Development of a multiple metric index for macroinvertebrates collected from lower Missouri River floodplain wetlands

By

Jason A. Koontz

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Dr. Donald Huggins Co-Chair

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Dr. Belinda Sturm Co-Chair

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Dr. Stephen Randtke

Date defended:_______________

The thesis committee for Jason Koontz certifies that this is the approved version of the following thesis**:**

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Committee:

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Dr. Donald Huggins Co-Chair

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Dr. Belinda Sturm Co-Chair

____

Dr. Stephen Randtke

Date approved:________________

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Abstract

The biological integrity of the aquatic ecosystem has become an important component for assessing wetland condition and quality. Aquatic Invertebrates 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. A study was designed to assess the quality of wetlands in the lower Missouri River floodplain using remote sensing technology, a rapid field 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 community, that influence wetland conditions.

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List of Abbreviations

ANOVA – Analysis of Variance

AP – Agricultural Pesticides

APHA – American Public Health Association

BU- Burrower

CN – Clinger

CPCB – Central Plains Center for BioAssessment

DA - Disturbance Assessment

DAR – Desethylatrazine to Atrazine Ratio

DEA – Desethylatrazine

DIA – Desisopropylatrazine

DOC - Dissolved Organic Carbon

DTF – Depth To Flood

EMAP – Environmental Monitoring and Assessment Program

EPA – Environmental Protection Agency

EPT – Ephemeroptera Plecoptera and Trichoptera

ETO – Ephemeroptera Trichoptera and Odonata

FC – Filterer-Collector

FQA – Florist Quality Assessment

FQAI – Floristic Quality Assessment Index

GC – Gatherer-Collector

GPS – Global Positioning System

HM – Heavy Metal

IBI – Index of Biological Integrity

IQR – Inner Quartile Range

KW - Kruskal-Wallace

MMI - Multiple Metric Index

MS - Microsoft

NCSS – Number Cruncher Statistical System

NOD – Nutrient and Oxygen Demanding chemicals

NTU - Nephelometric Turbidity Units

PA - Parasite

PI – Piercer

PL - Planktonic

POC – Persistent Organic Carbons

PR – Predator

SP – Sprawler

SSS- Suspended Solids and Sediments

STERR – Standard Error

TOC – Total Organic Carbon

1.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 a growing amount disturbance due to urbanization and extension of rural areas in 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 (Dahl 2000). Alterations to the Missouri River, including berms and levees, have undoubtedly disrupted the connectivity that once existed between the river and the surrounding floodplain wetlands that remained. However, it cannot be refuted that wetland loss is also due to natural succession caused by the changing course of the river, though this does occur within the confined boundaries imposed by man's need to control flooding and acquire the greatest benefit from the floodplain landscape. 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.

On a global scale disturbance to existing wetland systems has significant impacts on the cycling and fate of atmospheric carbon, nutrients such as nitrogen and phosphorus, and toxic contaminants that effect biota in many systems (Brigham et al.1995). For wetlands located in temperate and arid regions, global temperature rise has reduced wetland area, connectivity, and productivity. Fundamentally, high productivity in wetlands is attributed to their hydrologic condition of variable inundation that promotes aerobic and anaerobic processes responsible for the cycling and accumulation of nutrients and carbon (Mitsch and Gosselink, 2000). This high productivity is carried over in energy transfer up through the food web to larger and larger organisms, some of which depend on wetlands immensely, and some of which we value as consumers and observers.

