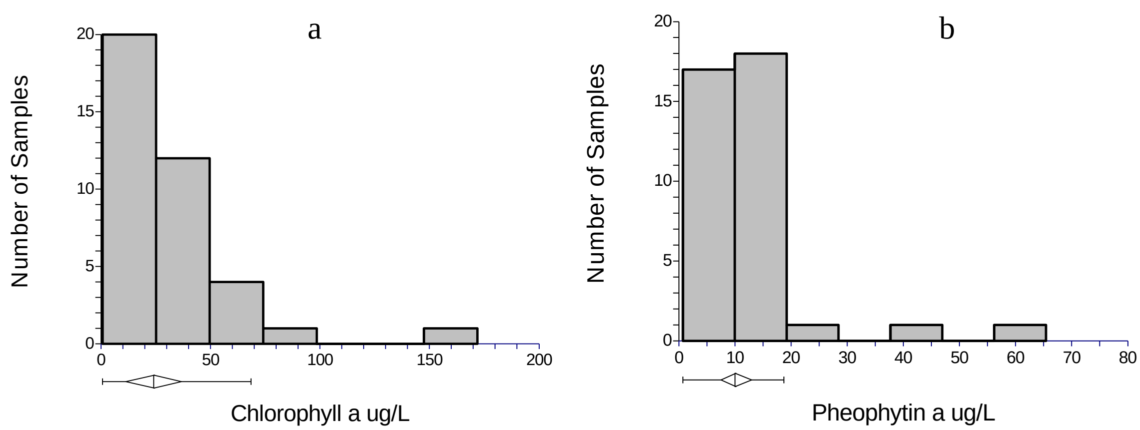

Figure 15. Frequency histograms showing sample distributions based on concentration of: (a) chlorophyll-*a* and (b) pheophytin-*a*.

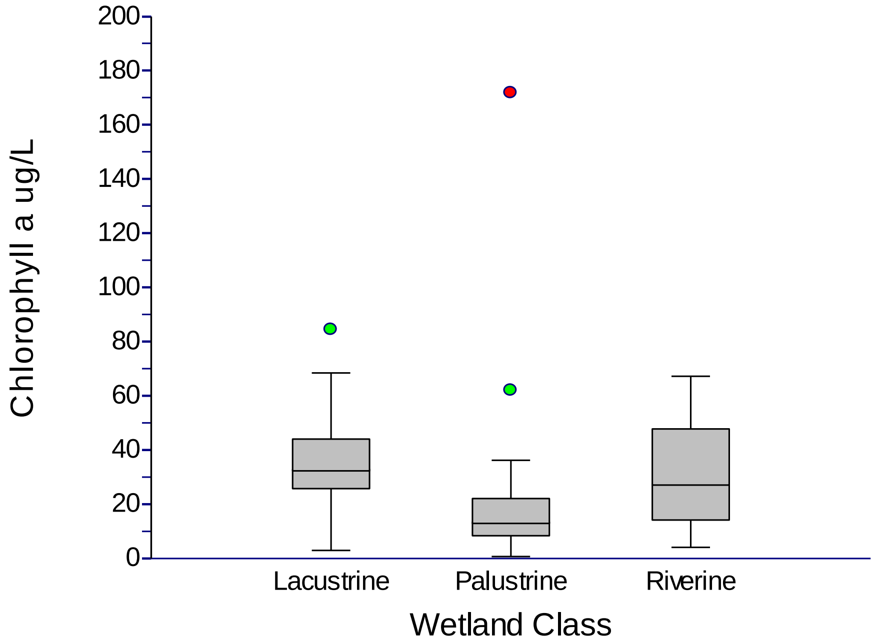

Figure 16**.** Box plots showing distribution of chlorophyll-*a* values for the lacustrine, palustrine, and riverine wetland classes.

<u>Herbicides</u>

Atrazine was found more frequently and typically in higher concentrations than all other herbicides. Atrazine metabolites (i.e. DIA and DEA) were often found at higher levels than the parent compound and median values for these metabolites exceeded the median for atrazine itself (Figure 17). No significant differences in concentrations or number of herbicides detected were found among wetland populations within ecoregion or hydrological class Most sites had measurable concentrations of six to seven of the eight herbicide analytes investigated. Simazine concentrations were typically the lowest for all herbicides detected in this study (Figure 17).

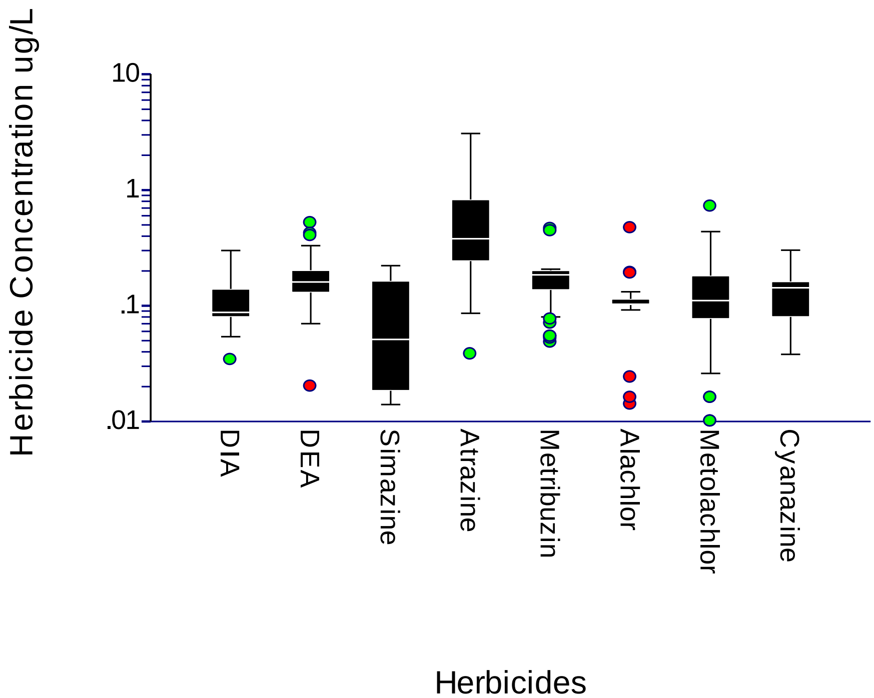

Figure 17. Box plots of herbicide concentrations for Phase II samples.

Comparisons between Phase I and II studies of the lower Missouri River floodplain wetlands

<u>Introduction and Background Information</u>
Before comparing results of both of our studies of the floodplain wetlands of the lower Missouri River some preliminary information is necessary. While we have referred to each of these studies as Phase I (see Kriz *et al.* 2007) and Phase II (this report) for reporting clarity, it is more accurate to refer to these two studies as reference and random population studies. The following section is meant to provide, in part, an assessment of the tools we developed in these studies as well as an assessment of the relative impairment of the randomly selected wetland population based on reference conditions identified in Phase I.

One of the conclusions of the reference wetland study (i.e. Phase I) was three of the 18 reference candidates (sites 7108, 7115, and 7116) were not of reference quality based upon their status as created or restored wetlands and their floristic quality assessment metrics. However, current evaluation indicated sites 7115 and 7116 had water quality and macroinvertebrate metric values that were within the range of the Phase I reference population and that site 7108 was an extreme outlier based on most water quality parameters, the floristic quality assessment, and macroinvertebrate data. Hence, site 7108 was excluded from our comparison studies and sites 7115 and 7116 were considered reference wetlands. In many analyses the numbers of samples (n) changed since not all sites had open water or an established macroinvertebrate community. All sites (n=64) were assessed for floristic quality (i.e. 21 reference candidates and 42 randomly selected wetlands). The final number of wetlands assessed using the developed Disturbance Assessment was 18 reference and 42 random. Water quality data was available for 17 and 38 wetlands, respectively. Macroinvertebrate collections were obtained from 54 sites, but the exclusion of one outlier (site 7108) reduced the number to 53, with 16 samples from Phase I and 37 samples from Phase II. Because disturbance assessment data were used in the macroinvertebrate selection process in developing a multiple metric index, one Phase I sample (7107) was excluded during the MMI development process because this information was not available. Because disturbance assessment information was used in development of the multiple metric index (MMI), only 52 sites were used in developing the MMI but all 54 samples were scored.

Floristic Quality Assessment Results

No significant differences were found between Phase I and Phase II sample populations when ANOVA and Tukey-Kramer multiple comparison test were performed on the FQI and Native plant FQI scores. However, significant differences ($p = 0.001$) in variance and mean total richness and native richness were found between the two study groups. Total plant richness mean value for reference sites was 41.05 (STDERR = 14.39) and 3.14 (STDERR = 2.34) for the random sites. Native plant richness mean value for reference sites was 36.10 (STDERR = 2.68) and 23.76 (STDERR = 2.00) for the random sites. Mean conservatism was also found to be significantly different between reference and random, with 3.64 (STDERR = 0.17) for the random population and 3.07 (STDERR = 0.17) for the reference population. A similar trend was seen in the measure of native mean conservatism where the random population had a significantly ($p = 0.014$) higher sample mean (4.05, STDERR = 0.15) than the reference population (3.44, STDERR = 0.16). Mean percent adventive species values were not significantly different between the two wetland populations. Further evaluation efforts in higher order delineation of sites should consider these groups separately.

ANOVA and Tukey-Kramer multiple comparison tests were performed using all study sites to examine possible differences associated with sample year. In the random population, mean FQI was statistically higher ($p = 0.04$) in 2009 ($n = 10$, mean FQI = 19.65, STDERR = 1.33) than in 2008 ($n = 32$, mean FQI = 16.41). No significant differences in FQI were found between 2005 and 2009. ANOVA testing of FQI scores for 2005 and 2008 samples showed significant yearly differences ($p = 0.04$) in FQI values. When all years were compared again using one-way ANOVA and Tukey-Kramer comparison tests, significant yearly differences were again noted ($p = 0.03$), though the *post hoc* comparison test did not clearly indicate group separations. ANOVA test using only the randomly selected wetland data indicated that the mean Native FQI values were significantly different ($p = 0.02$) between 2008 (17.34, STDERR = 0.72) and 2009 (20.83, STDERR = 1.29). When 2005 and 2008 were evaluated without 2009, significant yearly differences in were found with 2005 having a mean value of 19.99 (STDERR = 0.9); but when 2008 was excluded from analysis, 2005 and 2009 values were not significantly different from each other ($p = 0.62$). This further suggests that 2009 plant community samples were similar to those collected in 2005. It remains unclear if yearly conditions affected the FQI metric or if differences were merely serendipity. Overall, the wetlands in both study phases appear to exist on a continuum of floristic conditions as indicated by the overlapping FQI scores between and among collection years.

The CDFs for both the reference and random wetland groups were similar, but the CDF for reference wetlands indicated that most all of scores were above those of the random population

(Figure 19). This supports the contention that these groups are distinctly different from each other floristically.

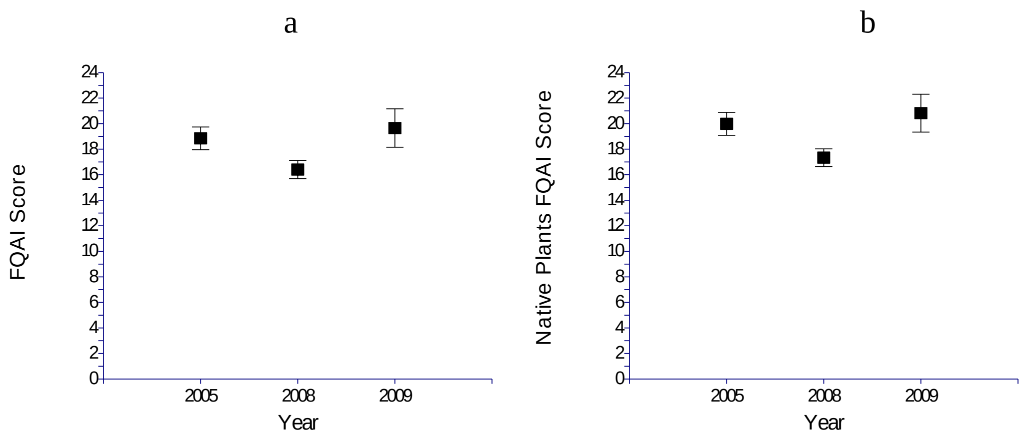

Figure 18. Mean error bar plots of florist quality assessment index scores for: (a) all species and (b) native species.

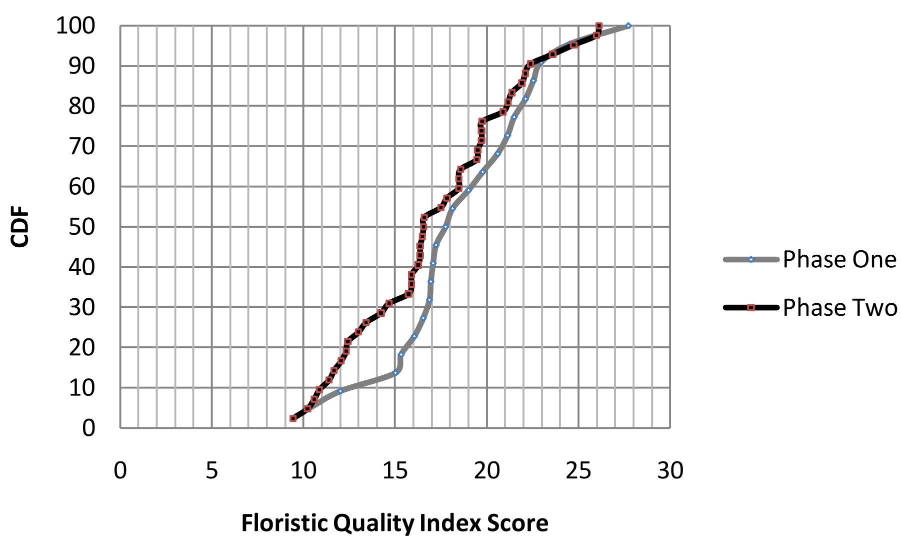

Figure 19. Cumulative distribution frequency (CDF) of FQI scores for reference and random populations of wetlands.

Plant Richness - All Species

Plant richness was significantly different (p = 0.001) among wetlands collected in each of the three study seasons. When all years were compared, 2005 had significantly higher mean plant richness value (41.05, STDERR = 3.14) than 2008 (24.91, STDERR = 2.61). When study Phase II was examined alone, no significant differences (p=0.09) were found between 2008 and 2009, with 2009 having a mean plant richness value of 34.2 (STDERR = 4.67). Exclusion of 2008 from analysis did not show significant difference ($p = 0.23$) in variance or mean plant richness between 2005 and 2009, however the differences in means and variance were found to be even more significant ($p = 0.000$) with the exclusion of 2009 samples during the comparison between 2005 and 2008. This indicates that 2009 plant richness values span the ranges of both the 2005 and 2008 samples and sites have similar plant richness qualities of both.

Plant Richness - Native Species

Significant differences ($p = 0.001$) in variance and mean native plant richness values were found between 2005 (mean = 36.10, STDERR = 2.73) and 2008 (mean = 21.81, STDERR = 2.21) when all years were included in the ANOVA and Tukey-Kramer multiple comparison test (Figure 20). The mean native plant species for 2009 was 30 (STDERR = 3.96) and was not significantly different ($p = 0.20$) from 2005 (Figure 20). Exclusion of 2009 showed that 2005 and 2008 variance and mean native plant richness values remained significantly different ($p < 0.000$). Because the number of lacustrine, palustrine and riverine wetlands sampled in each of the study years was so uneven, no meaningful ANOVA testing for yearly differences among these groups could be accomplished. The number of wetland types sampled in any one year varied from one to 22. No significant differences in native plant richness were found among wetlands when grouped by ecoregion (Western Corn Belt Plains n = 38, Central Irregular Plains n = 20, Interior River Valleys and Hills n = 5). Emergent macrophytes bed type (EM) differed significantly from both the mix (MIX) and unconsolidated bed (UB) types (see Beury 2010 for wetland type definitions). Further inspection of these types revealed that of the 33 EM sites, 25 sites were palustrine, 5 sites were lacustrine, and 3 sites were riverine. Native plant richness among lacustrine EM (30.6) was not significantly different from palustrine EM (36.72). It should be noted that all the lacustrine EM sites were littoral zone sites associated with large lakes. The MIX category consisted of two palustrine sites and four lacustrine sites (two limnetic and two littoral). All the MIX wetland types were observed as having native species richness from 15-16 species.

ANOVA and Tukey-Kramer tests revealed that the lacustrine UB types (n = 6, mean 12, STDERR 4.46) were significantly lower ($p = 0.011$) in native plant richness than the palustrine sites (n = 5, mean = 32.8, STDERR = 4.89). Within the lacustrine class, the littoral zone sites (n = 4) had higher mean native plant richness (13.25) than the limnetic zone (9.5), though these differences were not statistically significant. Other MIX types had higher native plant diversity; the riverine sites had a native plant richness value of 16 while the palustrine sites had 32.8. These distinct separations among the MIX category dramatically affect its perceived relationship among this and other parameters. When we look at the differences between types among palustrine and lacustrine sites we see no significant differences ($p = 0.08$). All palustrine sites appear to be similar in plant community structure. Within lacustrine sites, AB had significantly higher FQI native values ($p = 0.004$) and FQI total score ($p = 0.002$) than MIX and UB classes. Distinct differences exist between aquatic bed (AB) and both the MIX and the unconsolidated bed types of lacustrine sites. The UB wetlands and MIX categories of lacustrine sites are very similar to one another in vegetation attributes. AB sites appear to be higher quality wetlands. The littoral zone emergent macrophyte beds that were sampled from lakes were not significantly different from the aquatic bed, MIX, or unconsolidated bed classes. If the MIX class is a combination of all three types it appears that in the case of lacustrine sites it is most affected by the unconsolidated bed and that among the palustrine sites it is an arbitrary or non-distinct class, at least in terms of vegetative quality.

The CDF curves for reference and random wetland populations are very distinct with the vast majority reference wetland having much higher native plant richness values (Figure 21). Again these CDFs indicate that the two wetland groups are different from each other in regard to plant richness.

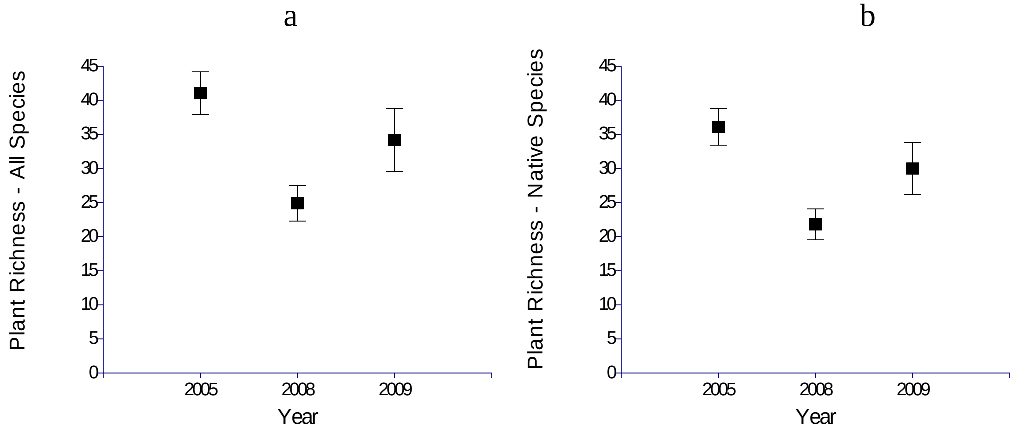

Figure 20. Error bar charts of the mean values and standard error for: (a) all plant species richness and (b) native plant species richness observed for each sampling season (2005, 2008, and 2009). Error bars are one standard error.

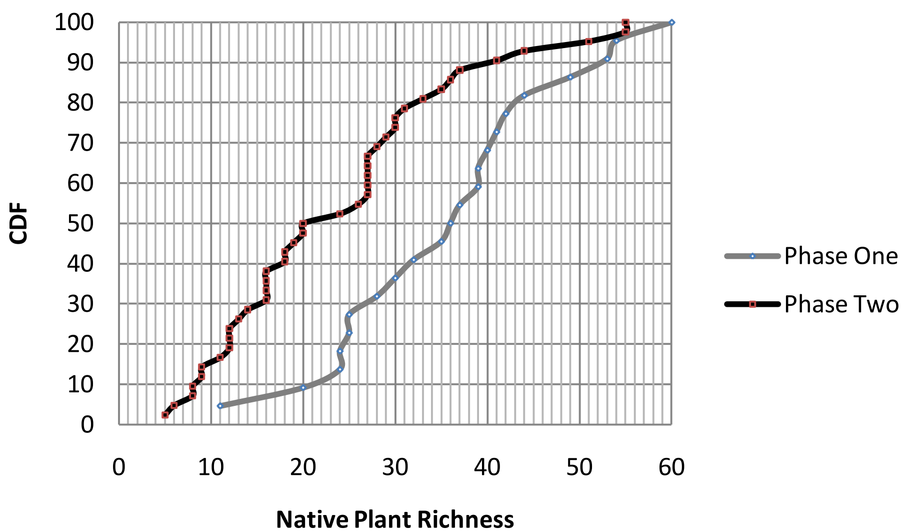

Figure 21. Cumulative distribution frequency (CDF) of native plant richness of reference (Phase I) and random selected (Phase II) wetlands.

Spatial attributes

Wetland area

Though a minimum size limit of ten acres was used in the site selection process, no maximum size limit was established. No statistical differences were found between years, ecoregion, or major wetland classes when ANOVA and Tukey-Kramer tests were performed using wetland surface area as a factor. However, size significantly differed between lacustrine and both palustrine and riverine sites as indicated by Kruskal-Wallace non-parametric analysis and Z tests ($p = 0.006$). When area (acres) was placed in a Pearson correlation matrix, it was found to correlate positively with orthophosphate (PO_4), total phosphorus, and atrazine concentrations with all relationships being significant ($p = 0.05$). When robust linear regression analysis was performed, the relationships between area and both PO_4 and total phosphorus concentrations were not significant and adjusted R^2 values were essentially zero. The relationship between area and atrazine concentrations remained significant, but the amount of variance explained was small ($p = 0.013$, $R^2 = 0.10$). Further analysis and discussion of the atrazine concentrations of the wetland population will consider the significance of this relationship.

Depth to Flood

Depth to flood (DTF) was used as a surrogate for flood return period. The value of DTF was calculated using the KARS floodplain model and defined as the river height above river channel height needed to create a surface connection to the wetland either by backfill or sidespilling at the topographically lowest wetland boundary (Kastens 2008). Kruskal-Wallace non-parametric medians analysis revealed that the riverine class ($n = 6$) was significantly lower in depth to flood than lacustrine ($n = 21$) or palustrine ($n = 32$) classes ($p = 0.043$, regular Z variables significant). This should be expected given that riverine sites are either backwater channels or sloughs that become connected with the Missouri River channel at much more frequent intervals than sites more set back from the channel. ANOVA and Kruskal-Wallace tests revealed significant differences in DTF between the sample populations within the Western Corn Belt Plains and the Central Irregular Plains. The mean DTF value for the CIP wetlands was 7.81 while the WCB wetland population had a mean value of 3.71. The Interior River Valleys and Hills sample population ($n = 5$) had a mean DTF value of 4.72, which was not significantly different from either the WCB or CIP wetland population values. The fact that DTF values positively correlated with the linear distance from the Missouri River channel ($R^2 = 0.91$, $p \leq 0.001$) reflects the fact that floodplain valley widens and contracts along its lower portion and that sites within the CIP have greater distances of overland flow and significantly less connectivity with the floodplain (Figure 22).

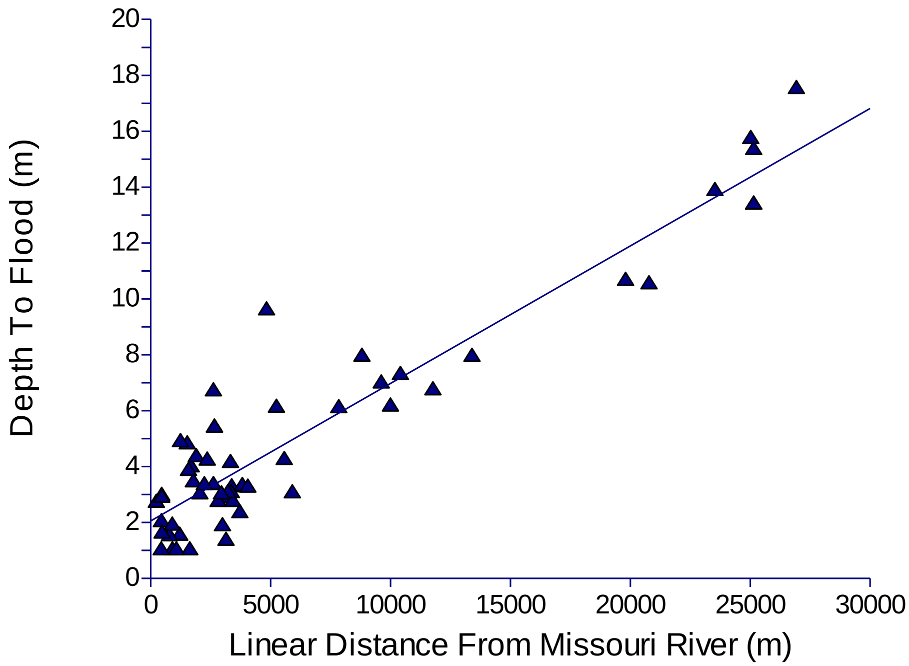

Figure 22. Scatter plot of depth to flood (DTF) values calculated from Kansas Applied Remote Sensing FLDPLN model and linear distance measures of site centroid to Missouri River Channel.

No significant differences were observed among the four dominant vegetation types (EM, UB, AB, MIX) and DTF suggesting that DTF is not a determinant factor in defining the vegetation of any particular wetland. However, DTF may have a significant effect on the condition or quality of a wetland because of the nutrient and hydrological regeneration that is provided by the flood pulse. Other studies have shown that sites with moderate connectivity, experiencing return floods of one to two years, have higher productivity and biotic richness and diversity than those receiving mostly groundwater or precipitation inputs and those that maintain greater connectivity with the river channel (Bornette *et al.* 1998, Smith *et al.* 2008). No significant difference in DTF among the sampling years was found using ANOVA or Kruskal-Wallace test procedures

Mean Depth and Maximum Depth
Mean and maximum depth means were not significantly different among sample years, despite the fact that the number of lacustrine, palustrine and riverine sites varied greatly between sample years and these wetland types often differed greatly in size. In fact, area of lacustrine sites (mean acres = 269.53, n = 21) were significantly different (p = 0.022) than palustrine sites (mean acres = 63.20, n = 34). Riverine sites were even smaller (mean acres 39.58, n = 6).

Early attempts to identify relationships between wetland type and depth overlooked the simple fact that lacustrine sites were sampled along littoral areas that assumed wetland-like conditions, though the site itself was a lake. However, some small, shallow lakes were also sampled in their limnetic area entirety, many having depths less than that constrained by Cowardin's 2-meter criterion. Caution should be given when categorizing wetland types based on prescribed rules that discriminate based on one measurement, especially when other factors influence that measurement. There are sites within our sample population that are considered lacustrine, but due to drought conditions in 2005, at least one of these sites was completely dry apparently due to water use on surrounding agricultural areas. Upon revisit during Phase II, this site had a maximum depth of 1.15 m and appeared to have lacustrine qualities, including an extensive aquatic bed habitat and a fish population. Given that rainfall, river flooding, and evaporative processes may significantly affect floodplain wetland water depth during any year or season, the use of water depth to define the difference between lacustrine and palustrine should be used with caution. In the EPA's National Lake Survey, lake sites that were less than 1 m deep were excluded. Perhaps 1 meter is a better threshold for helping distinguish between deepwater habitats (e.g. lacustrine) and palustrine wetlands. It should also be noted that the NWI dataset for wetland bodies assumes that wetlands with large surfaces areas are also deeper and thus are assigned to the lacustrine class. In our study, many discrepancies were found between the NWI classification and existing water body conditions. Because true lake sites did exist, and relationships were found between and among lacustrine, palustrine, and riverine wetlands, this major classification scheme was retained along with the dominant vegetation type and the lacustrine zone of the surveyed wetland.

Kruskal-Wallace non-parametric tests by CPCB type indicated that emergent macrophyte bed types ($n = 25$) have significantly ($p = 0.001$) shallower mean depths than aquatic bed types ($n = 9$), unconsolidated bed types ($n = 15$), and MIX types ($n = 7$), based on normal Z value differences. However, more discriminate Bonneferonni Z value tests only indicated that AB and EM differed significantly. Differences in maximum depth were found to be significant ($p = 0.001$) among types, though normal Z tests only showed that AB and EM were statistically different. When populations were separated by major classes (lacustrine and palustrine), no significant differences were found among the mean and maximum depth means. Mean and maximum depth positively correlated with mean Secchi depth, TN:TP, and each other, and were found to be negatively correlated with mean turbidity, total phosphorus, organic phosphorus, TOC, and DOC concentrations. Robust linear regression analysis showed that a significant positive relationship existed between mean depth and TN:TP ratio ($p = 0.001$, $R^2 = 0.183$). Maximum depth and TN:TP relationship was even stronger ($p < 0.000$, $R^2 = 0.28$). Maximum depth and mean Secchi depth relationship was significant and positive ($p < 0.000$, $R^2 = 0.28$), while mean depth and mean Secchi depth was also significant ($p < 0.000$, $R^2 = 0.25$). Mean turbidity was significantly correlated (negative) with mean depth but explained very little of the variance between these factors ($p = 0.029$, $R^2 = 0.069$). Maximum depth was also significantly correlated with mean turbidity ($p = 0.014$, $R^2 = 0.093$). Total phosphorus was negatively related to maximum depth ($p = 0.093$, $R^2 = 0.25$) and mean depth ($p <000$, $R^2 = 0.21$). Organic phosphorus was negatively correlated to mean ($R^2 = 0.20$) and maximum depth. TOC was negatively related to both maximum and mean depth ($p < 0.000$, $R^2 = 0.10$ and 0.20, respectively). DOC was also negatively correlated with maximum and mean depth and explained slightly more of the variance between these factors that did TOC ($R^2 = 0.22$ and 0.16, respectively).

Water quality

Dissolved oxygen

Variance in dissolved oxygen concentrations was similar among types, ecoregions, and across survey years and no significant differences were found when evaluated through one-way ANOVA testing of these factors. Dissolved oxygen significantly correlated with temperature and pH. Robust linear regression analysis indicated that a significant and positive relationship existed between dissolved oxygen and temperature ($p < 0.000$, $R^2 = 0.22$). This was not unexpected as dissolved oxygen saturation levels are temperature dependent. Differences in dissolved oxygen concentrations were found to vary within wetlands depending on where the *in situ* readings were taken. Densely vegetated areas had lower mean dissolved oxygen levels than open water areas in seven wetlands that were sampled in both areas (Table 8).

Table 8. Comparison of variation within seven wetlands according to differences in microclimates (open water vs. vegetated habitat) using paired t-test (NCSS 2004). * = $p < 0.10$, ** = $p < 0.05$.

	Sample means	
Variables	Open Water	Vegetated
pH**	7.33	6.91
Conductivity (µmS/cm)	0.271	0.268
Turbidity (NTUs)*	69	23
Dissolved Oxygen (mg/L)**	3.96	2.95
Temperature (^{o}C)*	25.6	24.6
Nitrate (mg/L)	0.01	0.02
Ammonia (µg/L)*	67	103
Total Nitrogen (mg/L)	0.92	1.25
Phosphate (µg/L)**	109	179
Total Phosphorous (µg/L)*	299	640
Chlorophyll a (µg/L)	25	75
Total Organic Carbon (mg/L)	10.4	16.1
TN:TP by weight**	4.7	3.6

Turbidity

Mean Secchi depth differed among the wetland types but not among classes (Figure 23a and b). Aquatic beds had significantly ($p = 0.028$) higher mean Secchi depths (0.82 m) than all other types except the MIX category (0.35 m). Due to the wide range of turbidity values across three orders of magnitude, mean turbidity data were log transformed to meet ANOVA assumptions of normality. Mean turbidity significantly differed among the wetlands types with AB being different than all other types (Figure 23 c). No significant differences were found between palustrine, lacustrine, and riverine classes (Figure 23 d). Mean Secchi depth and mean turbidity had a significant linear regression relationship ($R^2 = 0.52$). Typically the relationship between Secchi depth and turbidity is stronger, but many Secchi depths were limited by their occurrence on the bottom of the wetland. Because of the bottom limitations to accurate Secchi depth measurements, turbidity was considered the more appropriate measure of light penetration and water clarity.

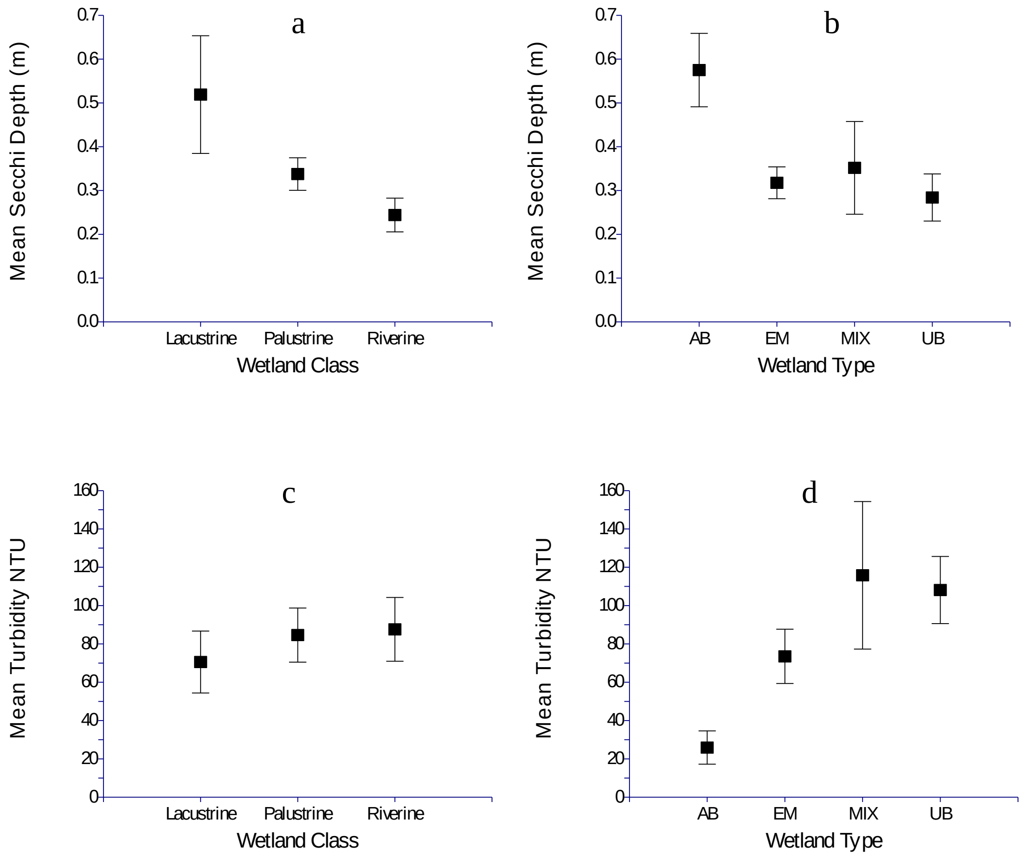

Figure 23. Error bar charts of mean Secchi depth by (a) wetland class and (b) type, and mean turbidity by (c) wetland class and (d) type. Error bars are one standard error.

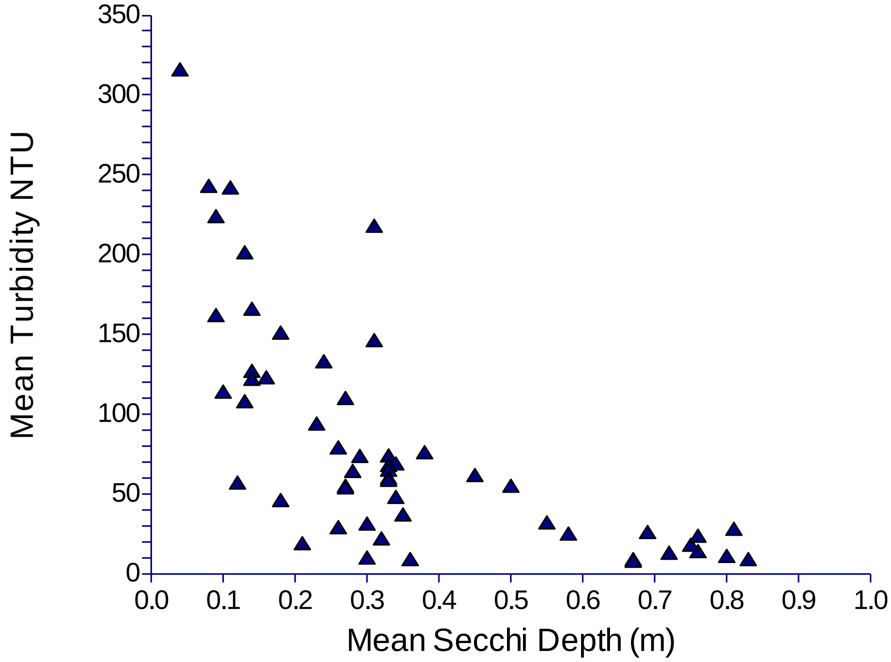

Figure 24. Scatter plot of showing relationship of mean turbidity (NTU) measures to mean Secchi transparency depths. One outlier was removed (site 7457).

Carbon

Mean concentrations and variance of total organic carbon (TOC) and dissolved organic carbon (DOC) were similar between both study phases. Only TOC values differed among wetland class ($p = 0.028$), with lacustrine sites having lower ($n = 20$, 1.07 mg/L) values than palustrine sites ($n = 30$, 2.10 mg/L, Figure 25). Though lacustrine sites were lower, we must consider that a large number of those sites were lake littoral zones that resemble palustrine sites. Riverine sites had a mean concentration of 1.06 mg/L, but the low sample size ($n = 5$) and high variance contributed to this group not being statistically different from the palustrine wetland group.

TOC was significantly ($p = 0.009$) higher in EM than in AB and UB, while DOC was significantly higher in EM than UB. MIX sites were found to be similar to all sites. Statistical testing for differences between MIX, EM, AB and UB (there were only two MIX samples) indicated that there were significant organic carbon (TOC, DOC) differences between types (ANOVA $p = 0.026$, Kruskal-Wallace $p = 0.034$) within lacustrine and palustrine classes. However, we must interpret this with caution because the Tukey-Kramer multiple comparison test did not indicate means to be significantly different and no groupings were identified. Within the lacustrine sites, no significant differences in carbon concentration were found among the six AB and UB and four EM and MIX sites. These tests indicate that organic carbon concentrations were similar between all classes and types tested. The error bar charts suggest that palustrine sites have higher TOC which seems to be driven by the high TOC in the two palustrine MIX sites.