Wetland conservation began in the middle of the 20th century because of concerns expressed by hunters and wildlife ecologists alike in response to the diminishing habitat provided to waterfowl (Mitsch and Gosselink 2000). Waterfowl and amphibians rely on the availability of wetland systems that can support diverse communities by providing habitat and food resources that ensure growth, development, and reproductive success (Euliss et al. 2004). Macroinvertebrates are the food source for many amphibians and carnivorous waterfowl, and the success of higher trophic organisms depend directly on the success of these secondary and tertiary consumers. As common inhabitants of both lakes and streams, benthic macroinvertebrates are a highly diverse group that represents an important link in energy transfer through food webs (Rosenberg et al. 1997). Across the U.S. almost 9000 benthic invertebrate species are known to occur in freshwater systems. Furthermore, the taxonomy of most macroinvertebrate groups is well documented and identification keys and well-developed methods of data analysis are readily available (Rosenberg et al. 1997). Though macroinvertebrates are numerous and diverse, field collections of benthic macroinvertebrates is relatively quick, easy, and less invasive than other biological integrity assessments using higher order organisms (eg. amphibians, fish, mammals, reptiles, and birds). From functional and ecological perspectives their abundance and sensitivity to nutrient eutrophication, anthropogenic toxins, and habitat disturbance are most desirable for the purpose of assessing biological integrity in aquatic systems.

Macroinvertebrates can show significant response to perturbations to wetland ecosystems that are not always apparent with chemical and physical characterizations. Recent advances in the development of indices for biological integrity have combined landscape features, habitat, and water chemistry to identify aquatic ecosystems of high and low integrity and determine macroinvertebrate community components that delineate the two populations (Bouchard et al. 1998, Chipps et al. 2006, Lougheed et al.2007, and Stoddard et al. 2008). There is a need for rapid assessment tools to quantify wetland conditions and identify perturbations to systems for ecosystem integrity management.

1.1 Wetlands Study Overview

In 2005, researchers set out to identify a reference set of Lower Missouri River Floodplain wetlands, using satellite imagery, land use, elevation data, and hydric soils classifications (Kriz et al. 2007). Wetlands observed as having high productivity and plant species diversity that can support diverse biological communities are considered reference. However, discrepancies in the classification and identification of hydric soils between state and county boundaries prevented the use of hydric soils information in helping define potential reference wetlands. Many sites identified by the National Wetland Inventory as wetlands for the reference study and the preceding random study of the lower Missouri floodplain did not exist as wetlands due to either natural or anthropogenic processes such as filling or draining (Kriz et al. 2007). Disrupting the connectivity of a wetland to the floodplain by levees, dikes, or berms combined with upland activity leads to sediment accumulation and eventually to the development of terrestrial landscapes, which in many cases were incorporated into farmland. Over 21 sites where surveyed in the summer season for florist quality, while only 18 contained water quality and macroinvertebrate samples (Figure 1). Fifteen sites were determined to be reference, based on the results of the floristic quality assessment index (FQAI) scores, water quality parameters, and cursory examination of the landscape-based disturbance assessment. Many important relationships were found between levels of nitrogen, phosphorus, and to a lesser extent the organic carbon content of the water and the floristic quality assessment index (FQAI) scores.