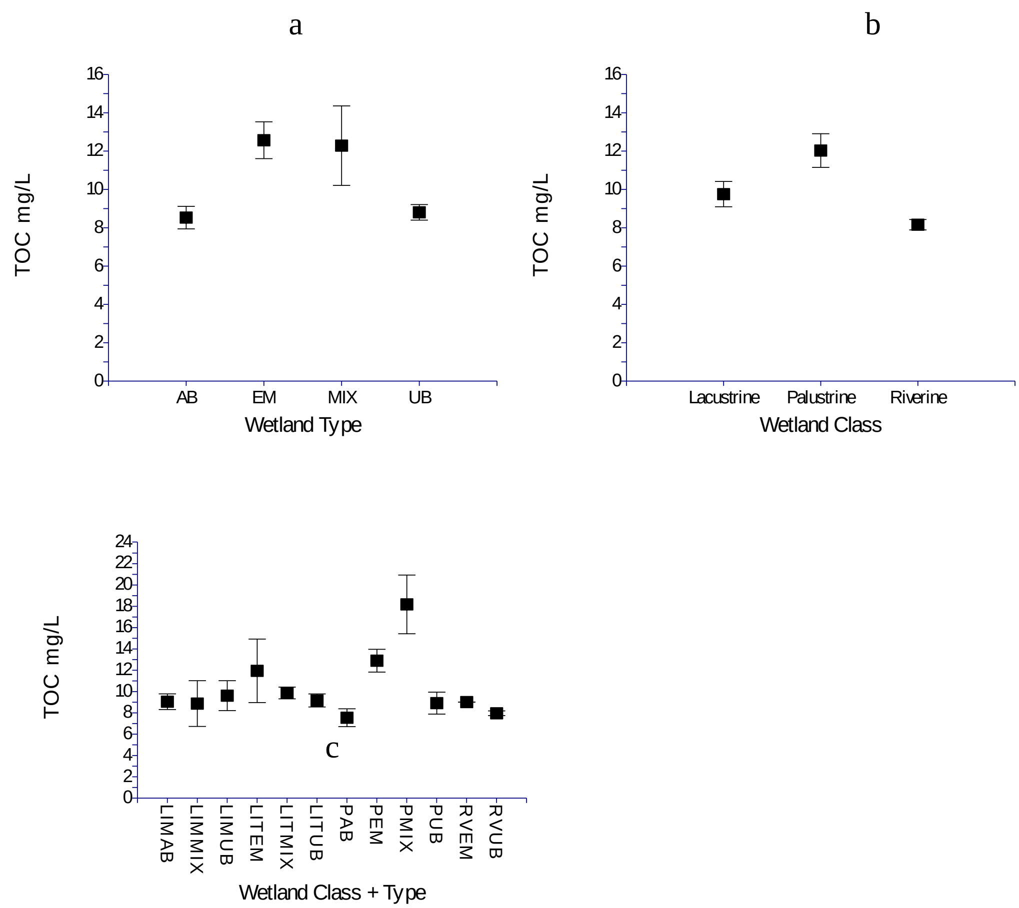

Figure 25. Error bar charts of total organic carbon concentration among (a) wetland class, (b) type, and (c) combined classifications. Error bars are one standard error.

Nitrogen

Ammonia (NH_3) concentrations were similar among all wetlands, regardless of population (reference vs. random), ecoregion, wetland class, or CPCB type. However, nitrate was found to be significantly different (Kruskal-Wallace, $p = 0.004$) between the reference and random wetland groups, with reference wetlands having higher nitrate (mean = 0.05 mg/L) in the 2005 samples than the 2008 and 2009 samples (men = 0.03 mg/L). Nitrite contributed the least significant fraction of the Nitrate+Nitrite measure, which might be expected since it is the first step in aerobic nitrification processes. On the other hand, transformation of nitrite to nitrate is the rate limiting step, and a comparison of nitrate to ammonia ratios revealed that these ratio values were very different between the reference and random groups (Figure 26a).

Total nitrogen concentrations were also found to be significantly higher (Kruskal-Wallace $p < 0.000$) in the reference wetland population (1.88 mg/L) than in the random population (1.14 mg/L, Figure 26b). The CDF curves also suggest that these two groups are different (Figure 27). Organic nitrogen, calculated as total nitrogen minus measured ammonia, nitrate, and nitrite concentrations, was also significantly higher (Kruskal-Wallace $p < 0.000$), but the ratio of dissolved nitrogen compounds to the organic nitrogen concentration was higher in the random population. Combined, these measures of nitrogen concentrations demonstrate that cycling of nitrogen in the reference sites is greater than in the random sites, indicating higher microbial productivity. Wetlands that have well established, diverse microbial communities are considered to be high quality, functional ecosystems that play a significant role in attenuation of the floodplain nutrients, especially in areas with agricultural runoff.

No significant differences and no interactions were found among ecoregions, classes, or types when multiple factor ANOVA's were used to examine those nitrogen measures that significantly differed among the sample populations. Some indication of difference (Kruskal-Wallace $p = 0.009$) in mean total nitrogen concentrations among the types was found when only the samples from the random population were examined (Figure 26c). Aquatic bed types had significantly lower mean total nitrogen (0.83 mg/L) than emergent macrophyte beds (1.43 mg/L). Organic nitrogen significantly differed between populations (ANOVA and Tukey-Kramer multiple comparison test, $p \leq 0.05$). Within the random population, the MIX and UB types had similar mean total nitrogen values, 0.92 and 0.99 mg/L respectively. Though no interactions were identified, total nitrogen levels within both populations appeared to be influenced by the distribution and number of wetland types in each grouping (multiple factor ANOVA). More than half the reference population was composed of EM sites (9); the rest of the sites being one AB, four MIX, and three UB. In the random population, fewer than half the sites were EM (16), while there were three MIX, eight AB, and 11 UB types.

Group composition by type becomes more significant when considering conductivity and its relationship to percent adventives and mean conservatism, and the distribution of these types along the floodplain corridor. Emergent bed types have the capacity to cycle and store larger amounts of nitrogen (e.g. Moshiri 1993). Shallow water depths allow for the establishment of dense stands of macrophytes that senesce and contribute large amounts of detritus to the wetland sediment and water column. Increased evapotranspiration along with the substantial biomass accumulation associated with these persistent species contribute significantly to EM type and its significant numbers among the sample population. Though many wetland managers attempt to control these dominant plants in hopes to provide more open water areas for waterfowl and obligate aquatic flora, the EM type does represent a large population of sites along the Missouri River that functionally can provide significantly to the cycling and retention of nutrients.

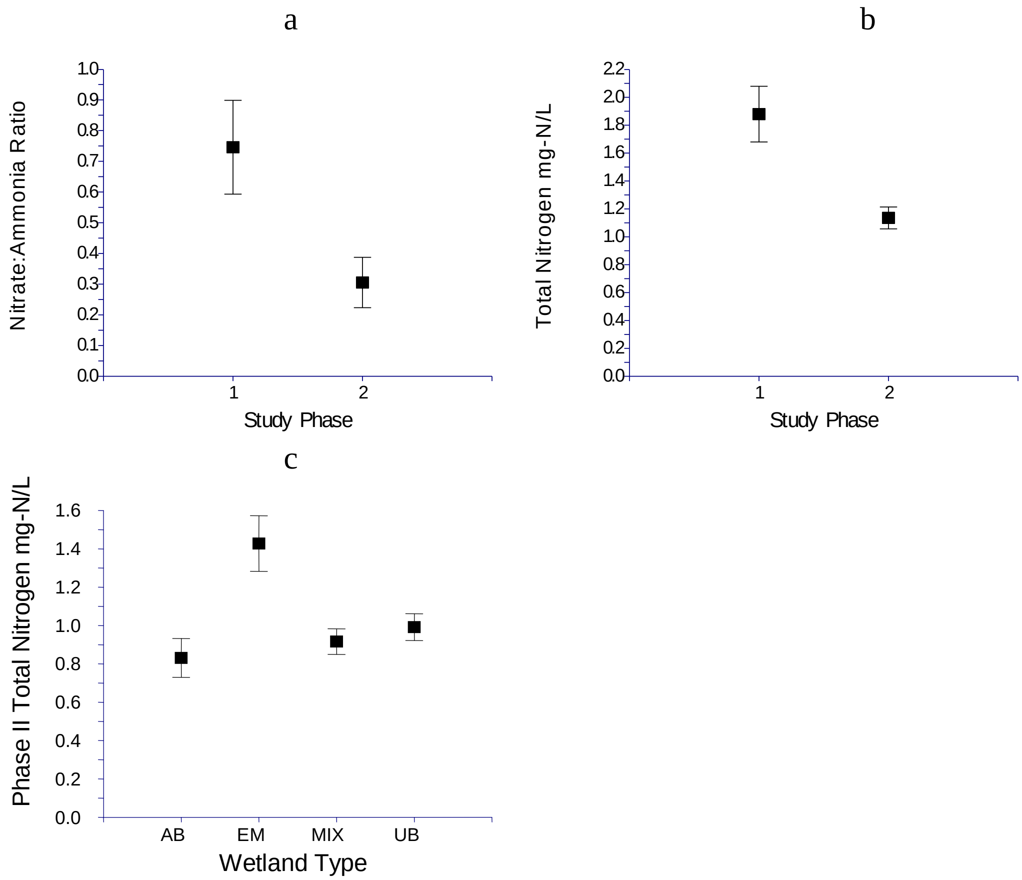

Figure 26. Error bar plots of (a) nitrate to ammonia ratio and total nitrogen concentrations (mg N/L) (b) in reference and random populations and by (c) wetland type. Error bar plots are one standard error.

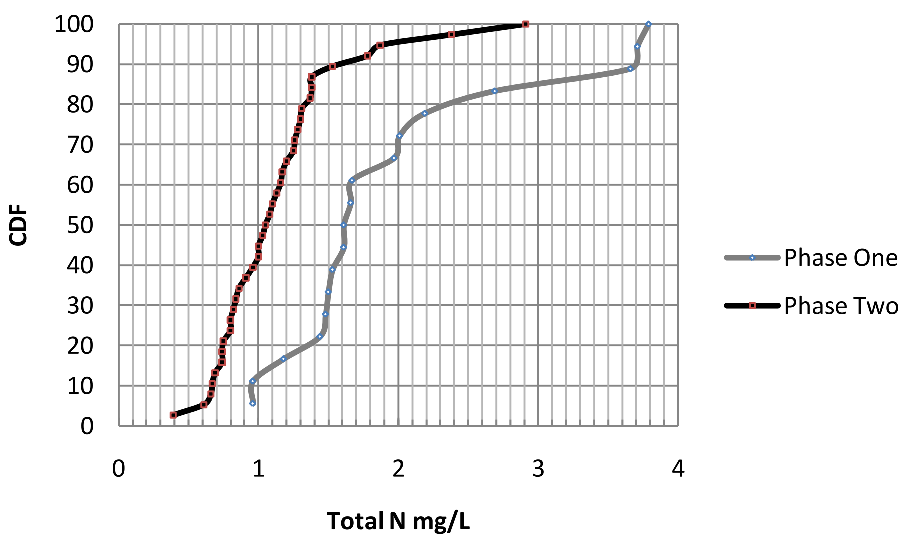

Figure 27. Cumulative distribution frequency (CDF) of total nitrogen for reference (Phase I) and random (Phase II) populations.

Phosphorus
Orthophosphate was significantly higher (Kruskal-Wallace $p = 0.036$) in the random population (mean = 171.41 µg-P/L) than in the reference group (93.28 µg-P/L, Figure 28a). Total phosphorus did not differ between study populations, with mean group values being somewhat over 400 µg/L. Total phosphorus was significantly (negatively) related to mean depth, but little of the variance in this relationship was explained ($R^2 = -0.07$, $p = 0.029$). Orthophosphate was not significantly related to mean depth. Total phosphorus and organic phosphorus held similar robust linear regression relationships with maximum depth, with total phosphorus exhibiting a stronger relationship.

Total phosphorus had significant ($p < 0.000$) positive linear relationships with both TOC ($R^2 = 0.25$) and DOC ($R^2 = 0.35$). The DOC fraction of TOC seems to be the largest contributor to organic carbon in these systems (Figure 28b). This indicates that adsorption processes are dominating the phosphorus speciation and location in the wetlands. This is further illustrated by the small but significant positive relationship between orthophosphate and DOC, and that no significant relationship was found for TOC. While total phosphorus was not significantly related to chlorophyll-*a* levels in the study wetlands, orthophosphate and chlorophyll-*a* were significantly correlated ($R^2 = 0.36$). The CDF curves for total phosphorus in the random and reference groups suggested that there is little difference in these groups based on phosphorus levels within the populations (Figure 29).

a b

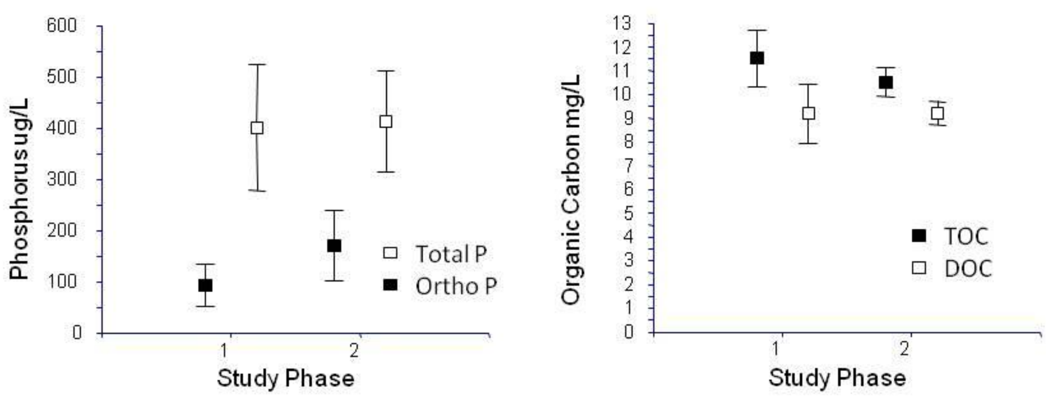

Figure 28. Error bar plots of (a) phosphorus and (b) carbon measures from study Phases I and II. Error bars are standard error.

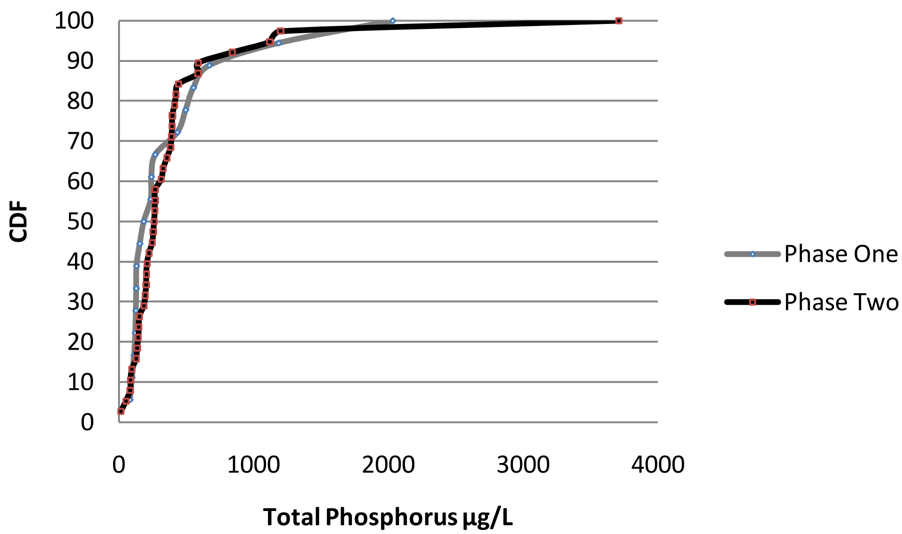

Figure 29. Cumulative distribution frequency (CDF) of total phosphorus for reference (Phase I) and random (Phase II) populations.

TN:TP ratio

The log mean ratio of total nitrogen to total phosphorus was higher ($p = 0.012$) in reference wetlands than in the random group due to the significantly higher total nitrogen values in the reference wetlands (Figure 30a). Mean TN:TP ratios for reference and random populations were 8.50 and 5.34, respectively. No significant differences were found among ecoregions, classes, or types for TN:TP. However, phosphorus had that most influence on the ratios. Robust linear regression indicated that total phosphorus explained 19% of the variation in TN:TP ratios while TN was not a significant independent variable (Figure 30c and d). Evaluating the study phases separately showed this relationship of total phosphorus to be substantially stronger within the reference population ($R^2 = 0.4$).

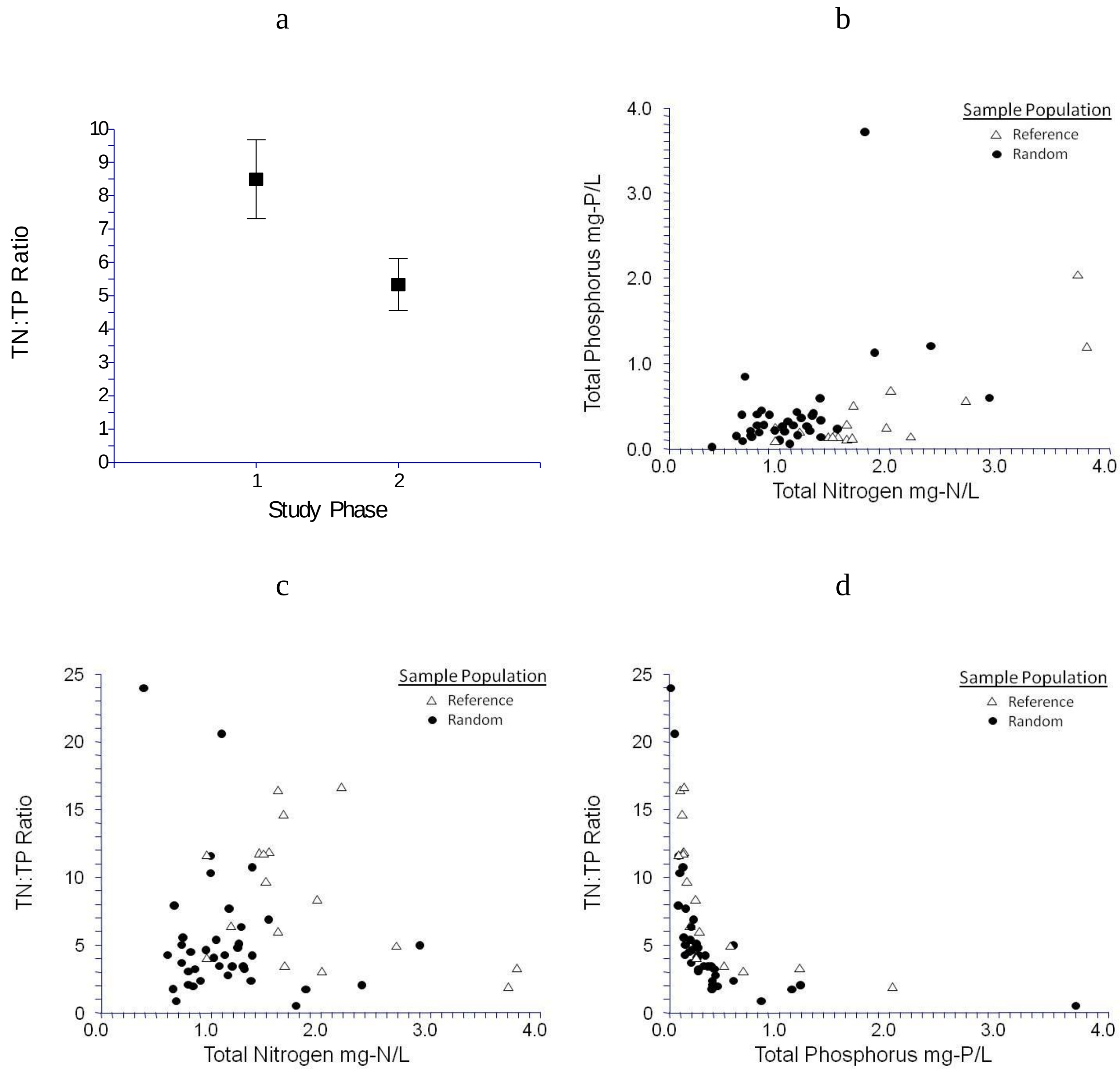

Figure 30. Relationships of total nitrogen and total phosphorus concentrations: (a) error bar plots of total phosphorus and orthophosphate concentrations, (b) scatter plots of total nitrogen and phosphorus, (c) TN:TP ratio and total nitrogen, and (d) TN:TP ratio and total phosphorus.

Chlorophyll-a

Kruskal-Wallace non-parametric medians analysis was used to examine the chlorophyll-*a* and pheophytin-*a* data which could not be normalized by transformation. Chlorophyll-*a* was higher in reference wetlands ($p = 0.001$, mean = 54.58 µg/L) than in the random population (mean = 30.68 µg/L) possibly indicating that productivity was higher within reference wetlands. Pheophytin-*a* concentrations did not differ between study populations. Wetland types differed ($p = 0.019$) in chlorophyll-*a* in when the entire study population was examined. Aquatic Bed types had significantly lower median chlorophyll-*a* value (17.18 µg/L) than either MIX or UB (Figure 31- Figure 33). Chlorophyll-*a* concentrations were significantly related to both mean turbidity ($p < 0.000$, $R^2 = 0.27$) and total nitrogen concentrations ($p < 0.000$, $R^2 = 0.32$). Organic phosphorus had a stronger relationship ($R^2 = 0.36$) with chlorophyll-*a* than did total phosphorus. The same was true for organic nitrogen ($R^2 = 0.37$) when compared to total nitrogen. Visual comparisons of the chlorophyll-*a* CDFs for both the reference and random populations suggested that these populations were related but distinct from each other (Figure 32).

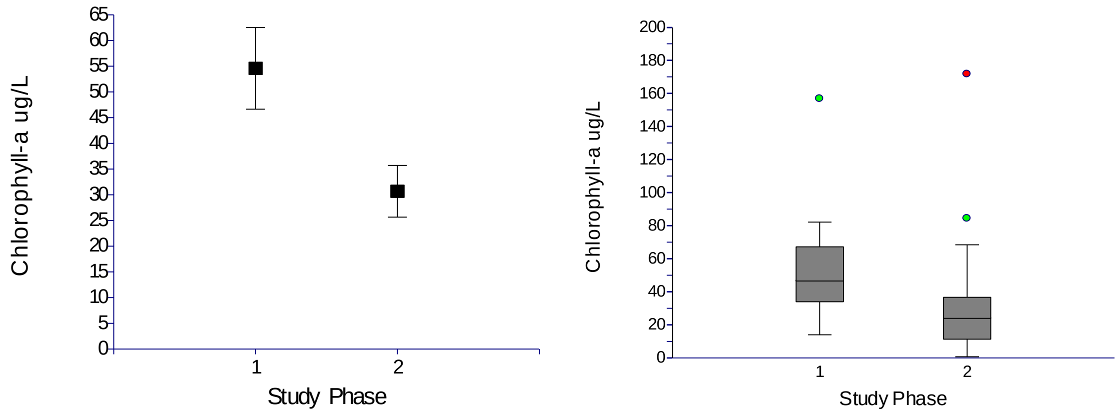

Figure 31. Mean and median chlorophyll-*a* values for each study group.

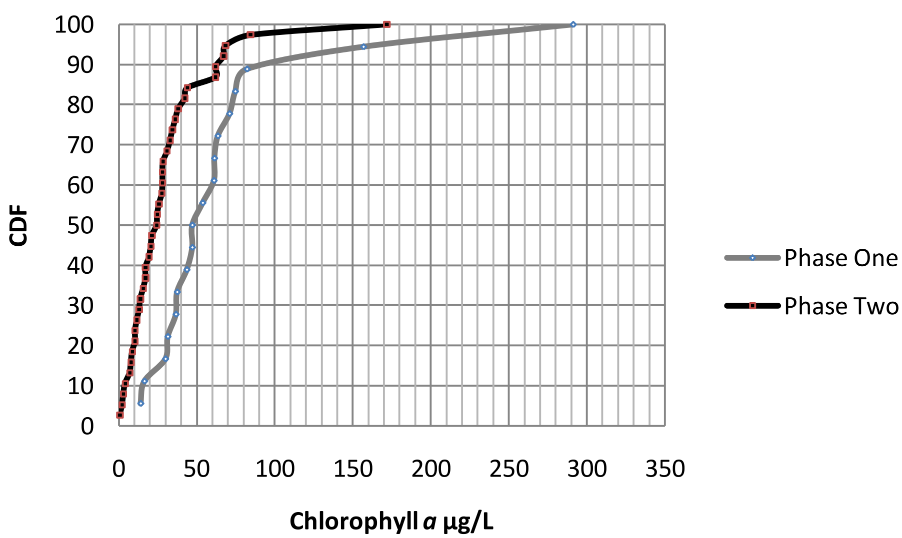

Figure 32. Cumulative distribution frequency (CDF) of chlorophyll-*a* for reference (Phase I) and random (Phase II) populations.

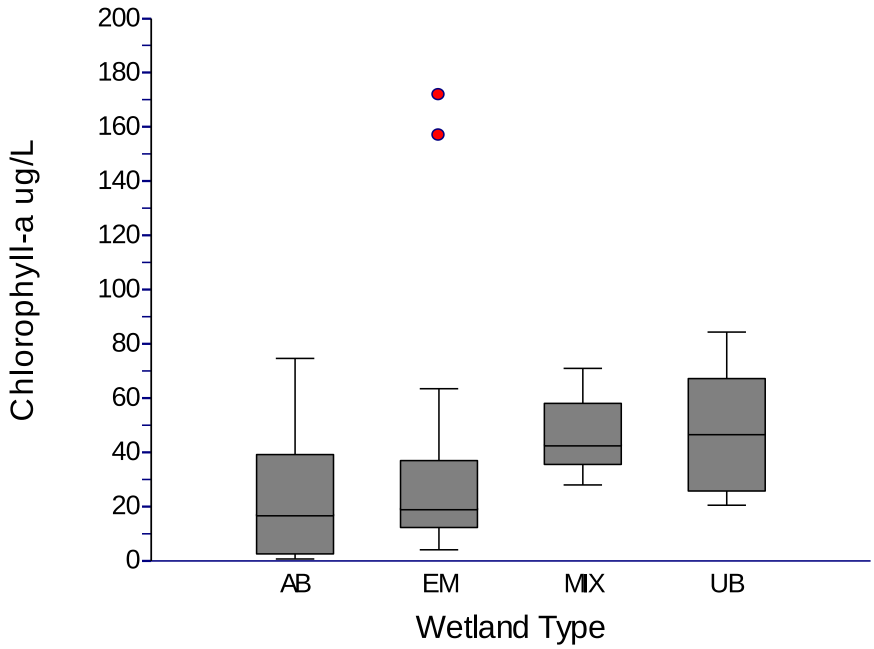

Figure 33. Median box plots of chlorophyll-*a* concentrations in the different wetland types.

Specific Conductance

Log transformed conductivity values were used in ANOVA testing. Mean conductivity in the reference population was significantly higher ($p = 0.005$) than in the random population. Kruskal-Wallace medians tests also showed that median conductivity differed between these two populations ($p = 0.007$). The mean and median conductivity values for reference wetlands were 0.51 and 0.57 mS/cm, respectively, compared to 0.31 and 0.28 mS/cm for the random population of Phase II. Log mean conductivity values differed among all three ecoregions. When two-way ANOVA tests were performed using study populations (reference vs. random) and ecoregion as factors, significant conductivity differences were found for both factors without significant interaction. This suggests the mean conductivity may be responding independently to both ecoregion effects and level of impairment (random vs. reference). Essentially four groups were identified: group 1 WCB random sites, group 2 WCB reference sites, group 3 IRV random sites, and group 4 consisted of both reference and random sites in the CIP. All sites in CIP had similar conductivity measures, whereas reference and random sites in WCB significantly differed. No significant differences among wetland classes or types were found.

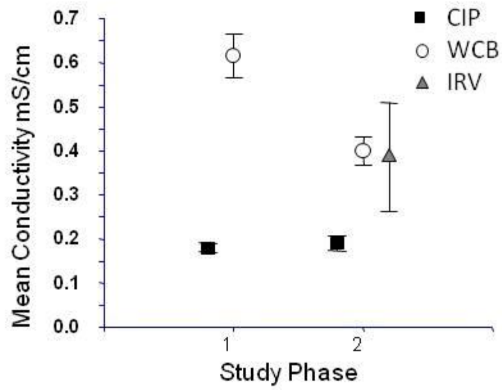

Figure 34. Error bar plots of mean conductivity values for samples grouped by ecoregion.

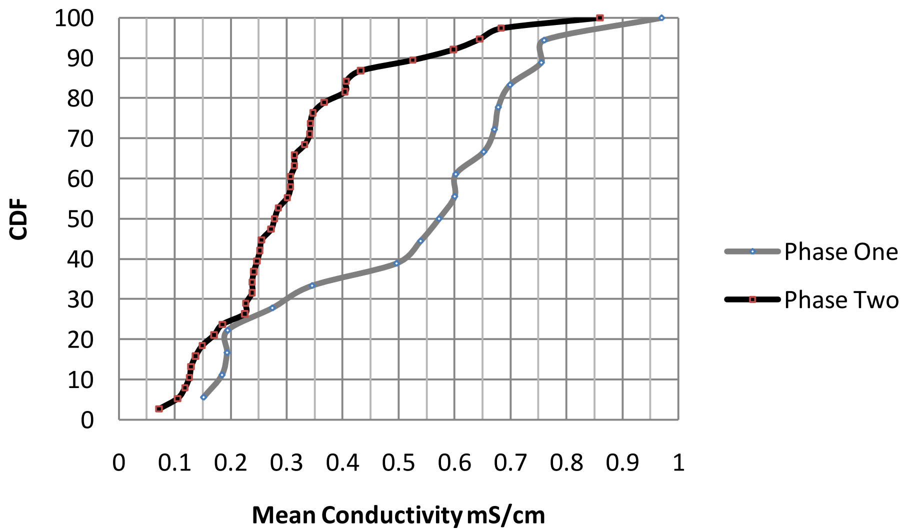

Figure 35. Cumulative distribution frequency (CDF) of mean conductivity for reference (Phase I) and random (Phase II) populations.

Mean conductivity correlated significantly with many other water quality and FQA parameters and spatio-temporal features (Table 9). If we consider that conductivity is the measure of the ionic strength of the water, then measurable concentrations of nutrients and contaminants would in theory define the ionic activity in the water (i.e. conductivity). Other correlations, though significant, may play some role in defining the conductivity, but only in as much as those parameters relate to inherent concentrations of ions measured. Herbicides levels go down as conductivity increases indicating that herbicides are not contributing to conductivity values but are merely related to conductivity levels.

Table 9. Liner regression results for a select number of wetland nutrients and herbicides variables where conductivity is the dependant variable.

Parameter	**$\lvert R^2 \rvert$**	**p**	**Correlation coefficient**	**Relationship**
NO3+NO2	0.179	0.001	0.423	Positive
NH3	0.217	< 0.000	0.448	Positive
Dissolved N	0.311	< 0.000	0.541	Positive
Desisopropylatrazine	0.168	0.001	-0.415	Negative
Metribuzin	0.153	0.002	-0.407	Negative
Alachlor	0.059	0.041	-0.291	Negative
Cyanazine	0.069	0.030	-0.293	Negative

Neither mean depth, maximum depth, temperature, pH, dissolved oxygen, nor turbidity had significant (robust) linear relationships with mean conductivity measures. Similarly, no significant regressions were produced between conductivity and total nitrogen, organic nitrogen, orthophosphate, or total phosphorus as independent variables. A single multiple regression model (robust linear regression, $R^2 = 0.49$) was composed of four independent variables Nitrate+Nitrite, NH3, Desisopropylatrazine and Cyanazine. The addition of DTF and mean conservatism of native plants to this model increased the R^2 to 0.71 indicating that much of the variance in conductivity values could be explained by these six variables. Conductivity measures the reciprocal of electron transfer caused by interference of typically mineral salts (Ca+2, Mg+2, Fe, etc.) and thus the relationships found in these models may be correlative and not causal.

Table 10. Parameters significantly correlated with mean specific conductivity.

Parameter	**R^2**	**p**	**Correlation coefficient**
Depth To Flood	0.314	< 0.000	-0.5540
Distance from Missouri River	0.216	< 0.000	-0.4667
Plant Richness (All)	0.165	0.001	+0.4312
Plant Richness (Native)	0.144	0.003	+0.4033
Percent Adventive	0.072	0.027	0.2824
Plant Mean Conservatism (ALL)	0.316	< 0.000	-0.5644
Plant Mean Conservatism (Native)	0.354	< 0.000	-0.5884

Herbicides
Detection in the reference wetlands of the eight analyzed herbicides was rare. Atrazine was detected in six sites, with metachlor in two of these. Metachlor was also detected at four other sites. One site had deethylatrazine (atrazine degradation byproduct). In one reference wetland atrazine concentration was 6.11 µg/L, with no degradation products present. Upon revisiting this site during Phase II, the atrazine level was much lower (0.98 µg/L), though desisopropylatrazine

and deethylatrazine were both present. Ten sites that had no detectable herbicide concentrations, including the statistical outlier site 7108.

Detectable levels of herbicides were found in most of the randomly selected wetlands of Phase II. Every site had atrazine and deethylatrazine. All but one site had detectable levels of desisopropylatrazine, all but one had metribuzine, all but four had alachlor, all but two had metachlor, and all but nine had cyanazine. Simazine occurred in only four sites.

All samples were scored by the number of herbicides detected as a way to account for possible combined effects and to overcome the variability in sample collection times, time of herbicide application, and losses due to degradation and other processes. Thus herbicide hits were tallied as present (+1) or absence (0) and the additive scores became independent of concentration. Examination of resulting CDFs clearly indicated that random and reference populations were different (Figure 36). However, CDFs indicated that these populations were very similar in atrazine concentrations (Figure 37). Because reference and random sites were collected across three summer periods there is the possibility that concentrations and detection hits were associated with differences in annual hydrological conditions. Comparison of sites that were sampled in both the Phase I and Phase II studies indicated yearly differences in hydrology.

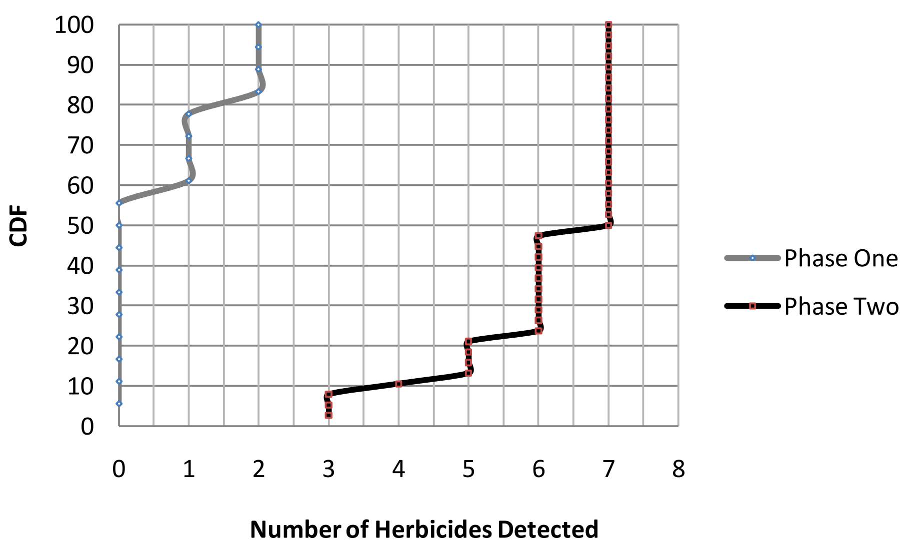

Figure 36. Cumulative distribution frequency (CDF) of number of herbicides detected in reference (Phase I) and random (Phase II) populations.

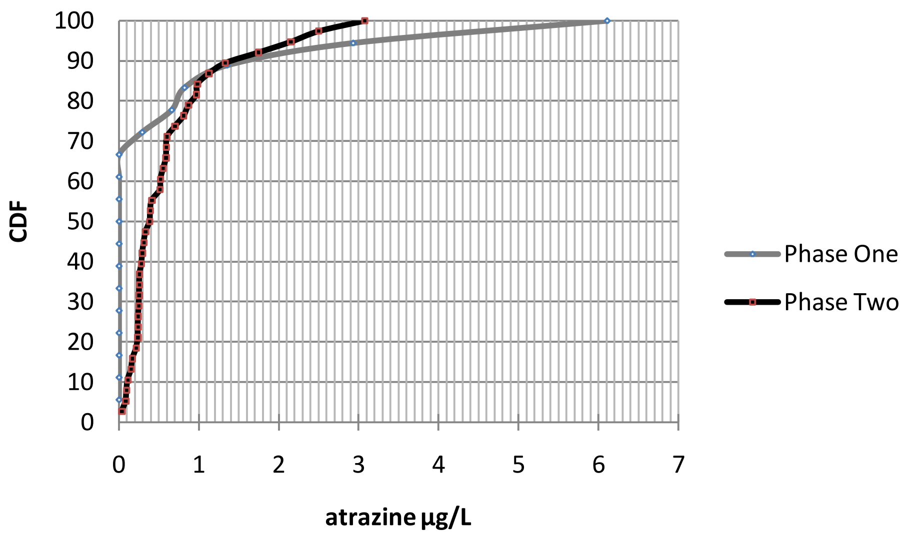

Figure 37. Cumulative distribution frequency (CDF) of atrazine of in reference (Phase I) and random (Phase II) populations.

<u>Resampled sites</u>
Four sites were resampled from Phase I (2005) and Phase II (2008); only three have all tiers of assessment data. Forney Lake, which lies in the Western Corn Belt Plains, was completely drained during summer 2005 when this area experienced drought conditions. Only disturbance assessments and FQA data were obtained there. The disturbance assessment score for 2005 and 2008 were very similar, 12 and 13 respectively. FQI was 12.02 in 2005 and 16.26 in 2008. Richness values in 2005 (40 all plants, 32 native plants) were considerably higher than in 2008 (18 for both). The dramatic difference in the scores is undoubtedly a consequence of the hydrological disturbance of drought and deluge, which promotes changes in plant community diversity. In 2005 there was only one hydrophyte species reported; there were six in 2008. The most dramatic shift in plant community structure was seen in the number of therophytes (annuals) present, which dropped from 20 in 2005 to one in 2008. Manipulation of wetland water level has been one of the most endearing best management practices for maintaining wetland floristic quality (Mitsch and Gooselink 2008).

The other three sites are located in the Central Irregular Plains ecoregion and all three assessment tiers were accomplished during both survey phases. Swan Lake is a lacustrine, unconsolidated bed wetland type. There were noticeable differences in floristic and water quality as well as macroinvertebrate MMI scores. Though there are differences in the disturbance assessment scores, the difference is limited considering that scoring was reported by a different evaluator each year. In 2005 the disturbance assessment score was 12; in 2008 it was 14, with the most changes occurring in the hydrological attributes section – a section that be expected to change somewhat due to differing climatic conditions. Floristic quality assessments and macroinvertebrate metric indices indicated that this site had become more degraded in overall quality over the three-year period. Water chemistry measures show a trend in higher concentrations of nutrients and lower pesticide concentrations in the 2005 sampling season. It is suspected that differences in sampling dates, and subsequent precipitation and runoff amounts might have affected concentrations of both nutrients and pesticides. Trends in herbicides seen in this wetland defy what was found in herbicide use trends and detections in this region. From 1996 to 2006, pesticide use and concentrations decreased; unless there was some dramatic increase in herbicide use after 2006, the herbicide data may be suspect. However, if we consider some significant relationships between water quality and observations made in the disturbance assessment, we can explain some of these discrepancies. First, a significant positive relationship exists between chlorophyll-a concentrations and turbidity (R^2= 0.46), which indicates that much of the solar adsorption interference can be accounted for by higher productivity by sestonic phytoplankton. In 2005 the survey was conducted in mid-July, the water table was quite low, organic nitrogen and phosphorus concentrations dominated the total nitrogen and phosphorus concentration, chlorophyll-*a* concentrations were high, and no indication of sedimentation was observed in the disturbance assessment. In 2008, sedimentation was indicated, though turbidity was lower, the water table was high, indicators of productivity (total nitrogen and phosphorus and chlorophyll-*a*) were lower, but herbicides had higher concentrations and more of them were detected. Alachlor, metachlor, and cyanazine have higher octanol water coefficients (Koc) yet higher solubility in water than atrazine, which was detected in significantly higher concentrations in 2005 than 2008. Metribuzine and atrazine have the longest aerobic soil half lives of all measured herbicides, and they were the only herbicides detected in 2005. These inherent qualities, coupled with differences in year to year and seasonal precipitation and runoff, may explain these significant water quality changes.