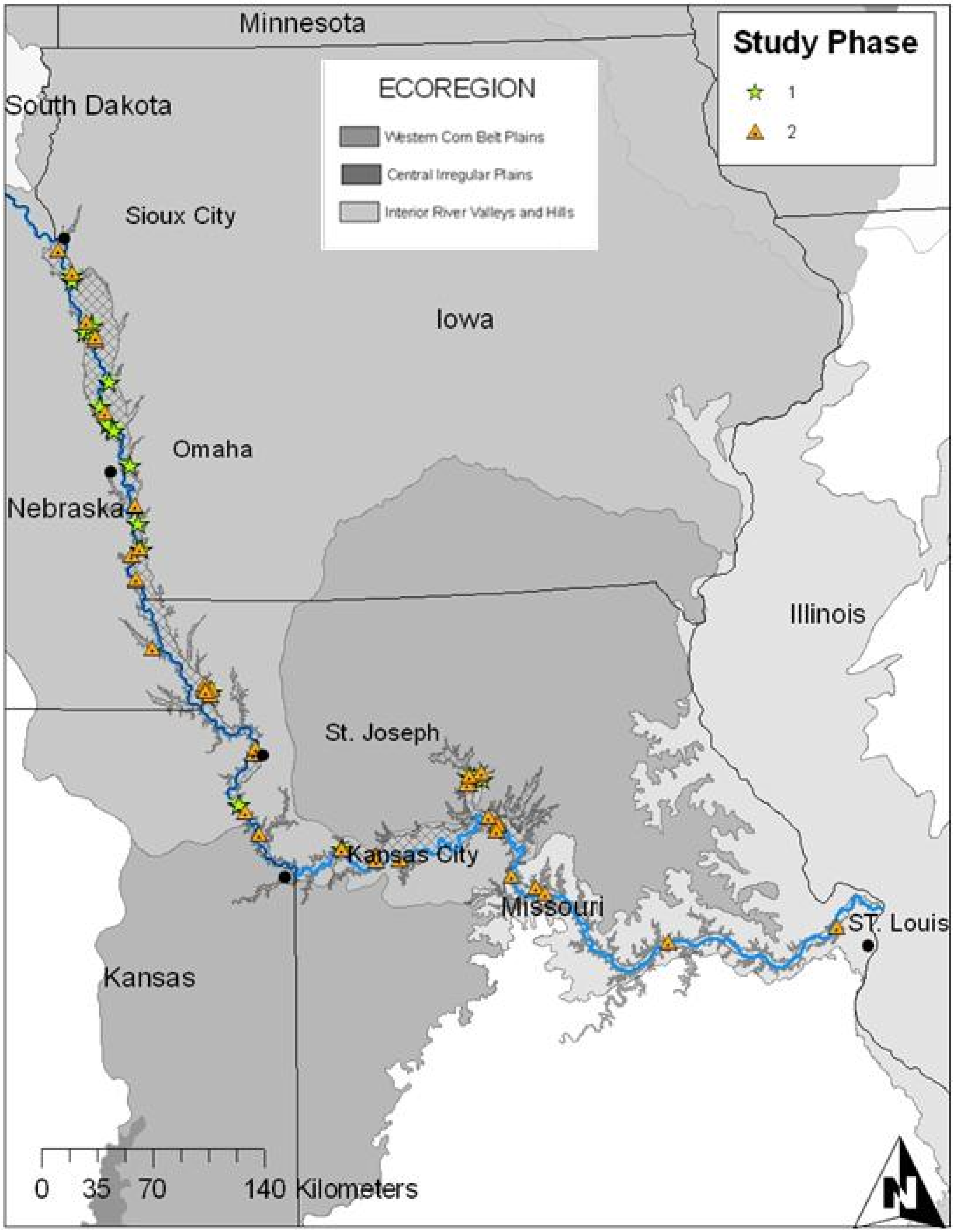


Figure 1: Area map of the Lower Missouri River floodplain wetland distribution.

The reference study was followed up by the assessment of randomly selected wetlands within the lower Missouri floodplain that aimed to complete the development of the Disturbance Assessment Tool developed by Kriz and Huggins for rapid assessment capabilities. A total of 42 sites were sampled during the summer seasons in 2008 and 2009, with 41 containing all water and biological samples and assessment measures (Figure 1). The disturbance assessment was refined so that scores could be obtained from visible landscape features observed by workers. Scores were compared with water quality parameters and FQAI values but few significant statistical relationships werefound. However, in the process of developing the disturbance assessment for this project another researcher found that identification of the dominant wetland types based on Cowardin et al (1979) contributed significantly to explaining variability found in FQAI values and water chemistry (Beury 2010). Many differences were found among the types, but the relationships overlapped and the significances were not always strong. Evaluation of these types indicated that wetlands found to have multiple dominant vegetation components were potentially sites in a state of transition or disturbance. Wetland type may be vital to calibrating the rapid disturbance assessment, and may also show significant influence in macroinvertebrate community structures. It is hypothesized that metrics derived from the macroinvertebrate collections can be quantified and related to the disturbance assessment, water chemistry parameters, and floristic quality metrics. Furthermore these metrics can be combined in 6060an additive model that can be used as an indicator of biological integrity that will validate the other assessment methods of this project. The objective of this study was to quantify the benthic invertebrate population using similar modeling approaches from previous research, toward the development of a macroinvertebrate multiple metric index for the lower Missouri floodplain wetlands.

1.2 Hydrodynamics

The United States Environmental Protection Agency has identified many stressors that affect the biological integrity of freshwater wetlands across the nation (Adamus et al. 2001). These include but are not limited to reduced dissolved oxygen, herbicides, sedimentation, dehydration, and fragmentation. USEPA recommends proper determination of wetland type by hydrogeomorphic and Cowardin classifications (Adamus et al. 2001). With hydrology and geology as the dominate components that define all types of wetland, floodplain wetlands can fall into two major categories: high and low gradient (Brinson et al. 1993). High gradient riparian systems tend to have higher flows associated with increased slope, courser bed materials, and stronger coupling between groundwater and surface water flows. Low gradient non-alluvial riverine wetlands have reduced slope and surface flow characteristics that encourage sedimentation and formation of natural levees (Brinson et al. 1993). Abiotic wetland hydrology features such as water depth, solute concentrations, temperature, and drying rate are influenced by altering of atmospheric and groundwater inputs from changing precipitation patterns (Euliss et al. 2004).

1.3 Vegetation

Hydrological variations influence the community composition of wetland plants, invertebrates, and vertebrates. Geomorphic setting, water source, and hydrodynamics making up the spatial and temporal components must be considered. Cycles of drought and deluge are crucial factors determining the diversity among the trophic community. The presence of woody and herbaceous vascular plants that are sensitive to wetland hydrology can be used to delineate wetland boundaries. Cowardin designated the upland limit of a wetland as the boundary between predominantly hydrophytic vegetation cover and mesophytic or xerophytic cover, but also as the boundary between hydric and nonhydric soils (Cowardin et al. 1979). Patterns of plant community structure can show response to human perturbations. Nutrient enrichment of wet meadows from agricultural fertilizer runoff results in lower species richness. Eutrophication increases dominance by a few species. *Typha*, *Praghmites*, *Lythrum salicaria*, and *Lemna* typify wetlands with eutrophic conditions. Enrichment also increases litter accumulation and stimulates phytoplankton and epiphytic algal growth that can smother or reduce the availability of light to submerged plants (Adamus et al. 2001).

1.4 Components of Macroinvertebrate Communities

Spatial distribution and water body permanence are important wetland dynamics, as shorter distances between ecosystems and increased water permanence has been associated with increased invertebrate diversity (Euliss et al. 2004). Though some insects survive unfavorable periods such as drying and freezing by means of resistant cysts, eggs, waterproof epigrams formation, aestivation, and diapauses, flight may be the most common dispersal mechanism among many insects.

Many state and federal agencies have developed biological assessment methods for aquatic lotic systems (rivers and streams) and, to a lesser extent, lentic systems including ponds, lakes and wetlands (Goodrich et al. 2004). A survey of 14 major monitoring protocols identified 10 primary macroinvertebrate metrics used in at least 25 % of the protocols reviewed: Percent Chironomidae, Percent Ephemeroptera, Percent Trichoptera, Percent Dominant Taxon, Hilsenhoff Biotic Index, Total Number of Taxa, Number of Dipteran Taxa, Number of Ephemeropteran Taxa, Number of Trichopteran Taxa, and Number of EPT Taxa (Goodrich et al. 2004).