Another scenario was observed where sites along the littoral zone of Browns Lake were sampled in both 2005 and 2008. The two zones are characteristically different in plant community structure and some water quality measures. In 2005, no herbicides were detected but there were six found in the 2008 water sample. The 2005 macroinvertebrate MMI score was slightly higher than in 2008, though there was only a 3 point difference between them. FQI and richness values were significantly higher in 2005 when the site had a more varied water depth regime with subsequent increased interspersion allowing for a greater diversity of plants. Both dissolved oxygen and nutrient concentrations were higher in 2005 than 2008 but dissolved oxygen levels can vary greatly just from the time of day of the measurement as well as from short-term climatic conditions such as cloud cover.

Cooley Lake, an AB site, was sampled during the 2005 and 2008 seasons. Comparison of sampling results from this wetland also illustrates that changes can occur as a result of temporal change and hydrological shifts. The 2005 sample year was very dry, and wetland water tables were low in comparison to the 2008 season. Though FQA values were similar overall, plant species richness was dramatically higher in 2005 than in 2008. Some water quality parameter shifts were thought reflected influence of hydrological or temporal change. Ammonia, orthophosphate, and herbicide concentrations were much higher in the 2008 season than in the 2005 season, suggesting that increased runoff from the surrounding landscape had occurred during 2008. However, total nitrogen, total phosphorus, and chlorophyll-*a* concentrations were higher in 2005 a time of overall drier conditions. The high nutrient levels in 2005 which would also be a normal part of runoff don’t support the increased runoff argument for ammonia, orthophosphate and herbicides.

Comparisons of data from those few sites that were revisited indicate that temporal and hydrological differences can affect both abiotic and biotic conditions within these floodplain wetlands. However, most of this study is based on the comparisons of two populations and the temporal and spatial variance within individual sites is part of the error that most be accept in one sample studies of populations.

<u>Disturbance Assessment</u>
A field-level disturbance assessment (DA) score system was developed during these studies (Appendix D). Initial development began in Phase I and continued through the early part of Phase II (see Kriz *et al.* 2007, Beury 2010). The initial field form of the DA was revised for Phase II and all sites scored with the early version were rescored.

The DA was developed as a Level 2 assessment tool to estimate the possible level of disturbance a site might be exposed to based on locally observed conditions and factors. The reference wetland population consistently had lower DA scores than the random population, although some wetlands in the random population are probably of reference quality. CDFs for DA scores for each study group clearly show population distinctions up through the 90 percentile (Figure 38).

In addition to scoring both the reference and random population to examine the DA's discriminatory ability we also used the DA to look at other wetland and landscape (i.e. ecoregions) factors. Only the Phase II wetlands were used in these tests as this population was thought to be the most variable in terms of levels of disturbance. Disturbance assessment scores were similar among the ecoregions, though means and standard error measures were slightly different. Means and standard errors for the final DA were 8 (STDERR = 0.94), 9.8 (STDERR = 1.69), and 10.38 (STDERR = 0.82) for the WCP, IRV, and CIP ecoregions, respectively. No significant differences were determined among the major wetland classes examined in this study, but lacustrine scores tended to be higher than palustrine scores. Unconsolidated Bed scores were significantly lower than Aquatic Beds, but all types were similar in means and variance (Figure 39). Though not significant statistically, mean scores for the UB wetland type were the lowest among all wetland types. Generally DA scores for each wetland type except MIX followed the same pattern as FQI and MMI (the macroinvertebrate multimetric index discussed in the next section) (Figure 39). The DA scores for MIX tend to be high, but the FQI and MMI scores suggest that the level of impacts are more moderate when compared to the other wetland types.

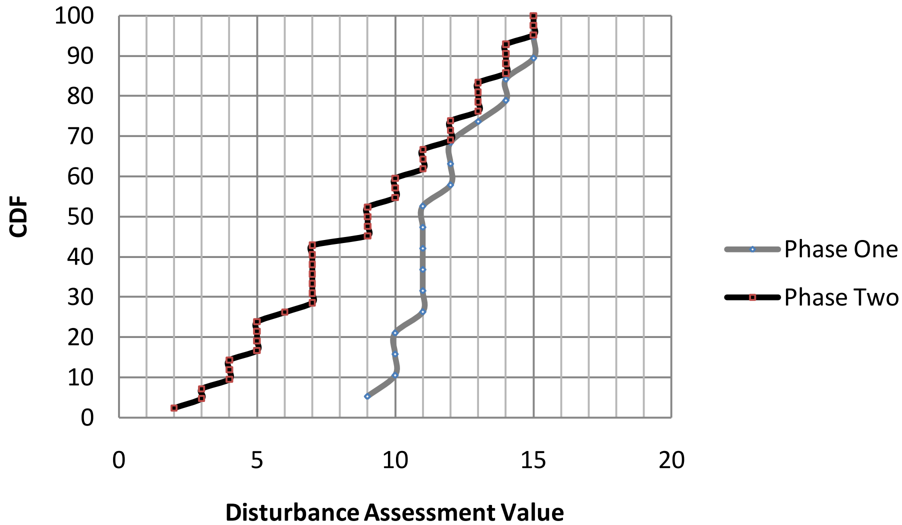

Figure 38. Cumulative distribution frequency (CDF) of Disturbance Assessment totals in reference (Phase I) and random (Phase II) populations.

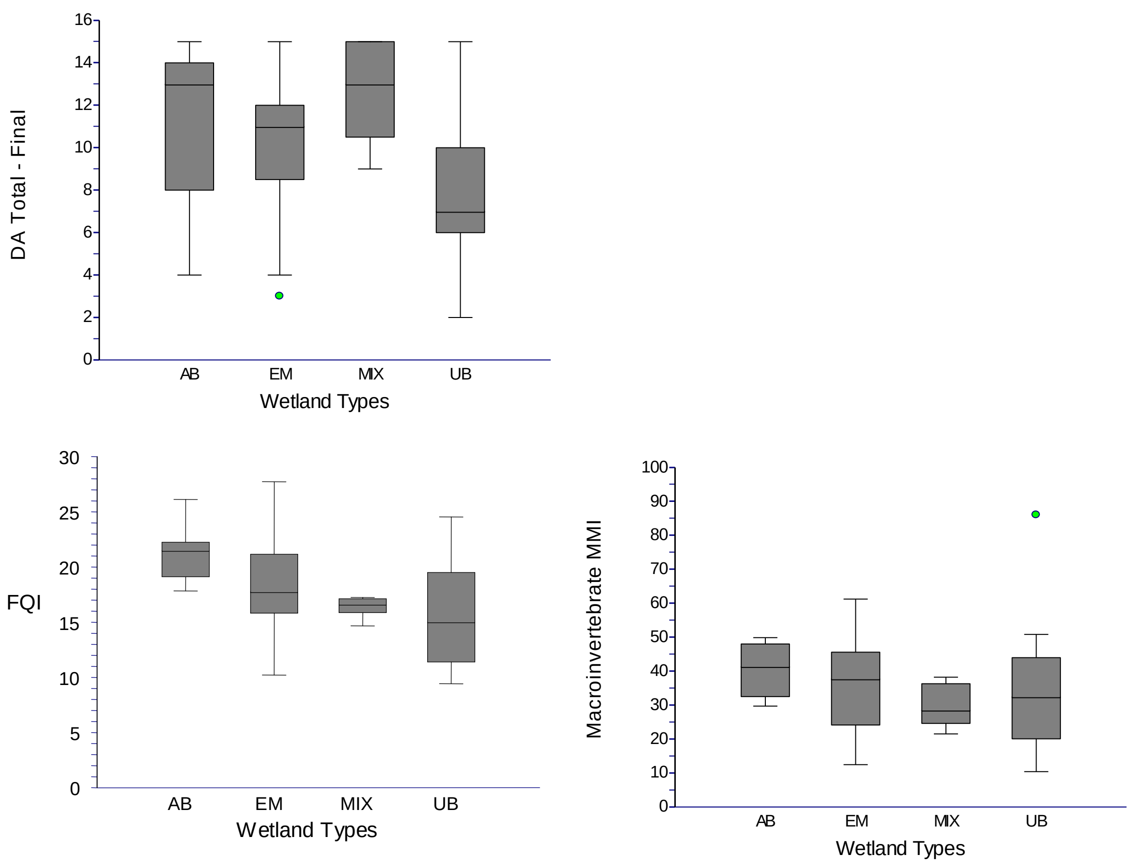

Figure 39. Median box plots of Disturbance Assessment (DA), FQI, and MMI scores for the different wetland types.

Macroinvertebrate MMI

Metrics

Of the 44 metrics evaluated in the development of various versions of the MMI; only 18 were statistically significant and of discriminatory value when evaluating *a priori* reference and non-reference groups using the t-test method described by Stoddard *et al.* (2008). Many of the metrics originally proposed for rivers and streams were inappropriate because the specific macroinvertebrate taxa used in the metrics are not a common part of the wetland fauna and were not found in our samples. Substitutions were made and 44 metrics were selected for evaluation using the macroinvertebrate samples collected during both studies (Table 11). The use of Hydrophilidae was adopted since Helophoridae were not present in any of the samples. Both families belong to the superfamily Hydrophiloidea and thus may provide the similar structural and functional information about the macroinvertebrate community. Other notable additions were the measures of intolerant species proposed by Huggins and Moffitt (1988). The count of intolerant taxa was derived by taking only those records with tolerance values < 3 (scale of 0-5). Huggins and Moffitt (1988) developed tolerance values for taxa relative to five major pollutant categories: agricultural pesticides (AP), heavy metals (HM), nutrient and oxygen demanding compounds (NOD), persistent organic carbons (POC), and suspended solids and sediments (SSS). A Percent Less Than Mean Regional Tolerance Value (RTV) metric was calculated from records with known regional tolerance values as the percentage of records having less than the calculated mean value for that specific site. Chironomidae diversity metrics and overall Margalef's Index were also evaluated as potentially robust measures of diversity among the samples. Count Collembola Taxa and Percent Parasitic Taxa were the only metrics that failed the range tests, with representation occurring in less than 25% of sample population (n = 52).

Table 11. Metrics used in the development of the macroinvertebrate MMI, grouped by richness and diversity measures, taxa proportions, taxa count, trophic guilds, and habitat behavior guilds.

Richness and Diversity Measures

- Taxa Richness
- Chironomidae Taxa Richness
- Chironomidae Total Abundance
- Percent Dominant 3 taxa
- Percent Dominant Taxa
- Margalef's Index
- Shannon's Index (H')
- Chironomidae Margalef's Index
- Chironomidae Shannon's Index (H')

Taxa Proportions

- Percent Amphipoda
- Percent Chironomidae
- Percent Coleoptera
- Percent Corixidae
- Percent Culicidae
- Percent Diptera
- Percent Hydrophilidae
- Percent Hydrophilidae
- Percent Leeches
- Percent Libellulidae
- Percent NonInsect taxa
- Percent Oligochaeta

Taxa Count

- Count Collembola Taxa
- Count Diptera Taxa
- Count Gastropoda Taxa
- Count Leech Taxa
- Count Odonata Taxa
- Percent Less Than Mean RTV
- Count ETO Taxa
- Count Intolerant Taxa AP
- Count Intolerant Taxa HM
- Count Intolerant Taxa NOD
- Count Intolerant Taxa POC
- Count Intolerant Taxa SSS

Feeding Guild Proportions and Counts

- Percent Collector-filterers
- Percent Omnivores
- Percent Predators
- Percent Scrapers
- Percent Shredders
- Count Parasitic Taxa
- Count Scraper Taxa

Habitat Behavior Proportions

- Percent Burrowers
- Percent Clingers
- Percent Sprawlers
- Percent Swimmers

a priori Groups and Metric Selection

The stressor-response metrics were selected using a Pearson correlation matrix (i.e. Pearson product-moment correlation coefficient) and linear regression test, except no single reference or random group was established *a priori*. In this study, *a priori* 'high' and 'low' groups were established for parameters that showed consistent significant responses to multiple macroinvertebrate metrics using the 25th and 75th percentile, since significant variability in response existed among landscape, plant community, and water quality measures. Macroinvertebrate metrics were placed in a correlation matrix along with floristic quality measures, water quality parameters, and surrogate spatial and temporal variables. All significant ($p \leq 0.05$) Pearson correlations were tested with linear regression and retained if still significant. Relationships were commonly found between various multiple macroinvertebrate metrics and one water quality measure, floristic quality metric, or other variable. Groups were created as 'least disturbed' or 'degraded' condition with samples having parameter values equal to and lower or higher than the 25th or 75th percentile value, respectively. The macroinvertebrate metrics that were significantly related to the other environmental parameters through linear regression analyses were assessed using the two-sample t-test method described by Stoddard *et al.* (2008), resulting in 39 macroinvertebrate metric responses to 11 groups, with two groups eliminated in this process. Many metrics also responded to various groups in the t-test analysis, thus it was necessary to define each metric by its greatest t-score, further eliminating many *a priori* groups.

Twenty-six metrics were retained, the greatest numbers of which were found in the Number of Herbicides Detected group, Native Plant Richness group, and Maximum Depth group, with a small representation of other groups having metrics with significant t-scores. Five macroinvertebrate metrics having the lower t-score between high and low *a priori* groupings were eliminated due to redundancy (Pearson $R \geq 0.70$) with another macroinvertebrate metric. Only the Native Plant Richness, Number of Herbicides Detected, and Maximum Depth groups were further evaluated because they had the greatest response from macroinvertebrate metrics when metrics also responded to other parameters and groups. These three groups represented hydrological and floristic wetland qualities as well as anthropogenic disturbance. The remaining 21 metrics were two sample t-tested in these groups.

T-test values remained significant for three metrics in the native plant richness group: Shannon's Diversity Index (+), Percent Burrowers (-), and Count Intolerant Taxa to Suspended Solids and Sediments (SSS) (+) (Table 12). Four completely different metrics in the maximum depth 'high' and 'low' groups were found to be significant in t-test scores: Percent Hydroptilidae (+), Count ETO taxa (+), Percent Sprawler Taxa (+), and Percent Intolerant based on mean Regional Tolerance Values (+). The metrics having significant t-test scores between the low and high Number of Herbicides Detected group were Percent Non-Insect Taxa (-), Percent Burrowers (-), Intolerant Taxa to Heavy Metals (+), and Count Intolerant Taxa to Suspended Solids and Sediments (+). These metrics were not significantly ($p<0.05$) correlated with each another. The Disturbance Assessment (DA) was developed to characterize both internal and external hydrological and landscape features that could affect wetland condition. Scores ranged from 2–15. Sites in the median 25th percentile with scores $\leq$ 7were deemed the 'low' group; sites with DA scores $\geq$ 13 (75th percentile) were regarded as the 'high' group. Two sample t-tests between the two groups determined two metrics to be significantly different when these groups were tested: Percent Clingers (+) (p=0.019) and Percent Diptera (+) (p=0.043), having t-scores of 2.48 and -2.12, respectively.

Table 12. Macroinvertebrate metrics determined to delineate between *a priori* groupings using two sample t-tests of high and low scores in the Disturbance Assessment (DA), native plant richness, maximum depth, and the number of herbicides detected.

DA	**Native Plant Richness**	**Maximum Depth**	**Number of Herbicides Detected**
% Diptera (+)	Shannon's diversity index (+)	Count ETO Taxa (+)	Shannon's diversity index (+)
% clingers (+)	% burrowers (-)	% sprawler taxa (+)	% burrowers (-)
	count intolerant taxa to SSS (+)	% intolerant based on mean RTV (+)	count intolerant taxa to SSS (+)
		% Hydroptilidae (+)	% Hydroptilidae (+)
			% non-insect taxa (-)
			Count intolerant taxa to HM (+)

Metric Testing

Reference and Random Population Comparisons

Significant differences were found between study phases, years, regions, and wetland types in the DA scores, FQA metrics, and water quality parameters from previous ANOVA tests of all 54 samples. When ANOVA tests were performed on the sample population (n = 52), many of the same significant differences among the other parameters and metrics remained, but congruency was also seen in the outcome of some of the MMI scores. Mean DA scores were significantly higher (p = 0.004) in the reference samples than in the random samples (Figure 40a). Mean native plant richness was also found to be significantly higher (p = 0.001) for the reference population, though FQI values were not (Figure 40b).

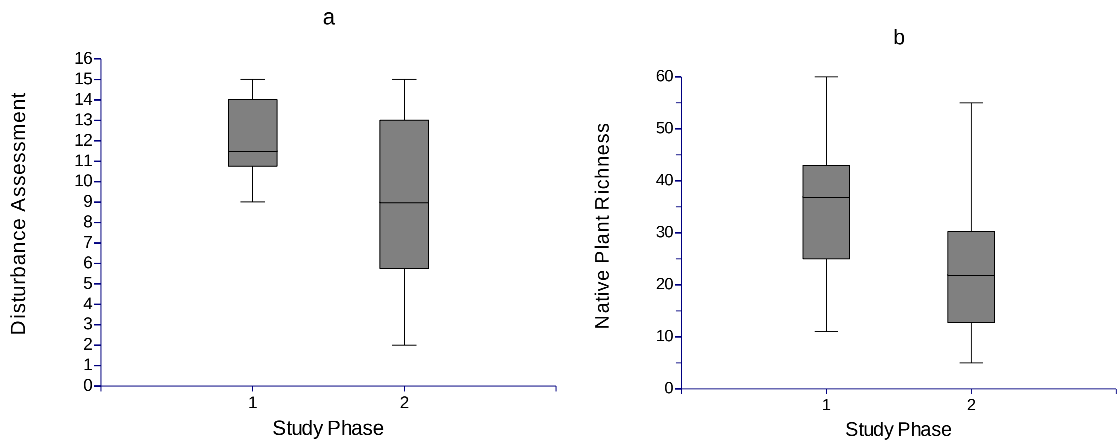

Figure 40. Box Plots showing the range and distribution of (a) Disturbance Assessment Scores and (b) Native Plant Richness by reference (Phase I) and random (Phase II) population. Box area represents inner quartile range, while "whiskers" represent the upper and lower observations.

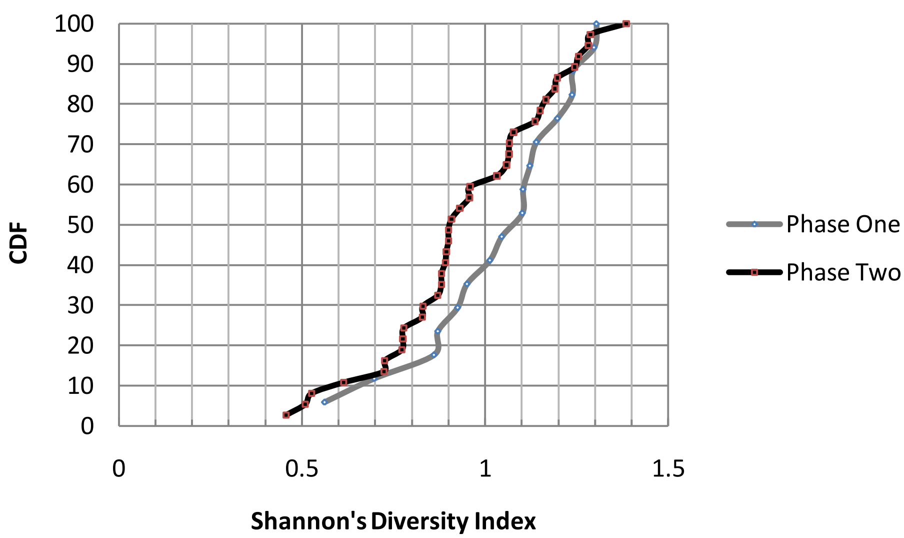

Figure 41. Cumulative distribution frequency (CDF) of Shannon's Diversity Index in reference (Phase I) and random (Phase II) populations.