1.5 Metrics

In developing multiple metric indices for the Ohio River, Applegate et al. used a number of the metrics commonly used among many agencies, yet further defined the rationale for using them (Applegate et al. 2007). As a primary component of ecological integrity, the total number of taxa is a major component for measures of species richness and diversity that correlate with adequate niche space, habitat, and food sources provided in the ecosystem surveyed. The number of dipteran taxa is indicative of homogenized habitats where increased dipteran individuals and reduction in species diversity are commonly found. Ephemeroptera, Plecoptera and Trichoptera are insect orders that are highly sensitive to abiotic conditions in aquatic habitats. Ephemeroptera are highly sensitive to pollutants, vulnerable to acidification, and exhibit a variety of feeding functions, whereas Trichoptera, though less sensitive are indicators of heavy pollution stress. Of these two, the Ephemeroptera are the first to disappear in the presence of pollution disturbance. Plecoptera are found in all unpolluted lotic systems and are intolerant of low dissolved oxygen concentrations (Applegate et al. 2007). Their presence in a lentic system is highly unlikely and will not be expected to occur in any wetland sample. Through panel discussions with many experts, Applegate and colleagues identified other biological indicator species that may show significant response to disturbance (Applegate et al. 2007). They noted that amphipods are generally restricted to cool, well-oxygenated, permanent water bodies and are also sensitive to many toxic heavy metals. Percent Oligochaeta was chosen for their multi-metric index development, because Oligochaetes are found to increase in abundance with increased pollution (Applegate et al. 2007). Though a multi-metric index was not formulated in a study of aquatic invertebrate response to agriculture and vegetation management of seasonal wetlands in Oklahoma, Davis and Bidwell (2008) identified many common and not so common metrics that showed significant response to human induced disturbance (Davis and Bidwell 2008). In a study of constructed and natural wetlands in various spatial relationships to the main channel of a river floodplain system, Gallardo et al. (2008) found many significant patterns between the connectivity of the wetlands to the river and the invertebrate types found therein. Specifically, those systems with higher connectivity showed increased dominance by crustaceans and Oligochaeta, and wetlands disconnected from flood water surface flow had higher numbers of Odonata, Ephemeroptera, and Diptera families (Gallardo et al. 2008). A list of metrics that were found to respond to various environmental stressors and used in final multi-metric indices is presented in Table 1.

Table 1. Potential metrics selected from previous studies for development of the MMI for the Lower Missouri Floodplain Wetlands. Plus and minus signs indicate the direction the metric is expected to respond with increasing wetland quality.

Metric	Expected Response	Source
Percent Hydroptilidae	+	Applegate et al. 2006
Percent Oligochaeta	-	
Percent non insect taxa	-	
number of Diptera taxa	+	
Percent leeches	-	
Percent Coleoptera	+	
Number of Coleoptera taxa	+	
Percent Amphipoda	+	
Percent burrower	-	Stoddard et al. 2008
Percent clinger	+	
Percent taxa with pollution tolerance value = 8-10	-	
No. of Collembola taxa	-	Chipps et al. 2006

Metric	Expected Response	Source
No. of Odonata taxa	+	
No. of parasitic taxa	+	
No. scraper taxa	+	
No. of ETO taxa	+	
Shannon's diversity index	+	
Proportion of Chironomidae	+	
Proportion of predators	+	
Proportion swimmers	-	
Proportion Libellulidae	+	
Proportion dominant taxa	-	
Proportion Culicidae	-	
Proportion sprawlers	+	
Proportion of Hydraenidae	-	
Proportion Helophoridae	-	
Proportion collectors-filterers	-	
Taxa richness	+	Hartzell et al. 2007
Proportion individual in dominant 3 taxa	-	
Proportion of Corixidae	-	
Proportion of Diptera	-	
Proportion of predators	+	
Proportion of shredders	+	
Proportion of omnivores	+	

Metric	Expected Response	Source
Proportion of grazers	+	
Proportion of gatherers	+	
Number of Chironomidae taxa	+	
Number of gastropod taxa	+	
Number of intolerant taxa	+	
Number of leech taxa	+	

1.6 Stressors

Many abiotic and biotic components of an ecosystem can affect the biological communities in wetlands.Quality of detritus and oxygen levels, which are influenced by the type of primary producers present were evaluated against macroinvertebrate community structure by Spieles and Mitsch (2003). Specific functional feeding groups and invertebrate biomass were related to primary production and allocthonous carbon matter in a simulated flow-through emergent marsh using effluent chemical oxygen demand data from two constructed wetland types in central Ohio. A wastewater treatment wetland and floodplain wetland receiving surface water from a third-order stream were used to calibrate the model. Primary productivity as metaphyton, macrophyte, and periphyton were simulated in the model as 50% macrophyte cover in a single square meter of wetland area. Metaphyton and periphyton were calibrated using substrate-attached and water-column chlorophyll *a* data collected in 1994 and 1997 respectively, while Chlorophyll *a* values were converted to dry algal biomass and kilocalories using Standard Methods 10200 H (APHA et al. 1998). It was determined that wetlands receiving inflows containing course particulate organic matter resulted in a larger standing crop of macroinvertebrates including collectors, shredders and predators. Low dissolved oxygen tolerant species such as *Chironomus*, *Physa*, and Oligochaeta were predicted using varying organic matter inflows to manipulate the dissolved oxygen levels in the wetland model. Other researchers observed dramatic increases in the percent of hypoxia-tolerant macroinvertebrates as the average daily dissolved oxygen decreased and determined that wetlands susceptible to severe organic input have a community majority composed of macroinvertebrates tolerant to low levels of dissolved oxygen (Spieles and Mitsch 2003).

In a study of eight high priority temporary ponds, one way analysis of variance (ANOVA) was tested on total abundance and taxon richness (Porst and Irvine 2009). Using Pearson product moment correlation, the relationship between the nutrient content of the systems and the invertebrate communities was compared using log transformed total nitrogen (TN) phosphorus (TP) and mean abundance and log transformed mean richness. Mean abundance and log transformed mean taxon richness were also compared with turbidity, chlorophyll a, and conductivity in a Spearman rank-order correlation. Porst and Irvine found that turloughs (i.e. temporary ponds) with high nutrient concentrations supported abundant Diptera and Gastropoda populations. Both taxonomic groups are known to be composed of taxa having high tolerances to conditions related to nutrient enrichment. Log transformed total abundance macroinvertebrate data was used in cluster analysis and multi-dimensional scaling (MDS) ordination to assess the similarity of samples (Porst and Irvine 2009).

A multi-metric index for macroinvertebrates has been used in many studies, and there are three overlying themes that exist among them: 1. responsiveness, 2. redundancy, 3. numbers (Chipps et al. 2006, Applegate et al 2007, Stoddard et al 2008). Responsiveness is the ability of a metric to differentiate between to a priori groups. Redundancy occurs when metrics respond to the same stressor in the same manner. Numbers of samples in the population having any particular metric must be sufficient to achieve accurate statistical measures. To create a multi-metric index capable of delineating wetlands from one another based on biological conditions it is important to eliminate metrics that do not contribute to this task. Determining the response variables that are significant to determining the structure wetland macroinvertebrate community is crucial to identifying the causative factors that affect the outcome of the index score obtained.

In a study conducted on the downstream impacts of wastewater outfalls on benthic macroinvertebrate communities in the Ohio River, two approaches were used to create separate indices (Applegate et al. 2007). The initial metric selection process was similar to that mentioned above where 55 potential metrics gathered from existing literature were evaluated for low numbers, low response, variable response, and redundancy. The first two criteria were similar to those used in the study by Stoddard et al. (2008); however, variable response was study specific in that metrics exhibited equal numbers of opposite and expected responses to those hypothesized in the five outfalls evaluated (i.e. two of five). Also, metrics were considered redundant when Pearson correlation coefficients with other more commonly accepted metrics were greater than or equal to 0.99 with a probability less than .0001. After this initial assessment, two indices were created, a ‘Panel Index’ and a ‘Percentage Index.’ The Panel Index was formed from a group of 12 selected metrics considered to be ecologically significant during conference with the Ohio River Valley Water Sanitation Commission (ORANSCO) Macroinvertebrate Advisory Panel (Table 1). The ‘Percentage Index’ was created by selecting only those metrics that produced the hypothetical response at more than 50 % of the outfalls. In this study the ‘Panel Index’ (based on best professional judgment) was observed as having a better response to outfall disturbance than the ‘Percentage Index’ (objective selection method). Applegate et al. also indicated that river flow affected macroinvertebrate community structure and that chemical water quality alone may not be sufficient in predicting biotic integrity. Furthermore, macroinvertebrate community structures in rivers must also be affected by flow regime, habitat structure, and energy flow (Applegate et al. 2006).