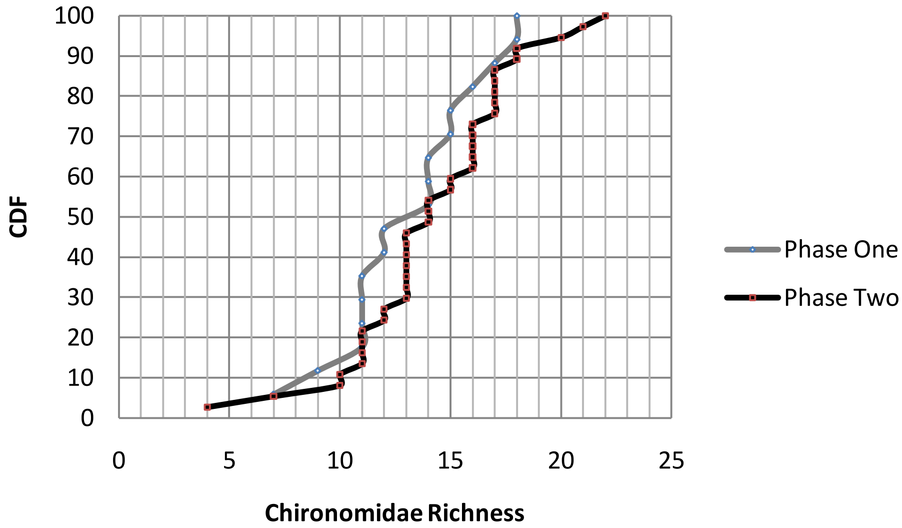

Figure 42. Cumulative distribution frequency (CDF) of Chironomidae Richness in reference (Phase I) and random (Phase II) populations.

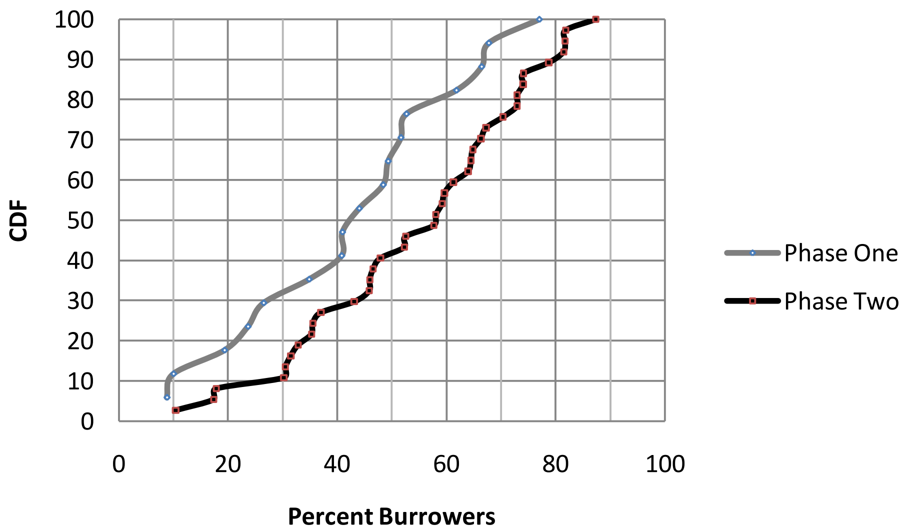

Figure 43. Cumulative distribution frequency (CDF) of Percent Burrowers in reference (Phase I) and random (Phase II) populations.

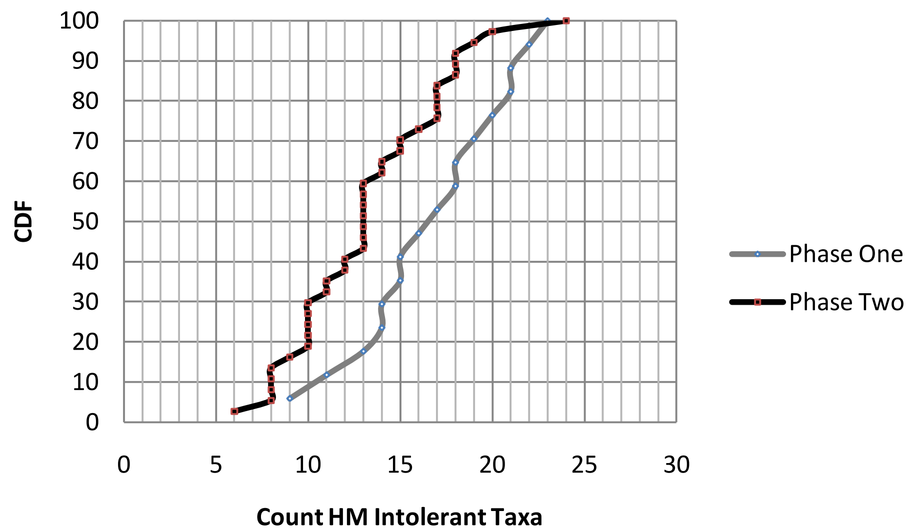

Figure 44. Cumulative distribution frequency (CDF) of HM Intolerant Taxa in reference (Phase I) and random (Phase II) populations.

To illustrate the multiple levels of congruency among assessment parameters, mean differences for study parameters earlier tested for differences between the reference (Phase I) and random (Phase II) populations also remained significant in this sample subset ($n = 52$). For example, log transformed total nitrogen mg/L, chlorophyll-*a*, log transformed mean conductivity, and number of herbicides detected again showed significantly different ($p < 0.05$) between the two study populations (Figure 45).

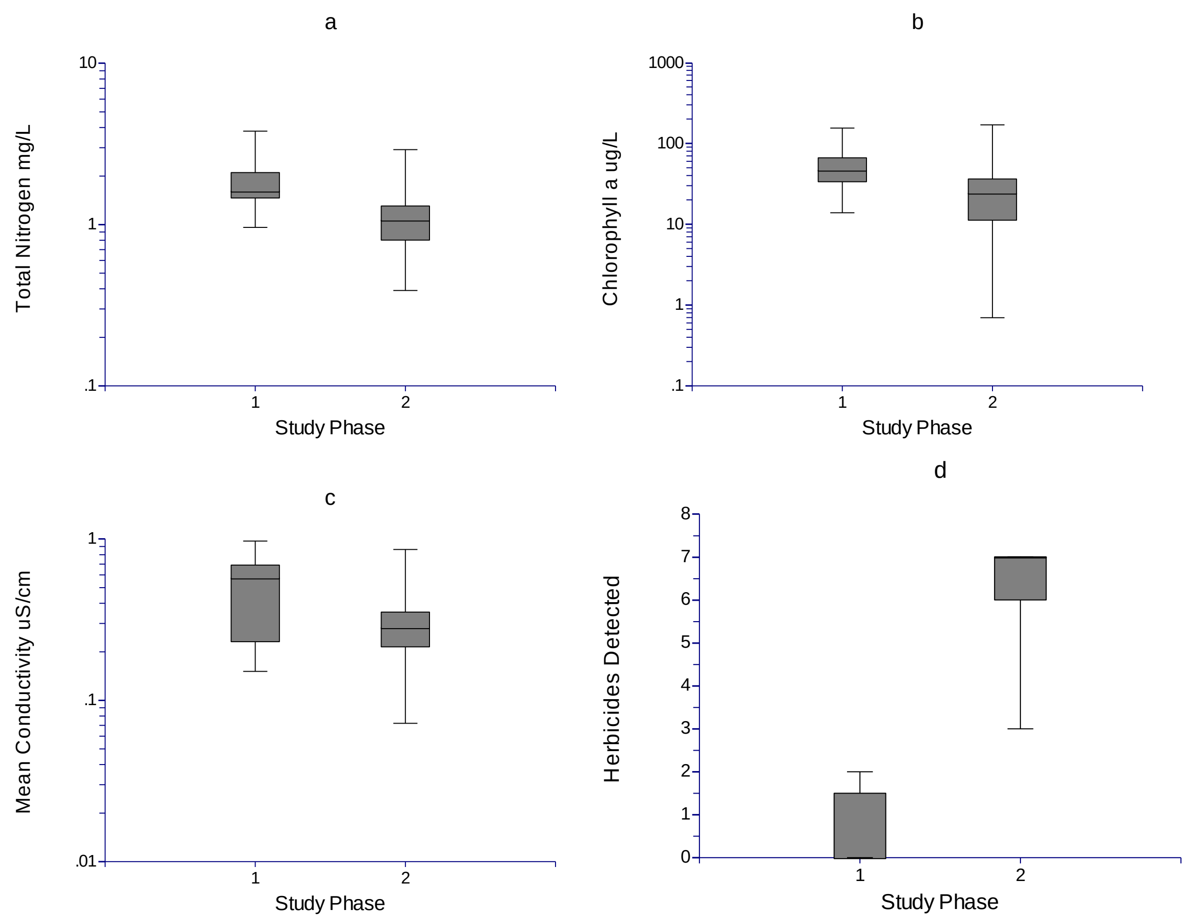

Figure 45. Median box plots of water quality parameters that were significantly different between study populations for (a) log total nitrogen mg/L, (b) log chlorophyll-*a*, (c) log mean conductivity mS/cm, and (d) the number of herbicides detected. Box area represents inner quartile range, while "whiskers" represent the upper and lower observations.

All metrics that discriminated between the identified *a priori* groups in the metric selection process were tested for congruency with other wetland assessment tools and water quality parameters using ANOVA or Kruskal-Wallace analysis. Metric scores for percent burrowers, count of heavy metal (HM) intolerant taxa, and count of taxa intolerant to suspended solids and sediments (SSS) were the only metrics found to be significantly different between the two study populations (Figure 46). However, the log transformed mean percent Hydroptilidae was significantly different ($p = 0.004$) between study populations suggesting that this metric could discriminate between the populations if the measurement scale was adjusted (use of log values or some other transformation). Counts of intolerant taxa to heavy metals and percent burrowers were normally distributed and were statistically different between populations. Percent burrowers (mean = 38.7) was significantly lower in reference sample than the random samples (mean = 54.3). Counts of intolerant taxa to heavy metals were highest in the reference (mean = 17.4) population when tested against the random population (mean = 13.3).

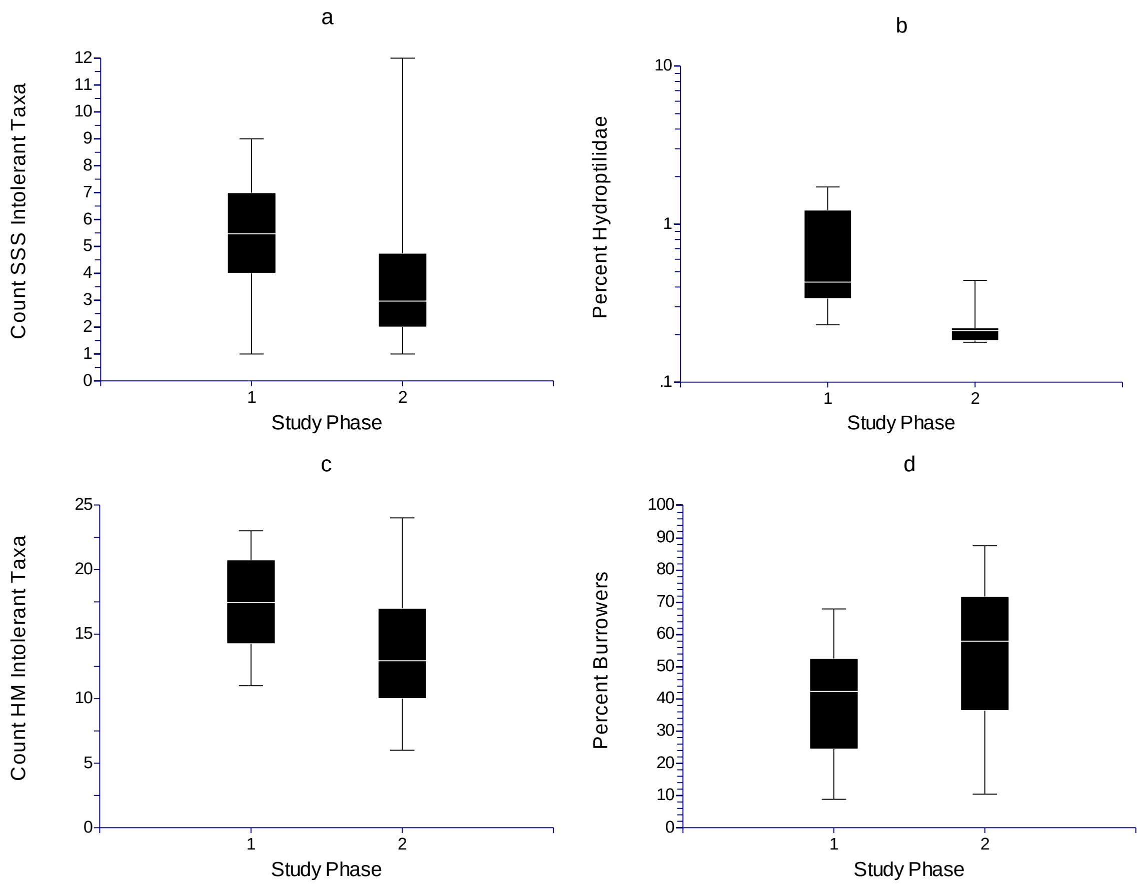

Figure 46. Box plots of macroinvertebrate metrics shown to be significantly different in ANOVA testing for population differences. (a) Count of Taxa Intolerant to Suspended Solids and Sediments (SSS). (b) Percent Hydroptilidae. (c) Count of Taxa Intolerant to Heavy Metals (HM). (d) Percent Burrowers. Box area represents inner quartile range, while "whiskers" represent the upper and lower observations.

Metric Correlations

The metrics selected after ANOVA testing were found to have significant relationships to many wetland water quality parameters and floristic quality values. While correlation does necessarily mean causation, most of the variability in the metrics were thought to be the result of either indirect or direct biological responses associated with these water quality and floristic factors. Many important water quality measures were correlated with multiple macroinvertebrate metrics, suggesting these metrics may have broad application as water quality indicators for wetland systems.

Percent Hydroptilidae was significantly correlated with depth to flood (DTF), mean specific conductivity, total organic carbon (TOC), dissolved organic carbon (DOC), and atrazine metabolite desisopropylatrazine (DIA), and desethylatrazine (DEA) (Table 13). However, for many samples collected during Phases I and II, the value of this metric was zero. Then these samples were removed from the analysis, only mean conductivity, TOC, and DIA were found to be significantly correlated to Percent Hydroptilidae. A robust regression model explained over 40% of the variation in Percent Hydroptilidae (adjusted R^2=0.41).

Percent Hydroptilidae = 1.463766 + 0.5988605 × MeanCond mS/cm - 0.1281936 × TOC mg/L - 2.909601 × Desisopropylatrazine µg/L

Table 13. Pearson product moment correlations for macroinvertebrate metrics and stressors. * $p \leq 0.05$, † $p \leq 0.001$. SSS - Taxa Intolerant to Suspended Solids and Sediments and Sediments. HM - Taxa Intolerant to Heavy metals.

	Macroinvertebrate Metric Response			
Stressor	**Percent Hydroptilidae**	**Percent Burrowers**	**Count HM Intolerant Taxa**	**Count SSS Intolerant Taxa**
Depth To Flood (DTF)	-0.30*			
Maximum Depth m		-0.32*		
Total Plant Richness		-0.38*		0.33*
Native Plant Richness		-0.38*		0.33*
Mean Total Plant Conservatism				-0.35*
Mean Native Plant Conservatism				-0.37*
Mean Conductivity mS/cm	0.39*			0.35*
NH3 µg-N/L				0.49†
Total N mg-N/L			0.28*	0.33*
TN:TP ratio		-0.37*		
Available N:P ratio			0.31*	0.35*
TOC mg/L	-0.28*			
DOC mg/L	-0.32*			
DIA µg/L	-0.32*	0.37*	-0.30*	
DEA µg/L	-0.30*	0.32*	-0.29*	-0.30*
Metribuzin µg/L		0.29*	-0.37*	-0.36*
Alachlor µg/L		0.32*	-0.40*	
Cyanazine µg/L			-0.39*	-0.30*
Number of Herbicides Detected		0.35*	-0.44†	-0.36*

Percent Burrowers correlated with fewer than half of water quality and plant variables listed in Table 13. Two of the listed stressors were retained in a significant robust regression equation (adjusted R^2=0.33).

Percent Burrowers = 79.74749 - 0.677929 × Native plant richness - 10.21359 × Maximum Depth

Count Intolerant Heavy Metal Taxa was significantly correlated with total nitrogen, available N:P ratio, DIA, DEA, metribuzin, alachlor, cyanazine, and Number of Herbicides Detected. In addition, a significant robust regression model was produced having a single independent variable, Number of Herbicides Detected (adjusted R^2 = 0.16).

Count Heavy Metal Intolerant Taxa = 36.04802 + 3.058258 × Number of Herbicides Detected

Count Intolerant Taxa to Suspended Solids and Sediments (SSS) was significantly correlated with total plant richness, native plant richness, mean plant conservatism, mean native plant conservatism, mean specific conductivity, ammonia-NH_3, total nitrogen, dissolved nitrogen, available N:P ratio, atrazine metabolite desethylatrazine (DEA), metribuzine, cyanazine, and Number of Herbicides Detected. Robust regression analysis of Count SSS Intolerant Taxa and the stressor variables in Table 13 showed that NH_3 and Number of Herbicides Detected as the only significantly correlated variables. The equation explained about 36% of the observed variance in the Count SSS Intolerant Taxa metric.

$$\text{Count SSS Intolerant Taxa} = 4.284377 + 12.98026 \times NH_3\ (\mu g/L) - 384267 \times \text{Number of Herbicides Detected}$$

The Macroinvertebrate Multiple Metric Index (MMI)

The above metrics were determined to be useful for assessing the biological condition (i.e. integrity) of the lower Missouri River floodplain wetland study population and were combined in a multiple metric index (MMI). In the metric development process, scoring the index is the most simple and straight forward task. Because both Stoddard et al. (2008) and Chipps et al (2006) referenced the continuous scoring technique for multi-metric indices described by Blocksom (2003), the following scoring calculation adapted from Minns et al. (1994) was used for metrics that increase in value (indicating positive wetland quality) with decreasing disturbance (Chipps et al. 2006):

$$M_s = M_r / M_{max} \text{ x } 10$$

Where M_r is the raw metric score and M_{max} is the maximum score found in the sample population, and M_s is the resulting individual metric score for each sample. Metric values that increase with increase disturbance, meaning those that indicate negative wetland quality, were calculated as:

$$M_s' = 10 - (M_r/M_{max} * 10)$$

The final multiple metric score for each site was calculated as:

$$MMI = (\Sigma M_{si}/n) * 10$$

M_{si} are the individual metric scores and n is equal to the number of individual metrics used to calculate the final index (Table 14 - Table 16).

Table 14. Descriptive statistics for the lower Missouri River floodplain wetlands (n=53) individual metric scores. Standard deviation = STDEV, Standard Error = STDERR.

Metric	Mean	STDEV	STDERR	Min	Max	Median	25th Percentile	75th Percentile
Count SSS Intolerant Taxa	3.07	2.3	0.32	0	10	2.5	1.25	5
Percent Hydroptilidae	0.81	1.93	0.27	0	10	0	0	1.09
Count HM Intolerant Taxa	6.05	1.77	0.24	2.5	10	5.83	4.58	7.5
Percent Burrowers	4.26	2.35	0.32	0	8.99	4.08	2.4	5.99

Table 15. The Final MMI Score descriptive statistics showing mean, median and range of values over the sample population. Standard deviation = STDEV, Standard Error = STDERR.

Count	Mean	STDEV	STDERR	Median	Minimum	Maximum	Range
53	35.36	14.45	2	33.69	10.42	86.02	75.61

Table 16. Descriptive statistics of Phase I and II MMI Scores. Scores for sites 7107 and 7108 were not part of the development process.

Phase	25th Percentile	Median	75th Percentile	7107	7108
I	37.18	45.13	53.97	40.82	14.41
II	20.56	29.94	38.94		

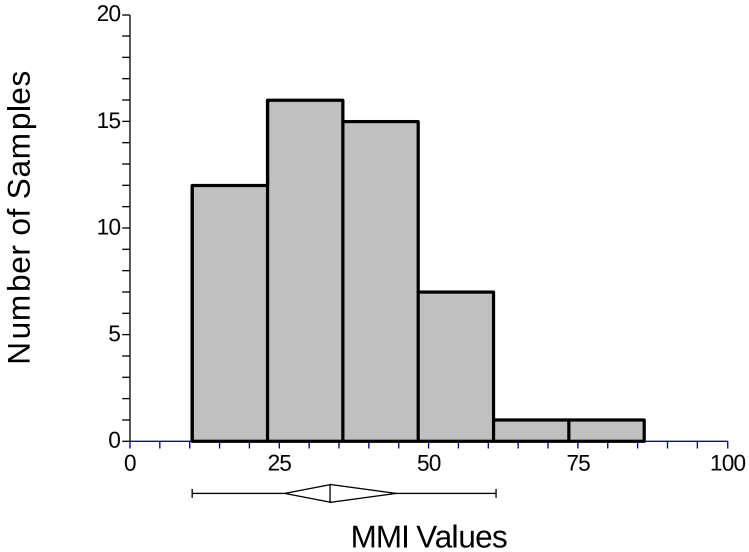

Figure 47. Distribution of MMI values within the sample population, with median value, interquartile range, and upper and lower observations.

The MMI assumes a normal distribution (Figure 47) due to metric scoring. When study population differences were evaluated with ANOVA, a higher mean value was observed in the reference population than in the random population ($p < 0.001$). Kruskal-Wallace non-parametric medians analysis found similar results ($p < 0.001$, Figure 48). One outlier (Site 7111) had a significantly higher MMI score than all other sites among the study Phase I samples. However Phase I and Phase II inner quartile ranges of the 25th and 75th percentile overlap. Site 7107 of the Phase I sample population was included in the population represented in Figure 48. Site 7107 was removed earlier because disturbance assessment data were not available. Though site 7108 had been excluded from this project, it was scored and found to have a significantly low MMI score in comparison to both sample populations.

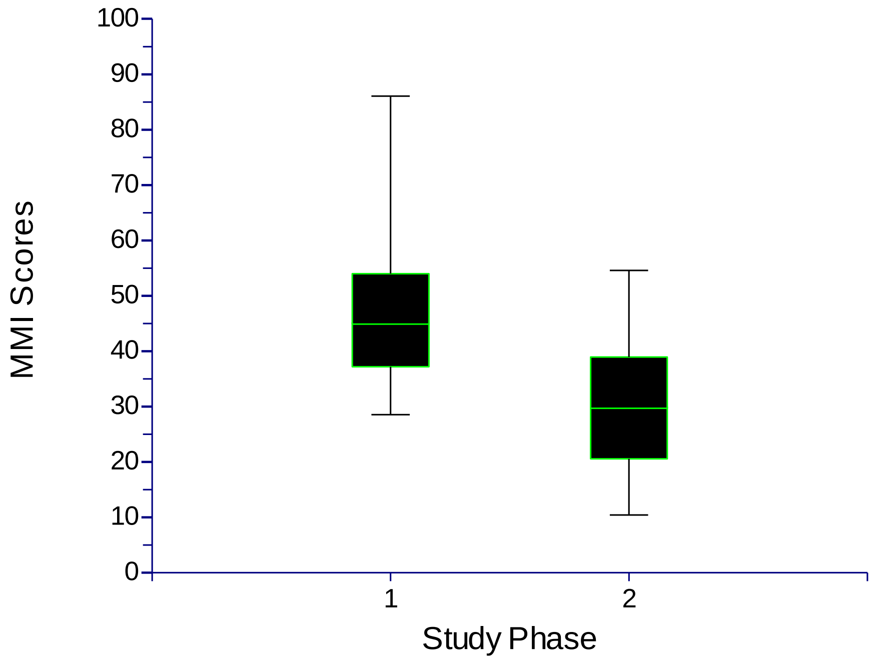

Figure 48. Median Box plots of MMI scores for reference and random populations. Box area represents inner quartile range, while "whiskers" represent the upper and lower observations.

The CDFs produced for some of the MMI metrics and the MMI itself indicated that these measurements do separate reference and random population along the length of the distribution curve (example Figure 44, Figure 49). This separation is broadest and most evident in Percent Burrowers, HM Intolerant Taxa, and the MMI itself.

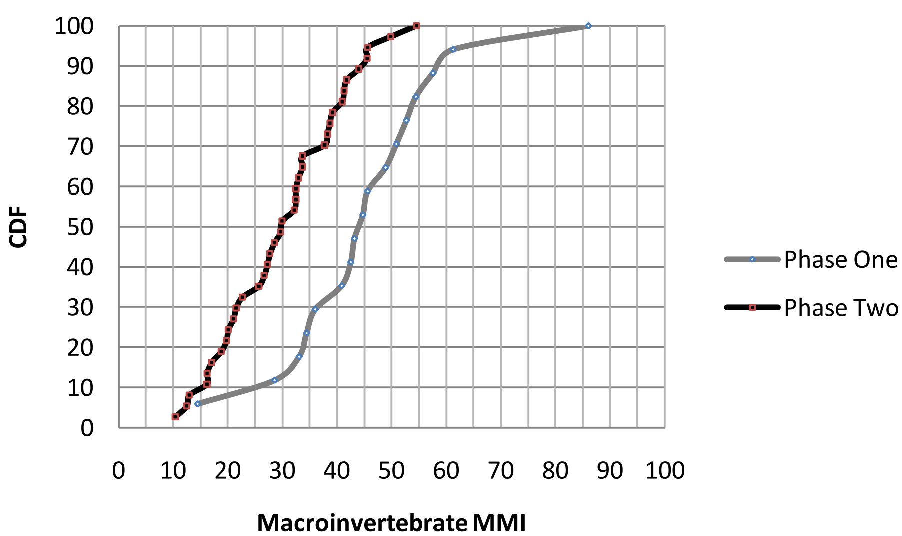

Figure 49. Cumulative distribution frequency (CDF) of macroinvertebrate MMI in reference (Phase I) and random (Phase II) populations.

MMI in Relation to Other Measures

<u>Responses to Ecoregion</u>

While many wetland assessment values appeared to differ with ecoregion location along the Missouri River Channel from Sioux City, Iowa, to St. Louis, Missouri, few parameters were found to differ significantly between the river floodplain portions of the WCB and CIP ecoregions. Observed ecoregional differences may, in part, be due to land-use activities and geomorphologic differences in the landscapes. The floodplain throughout the CIP is typically wider than it is in the other two ecoregions. The differences among the sample populations may be due to topography, flood control alterations, differing agriculture practices, and patterns of precipitation. Estimated flood depths (DTF) for each site were calculated using the KARS floodplain model as developed by Kasten (2008). This measure was acquired through a model that simulated river level rise with back flooding and forward flooding features that determined the river stage at which each site would become connected to the surrounding river valley floodplain. Significant mean differences between sites grouped by ecoregion ($p = 0.006$) were observed in DTF values, with the greatest mean DTF values associated with the CIP region which was significantly different WCP values based on a Kruskal-Wallace non-parametric test (Figure 50).

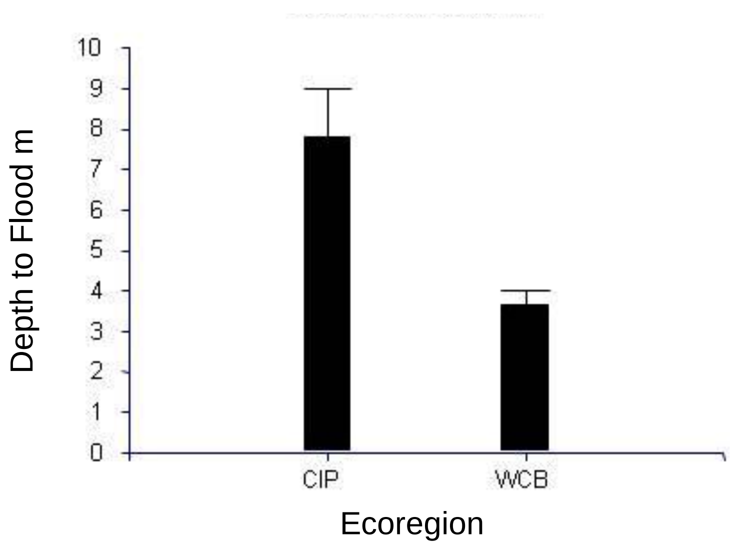

Figure 50. Error-bar plot of the mean depth to flood (DTF) values for the Central Irregular Plains (CIP) and Western Corn Belt Plains (WCB). Error bars represent standard error.

Only the mean conservatism measures for all the plants and native plants ($p < 0.001$) was found to be significantly different among the FQI metrics. Mean conservatism was lower in the Western Corn Belt Plains than in the Central Irregular Plains. The differences in mean conservatism may be inherent differences between the ecoregions, influenced by temperature, precipitation, or land use practices. Log mean conductivity mS/cm means were different among ecoregions, with the CIP having a significantly ($p < 0.001$) lower mean values than the other two ecoregions. Mean pH was also found to be significantly different (p = 0.030) between the CIP and WCB. Mean pH among the wetland sites in the CIP was approximately 0.5 pH lower than the Western Corn Belt Plains (mean pH = 8.06). Despite these findings, no ecoregional differences were observed in the Macroinvertebrate MMI and no interactions were observed when a multiple factor ANOVA was performed between study populations and ecoregion factors. While no significant ecoregional differences in the MMI scores were found the general scoring tends for ecoregions indicated that higher scores were associated with WCB, then CIP and lastly the Interior River Valleys and Hills (Figure 51).

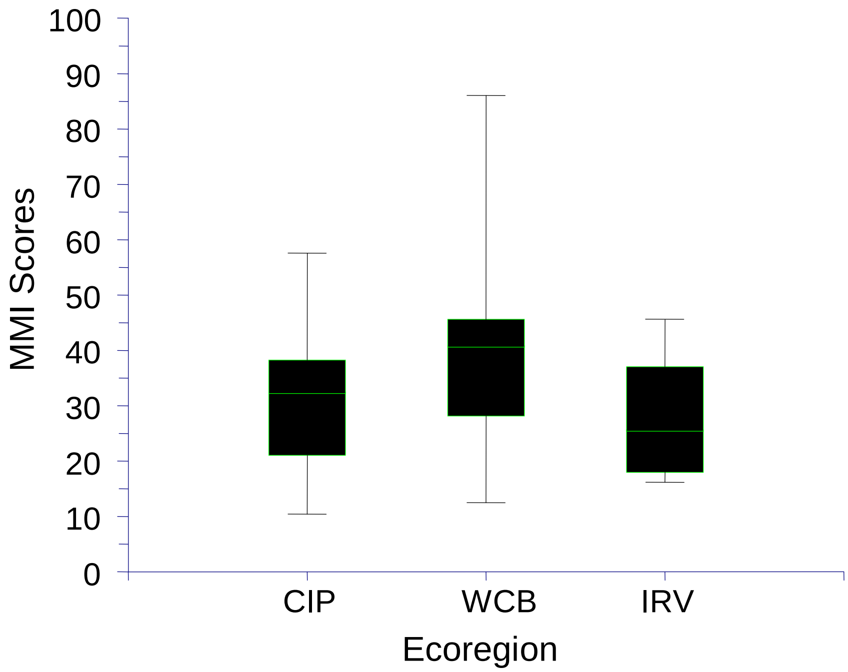

Figure 51. Median Box plots of the MMI scores for the entire sample population (n = 53) by ecoregion: CIP = Central Irregular Plains, WCB = Western Corn Belt Plains, and IRV = Interior River Valleys and Hills. Box area represents inner quartile range, while "whiskers" represent the upper and lower observations.

Differences in Wetland Types

Many significant differences were found between the wetland types for many of the FQA metrics, DA scores, and some water quality parameters. Total organic carbon concentrations (TOC), log Secchi depths (m), and log total nitrogen concentrations (TN) also showed similar significant separations between wetland types. ANOVA and Kruskal-Wallace non-parametric tests identified significant differences between palustrine and lacustrine sites in many of the FQA metrics and depth, though riverine wetlands seemed to separate with indicators of degradation, such as increased percent adventives species, lower native richness, and overall FQI scores. Between class and type differences were observed, but not all were statistically significant (Figure 52a and b). Examination of water quality, FQA, and Macroinvertebrate MMI variables and their values suggest that the MIX most closely related to UB and these sites probably should be re-classed as UB sites. FQ I means and mean native plant richness differences were not observed between reference and randomly selected EM sites. Significant differences in native plant richness were observed between study populations when lacustrine and palustrine sites were evaluated separately (Figure 52c and d). Only one Riverine type was observed in the reference samples, and thus ANOVA testing could not include this group.

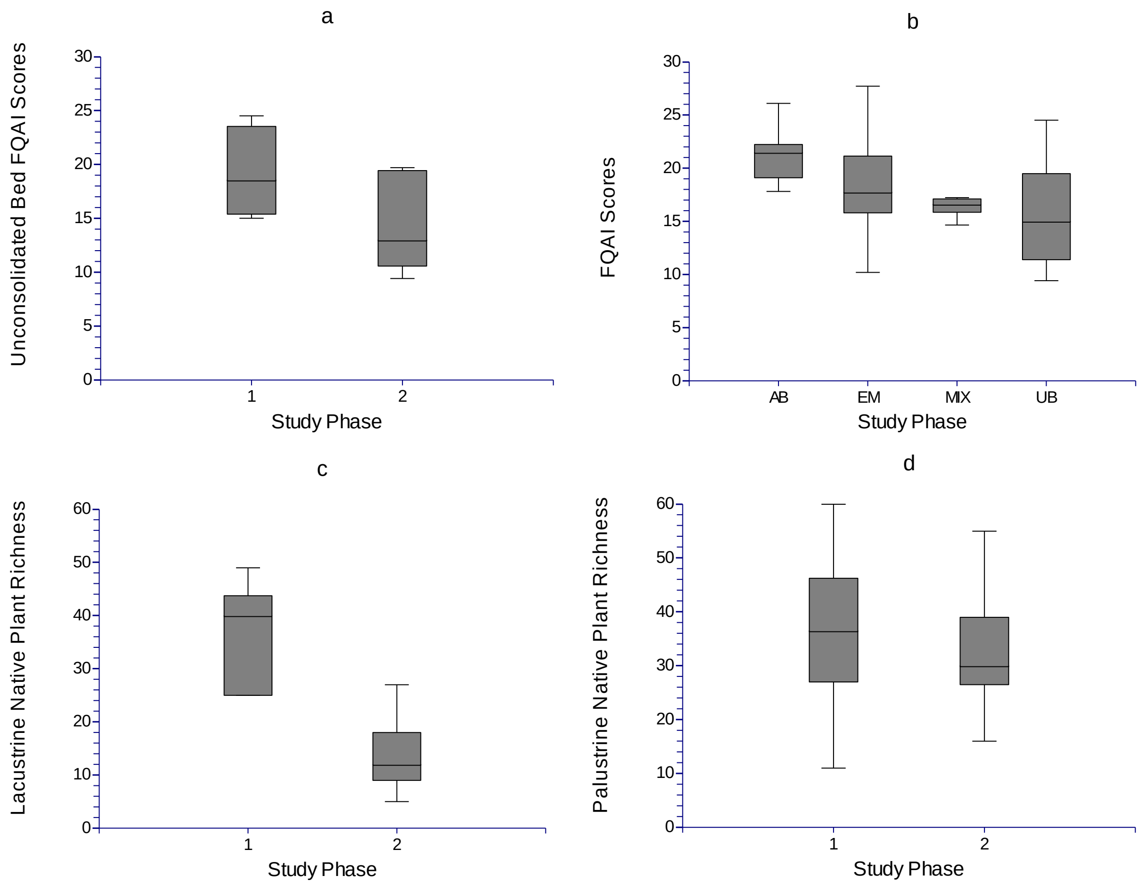

Figure 52. (a) Median box plots of floristic quality index scores for Unconsolidated Bed wetlands and (b) All wetland types among the entire study population (n = 53). Median Box plots in graph (c) and (d) show differences in native plant richness. Box area represents inner quartile range, while “whiskers” represent the upper and lower observations.

Wetland Types and MMI Scores

The macroinvertebrate MMI was evaluated with ANOVA tests, and no significant differences were found between wetland types or classes (Figure 53a and Figure 54a). However, when samples were grouped within reference and random populations both EM and UB types showed significant between population differences (p = 0.001 and 0.004, respectively) (Figure 53b and c). Others types lacked sufficient sample size within each study phases to warrant testing. ANOVA tests for class differences by study population revealed that there were significant difference between MMI class scores (Figure 54b and c). This supports the idea that the reference palustrine and lacustrine sites do support better macroinvertebrate communities than the random sites.

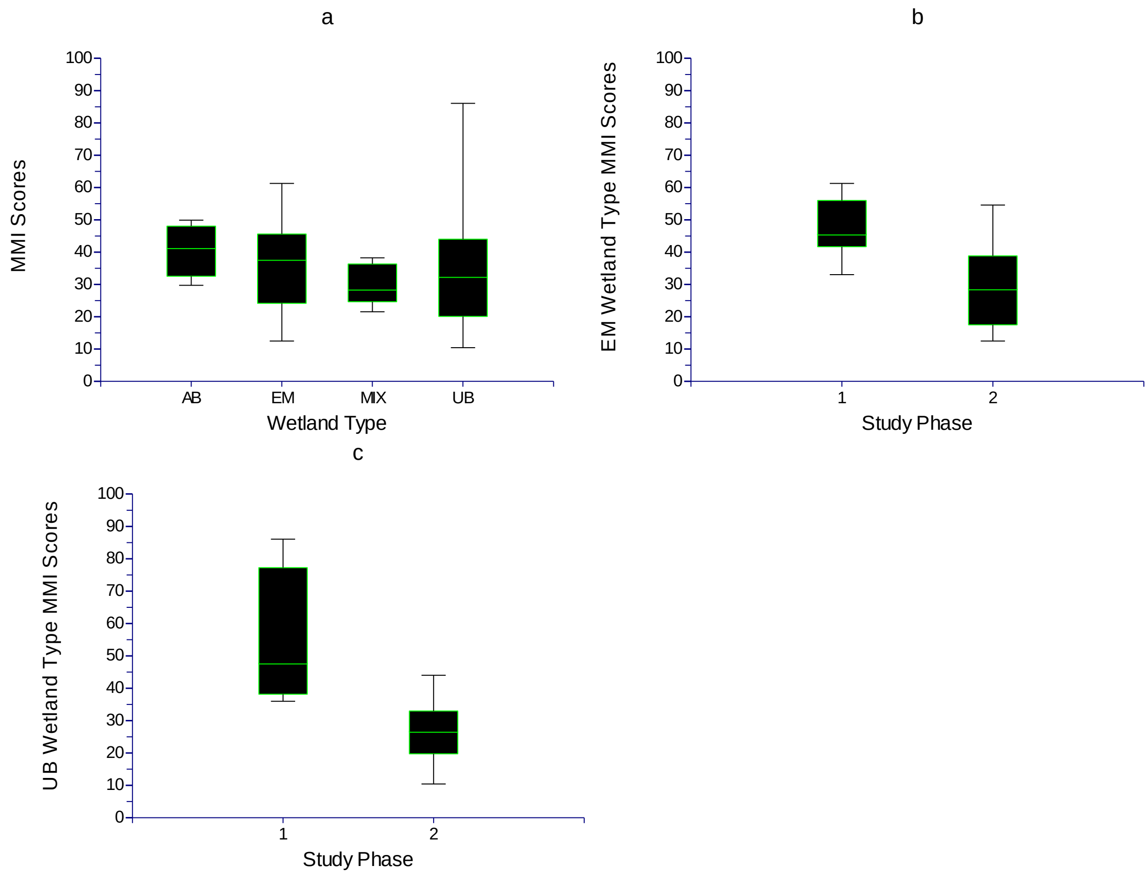

Figure 53. Median box plots of MMI scores for all wetland types and comparisons between Phase I and Phase II samples within types. EM = Emergent Macrophyte Beds, UB = Unconsolidated Beds. Box area represents inner quartile range, while "whiskers" represent the upper and lower observations.

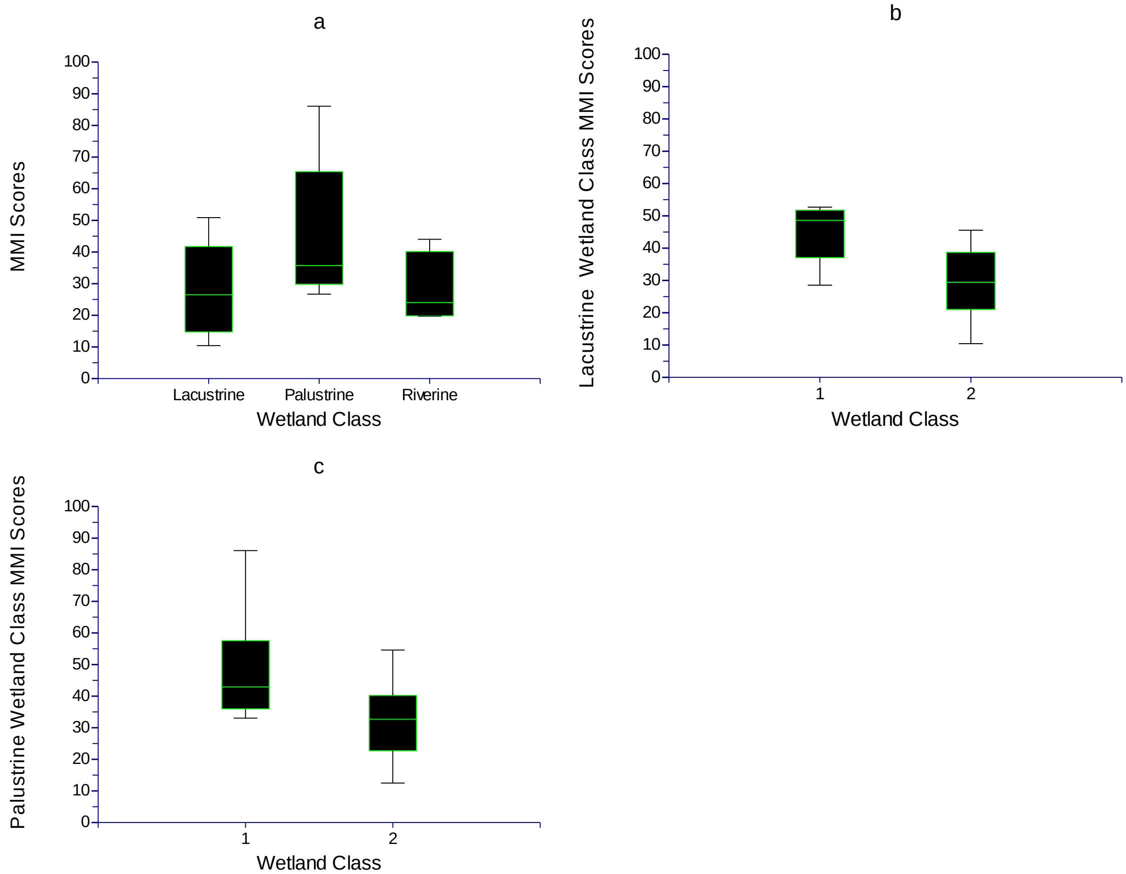

Figure 54. Median Box plots showing the distribution of MMI values among wetland classes and statistically significant differences within classes between Phase I and II. Box area represents inner quartile range, while "whiskers" represent the upper and lower observations.

MMI Result Conclusions

Tests of MMI's response to measures of floodplain connectivity including the DTF, distance from the Missouri River Channel, and measured distance between the sample wetlands did not reveal any significant relationships between the MMI and measures of connectivity. The MMI's significant correlation to the mean conductivity mS/cm measure was the only indirect evidence that hydrological connectivity might be affecting wetland macroinvertebrate community structure, given that mean conductivity also had significant relationships to the DTF and distance from the Missouri River channel measure. Despite this the MMI did show consistent congruency with the other wetland assessment indices and water chemistry metrics, providing evidence that the Phase I reference sample population overall had greater wetland quality. The strongest feature of this MMI is that does not significantly respond to potential ecoregion, class, or type differences, yet it can discriminate reference candidates from the random population regardless of the spatial location of the wetland or classification. The combination of highly responsive individual macroinvertebrate metrics to multiple stressors contributes to a robust measure of biological integrity across a variety of wetland types and classes within this study population.

Acknowledgements

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Appendix A. Goals and objectives of EPA Award R7W0812.

I. Wetland identification – *This information is available as a map and database on the project webpage.*

1. Commitments
    a. Create map of appropriate wetlands in the Missouri River floodplain.
    b. Randomly select sites using EMAP.
    c. Evaluate selected sites – permissions, access, size requirement, etc.
2. Outputs
    a. Map of river, 500-year floodplain boundary, and wetlands.
    b. A database of wetlands sites found in the Missouri River floodplain in USEPA Region 7.
3. Outcome
    a. A wetland map and database resource accessible by others.
4. Measurement
    a. Identification of 35–45 sampleable wetlands.

II. Wetland monitoring - *This information is available in the project database.*

1. Commitments
    a. Revise the Phase I quality assurance project plan (QAPP).
    b. Devise and implement a strategy to sample 35 – 45 wetland sites.
    c. Acquire and organize equipment to sample 35–45 wetland sites.
    d. Collect water samples, *in situ* measurements, and macroinvertebrates from the sites.
    e. Perform a floristic quality assessment (FQA) at each site.
    f. Process samples in the lab.
2. Outputs
    a. A database of field, chemistry, and macroinvertebrate data for wetland sites.
    b. Baseline floristic data for each wetland site.
3. Outcome
    a. A wetland database accessible by others.
4. Measurement
    a. Completion of sampling.
    b. Completion of lab work.
    c. A complete database.

III. Wetland assessment – *This is available as the final report or on the project webpage.*

1. Commitments
    a. Calculate floristic quality assessment metrics for each site.
    b. Quantify local land use and soil characteristics for each site.
    c. Perform basic statistical analyses to summarize chemistry and macroinvertebrate data.
    d. Examine relationships between water quality, FQA, macroinvertebrates, and surrounding landscape.

2. Outputs

    a. Land and soils coverage maps.
    b. Addition of landscape and soils features to the database.
    c. A report on the data and analyses.

3. Outcome
    a. A document for others to follow.

4. Measurement
    a. Review by EPA and others of the assessment document.

Dissemination of information - *This information is available on the project webpage. The workshop is being planned as a webinar. We have requested EPA feedback about fitting the webinar in with EPAs needs.*

1. Commitments
    a. Create a project webpage.
    b. Plan and host a workshop.
    c. Submit 4 semi-annual progress reports and 1 final report to EPA.
2. Outputs
    a. A webpage that holds wetland maps, database, and reports.
    b. A workshop at the Kansas Biological Survey.
    c. Progress reports and a final report.
3. Outcome
    a. The webpage will serve as an information resource for stakeholders, managers, researchers, etc.
    b. The workshop will be a forum for stakeholders and others to learn about this project.
    c. Communication of our progress to the EPA.
4. Measurement
    a. Feedback about the website.
    b. Number of attendees at the workshop.
    c. Feedback from the EPA.

Appendix B. Study sites for Phase II.

Code	Phase	Longitude	Latitude	Site Name	Date	Eco-region	County	State
7100	1	-95.02899	39.50008	Little Bean Marsh	11-Jul-05	WCB	Platte	MO
7101	1	-95.23602	40.0962	Squaw Creek	12-Jul-05	WCB	Holt	MO
7102	1	-95.26411	40.0698	Squaw Creek	12-Jul-05	WCB	Holt	MO
7103	1	-93.203	39.61183	Swan Lake	14-Jul-05	CIP	Chariton	MO
7104	1	-93.15128	39.60701	Swan Lake	14-Jul-05	CIP	Chariton	MO
7105	1	-93.23465	39.62194	Swan Lake	14-Jul-05	CIP	Chariton	MO
7106	1	-96.03905	41.52168	Desoto Sand Chute	21-Jul-05	WCB	Harrison	IA
7107	1	-96.00577	41.49416	Desoto Sand Chute	21-Jul-05	WCB	Pottawattamie	IA
7108	1	-95.86308	41.29599	Big Lake	20-Jul-05	WCB	Pottawattamie	IA
7109	1	-96.33112	42.30553	Browns Lake	27-Jul-05	WCB	Woodbury	IA
7110	1	-96.33191	42.27663	Snyder Bend Lake	29-Jul-05	WCB	Woodbury	IA
7111	1	-96.00095	41.4814	Wilson Island	26-Jul-05	WCB	Pottawattamie	IA
7112	1	-96.17571	42.04803	Blue Lake	27-Jul-05	WCB	Monona	IA
7113	1	-96.19015	42.00844	Middle Decatur Bend	27-Jul-05	WCB	Monona	IA
7114	1	-96.03114	41.74194	Round Lake	26-Jul-05	WCB	Harrison	IA
7115	1	-96.23383	42.00829	Tieville-Decatur Bend	28-Jul-05	WCB	Monona	IA
7116	1	-95.8053	40.98954	Keg Lake	04-Aug-05	WCB	Mills	IA
7117	1	-94.23274	39.25611	Cooley Lake	26-Aug-05	CIP	Clay	MO
7118	1	-95.24734	40.09355	Squaw creek	12-Jul-05	WCB	Holt	MO
7119	1	-96.11201	41.61032	Tyson Bend WMA	05-Aug-05	WCB	Harrison	IA
7120	1	-95.78052	40.85327	Forney Lake	20-Jul-05	WCB	Fremont	IA
7121	1	-96.17746	42.03449	Blue Lake	27-Jul-05	WCB	Monona	IA
7433	2	-95.84749	40.82027	FRW	28-Jul-08	WCB	Cass	NE
7434	2	-92.93709	39.0842	Big Muddy NWR	23-Jul-08	IRV	Saline	MO
7435	2	-93.24189	39.57662	Bosworth Hunt Club	11-Aug-08	CIP	Chariton	MO
7436	2	-94.90613	39.75889	Browning Lake	25-Jul-08	WCB	Doniphan	KS
7437	2	-96.32427	42.31215	Browns Lake	30-Jul-08	WCB	Woodbury	IA
7438	2	-95.68838	40.3287	Bullfrog Bend	31-Jul-08	WCB	Nemaha	NE
7439	2	-94.23274	39.25611	Cooley Lake CA	07-Jul-08	CIP	Clay	MO
7440	2	-94.23288	39.24842	Cooley Lake CA	24-Jul-08	CIP	Clay	MO

Code	Phase	Longitude	Latitude	Site Name	Date	Eco-region	County	State
7441	2	-96.05734	41.57493	Cornfield NRCS	29-Jul-08	WCB	Harrison	IA
7442	2	-90.4699	38.73339	Crystal Springs GC	14-Aug-08	IRV	Saint Louis	MO
7443	2	-93.02812	39.36448	Cut-off Lake	23-Jul-08	CIP	Chariton	MO
7444	2	-93.03012	39.37474	Cut-off Lake	23-Jul-08	CIP	Chariton	MO
7445	2	-93.03266	39.35659	Cut-off Lake	07-Jul-08	CIP	Chariton	MO
7446	2	-93.04834	39.32547	Forest Green	11-Aug-08	CIP	Chariton	MO
7447	2	-95.78646	40.85321	Forney Lake	28-Jul-08	WCB	Fremont	IA
7448	2	-93.25825	39.58086	Grassy Lake	12-Aug-08	CIP	Chariton	MO
7449	2	-96.13304	41.95692	Louisville Bend	29-Jul-08	WCB	Monona	IA
7450	2	-96.13594	41.97426	Louisville Bend	29-Jul-08	WCB	Monona	IA
7451	2	-92.75496	39.02148	MKT Lake	11-Aug-08	IRV	Howard	MO
7452	2	-91.75686	38.70043	Mollie Dozier Chute	15-Aug-08	IRV	Callaway	MO
7453	2	-95.81085	40.68384	NRCS	28-Jul-08	WCB	Fremont	IA
7454	2	-95.81622	40.69553	NRCS	28-Jul-08	WCB	Fremont	IA
7455	2	-95.28514	40.13354	Old Channel	24-Jun-08	WCB	Holt	MO
7456	2	-96.21407	42.05731	Casino	30-Jul-08	WCB	Monona	IA
7457	2	-96.43845	42.4351	S. Sioux City	30-Jul-08	WCB	Dakota	NE
7458	2	-93.15744	39.62371	Silver Lake	12-Aug-08	CIP	Chariton	MO
7459	2	-95.22478	40.10962	Squaw Creek NWR	24-Jun-08	WCB	Holt	MO
7460	2	-95.23213	40.07662	Squaw Creek NWR	24-Jun-08	WCB	Holt	MO
7461	2	-95.27962	40.10469	Squaw Creek NWR	23-Jun-08	WCB	Holt	MO
7462	2	-95.27493	40.0939	Squaw Creek NWR	23-Jun-08	WCB	Holt	MO
7463	2	-93.14423	39.6398	Swan Lake NWR	12-Aug-08	CIP	Chariton	MO
7464	2	-93.23518	39.62242	Swan Lake NWR	12-Aug-08	CIP	Chariton	MO
7467	2	-93.97916	39.20817	Sunshine Lake	07-Jul-09	CIP	Ray	MO
7468	2	-93.78772	39.18867	Kerr Orchard	23-Jul-09	CIP	Lafayette	MO
7469	2	-94.97184	39.4546	Lewis and Clark Wetland Reserve	22-Jul-09	WCB	Platte	MO
7470	2	-95.82191	41.07535	Folsom Lake	21-Jul-09	WCB	Mills	IA
7471	2	-92.68753	38.98735	Franklin Island	06-Jul-09	IRV	Howard	MO
7472	2	-93.10271	39.40514	Trophy Room	06-Jul-09	CIP	Chariton	MO
7473	2	-93.9696	39.18112	Sunshine Lake	07-Jul-09	CIP	Ray	MO

Code	Phase	Longitude	Latitude	Site Name	Date	Eco-region	County	State
7474	2	-94.87099	39.33801	Mud Lake	22-Jul-09	CIP	Platte	MO
7475	2	-94.88828	39.79213	French Bottoms	07-Jul-09	WCB	Buchanan	MO
7476	2	-95.82133	41.08235	Folsom Wetland	21-Jul-09	WCB	Mills	IA

Appendix C. Laboratory measurements and analyses.
DL = detection limit, TOC = total organic carbon, DOC = dissolved organic carbon.

Parameter	Container	Instrument/Method	Method Citation	DL	Holding Time	Preservation
Total Phosphorus	1L Amber Glass	Persulfate digestion @ 250°F and 15 psi, followed by colorimetric method using automated flow injection analyzer (Lachat QuikChem 8500)	Ebina *et al.* 1983 & 20th Ed. Standard Methods (4500-P G)	5 µg/L	5 days	4°C
Total Nitrogen	1L Amber Glass	Persulfate digestion @ 250°F and 15 psi, followed by colorimetric method using automated flow injection analyzer (Lachat QuikChem 8500)	Ebina *et al.* 1983 & 20th Ed. Standard Methods (4500-NO3- F)	0.01 mg/ L	5 days	4°C
Ammonia (NH3-N)	1L Amber Glass	Automated phenate method using flow injection analyzer (Lachat QuikChem 8500)	20th Ed. Standard Methods (4500-NH_3 H)	1 µg/L	24 hours	4°C
Nitrate-N	1L Amber Glass	Automated cadmium reduction method using flow injection analyzer (Lachat QuikChem 8500)	20th Ed. Standard Methods (4500-NO_3^- F)	0.01 mg/ L	48 hours	4°C
Nitrite-N	1L Amber Glass	Colorimetric method using automated flow injection analyzer (Lachat QuikChem 8500)	20th Ed. Standard Methods (4500-NO_2^- B)	0.01 mg/ L	48 hours	4°C
Chlorophyll-*a*	1L Amber Glass	Optical Tech. Devices, Ratio-2 System Filter Fluorometer	20th Ed. Standard Methods (10200-H)	1.0 µg/L	30 days	4°C
Atrazine	1L Amber Glass	Gas Chromatography/Mass Spectrometry	Thurman *et al.* 1990	0.05 µg/L	7 days	4°C
Alachlor	1L Amber Glass	Gas Chromatography/Mass Spectrometry	Thurman *et al.* 1990	0.05 µg/L	7 days	4°C
Metolachor	1L Amber Glass	Gas Chromatography/Mass Spectrometry	Thurman *et al.* 1990	0.05 µg/L	7 days	4°C
Cyanazine	1L Amber Glass	Gas Chromatography/Mass Spectrometry	Thurman *et al.* 1990	0.1 µg/L	7 days	4°C
TOC/DOC	1L Amber Glass	Shimadzu TOC Analyzer (TOC-5000A)	20th Ed. Standard Methods (5310 B)	0.1 mg/ L	7 days	4°C, add H_3PO_4 pH < 2

Appendix D. Disturbance assessment scoring form.

CPCB WETLAND DISTURBANCE ASSESSMENT	R7W08712 - _______
I. Wetland Attributes. *Score to a maximum of 15 points.*	
1. Wetland Size. Wetland boundaries for delineation are defined by evidence of changes in hydrology and may be fairly wide, especially in areas where there is gradual relief.	
1 pts <25 acres 2 pts 25-50 acres 3 pts >50 acres	
2. Natural Buffer Width. Natural wetland buffer includes woodland, prairie, surrounding wetlands and water bodies. The buffer width should be estimated by taking the average of buffer widths in each cardinal direction from the center of the wetland.	
1 pts <10m 2 pts 10-50m 3 pts >50m	
3. Land Use. Surrounding land-use is defined as dominant visible land-use adjacent to and upland from the wetland area, including the natural buffer.	
1 pts Intensive urban, industrial or agricultural activities	
2 pts Recovering land, formerly cropped or a mix of intensive and natural uses	
3 pts Landscape is relatively undisturbed by human activities	
4. Hydrology. Determine the dominant water source based on direct observation of the wetland and its position in the landscape relative to other water bodies or hydrologic features.	
1 pts Precipitation fed wetland, no recognizable inflowing water	
2 pts Fed by seasonal surface water, stormwater drainage and/or groundwater	
3 pts Source is clearly an adjacent lake or an unobstructed inflowing stream	
5. Vegetation Coverage. Refers to aerial coverage of wetland flora or the proportion of vegetated area to open water. Open water area does not include adjacent lakes.	
1 pts <20% 2 pts 20-40% or >70% 3 pts 40-70%	
Wetland Attributes Total	
II. Reference Indicators. *Score one point for each (to be added).*	
Wetland located in a National Wildlife Refuge, Conservation Area or otherwise protected by local, state or federal laws	
Amphibian breeding habitat quality is pristine	
Waterfowl habitat quality is pristine	
Endangered/Threatened Species present	
Interspersion as macrohabitat diversity characterized by a high shore to surface area ratio	
Connected to water bodies (and wetlands) during high-water, located within a natural complex and/or part of a riparian corridor.	
Reference Indicators Total	
III. Disturbance. *Score one point for each (to be subtracted).*	
Sedimentation suggested by sediment deposits/plumes, eroding banks/slopes, and/or turbid water column	
Upland soil disturbance such as tilled earth or construction activities	
Cattle present within or on lands adjacent to the wetland	
Excessive algae present in large, thick mats	
>25% invasive plant species	
Steep shore relief (score 2 pts if more than 50% of wetland edge)	
Altered hydrology shows deviation from historical regime and does not attempt to preserve/restore it	
Wetland is managed as a fishery or hunting club (i.e. water level is manipulated to limit growth of emergents)	
Disturbance Total	–
Total Score (Wetland Attributes + Reference Indicators – Disturbance) =	