Biological attributes of seasonally flooded wetlands in the upper Missouri River floodplain were analyzed for their response to anthropogenic disturbance (Chipps et al. 2006). Five low impact and five high impact wetlands were classified based on the condition of non-disturbed and disturbed based on local land-use surrounding each wetland. A wetland condition index (WCI) was developed from six biological metrics including three macroinvertebrate metrics. Stepwise discriminating function analysis (DFA) was used to identify from nineteen candidate macroinvertebrate metrics, those that discriminated between low and high impact sites. Seasonal and annual variation were also evaluated using correlation analysis, as well as canonical analysis between environmental conditions (also found through DFA) and the WCI scores. Individual metric scores were calculated for metric values that decreased or increased with wetland disturbance and combined into an additive model resulting in scores ranging from 0 to 100 on a continuous basis. Seasonal variation between samples was higher than the annual variation for the three macroinvertebrate metrics used in the final WCI as indicated by the Pearson correlation coefficients. Environmental variables were found to be significantly related to WCI scores through canonical analysis, especially those found for potassium total phosphorus and sediment phosphorus. However, weak correlations were found for total Kjeldahl nitrogen, alkalinity, and water conductivity. Chipps and coworkers also found that the Chironomidae abundance was greater and that Culicidae larvae were absent or negligible in low impact wetlands. Both of these groups have been found to be important components of macroinvertebrate communities, where Chironomidae generally decrease in response to increased wetland disturbance, and Culicidae tolerate eutrophic waters with low available oxygen (Chipps et al. 2006).

1.7 Index development

In the development of multi-metric indices for macroinvertebrates, Stoddard et al. (2008) suggested that metrics be grouped into six major categories: taxa richness, evenness/diversity, relative abundance, functional feeding groups, habitat behavior, and published tolerance values of known water contaminants. These metrics are believed to be ecological attributes that characterize inherit qualities of aquatic assemblages that are able to capture biotic condition. Metrics must pass a number of tests to filter out those having weak response gradients. The first of these tests is the range test; metrics with very small ranges must be eliminated because this indicates that it may not vary sufficiently to allow discrimination among sites having different conditions. Stoddard et al. (2008) stated that if one-third of the samples have zero values for a particular metric, it is generally eliminated, which reduces the number of potentially poor metrics for assemblages with fewer taxa such as fish. A metric must be measured among a large portion of sample population to ensure that it is reproducible and that between-site differences (temporal or spatial) are associated with the inherent quality of the sites and not from laboratory or sampling variation. This is generally tested by repeated sampling at the same site. Metric reproducibility is quantified by the ratio of variance among all sites (Signal) to the variance in the repeated visits to the same site (Noise) or the Signal to Noise (S/N) ratio. A metric with a high S/N ratio is more likely to show consistent response to a hypothetical stressors, and an S/N threshold equal to 2 is recommended for rejecting potential metrics (Stoddard et al. 2008). It is also important to identify variability in metrics that is caused by natural rather than anthropogenic gradients. This is done by using reference site data and the difference between the observed and expected metric values for calibration. Ultimately, it is the responsiveness of metrics that allows them to distinguish between least (reference) and most disturbed conditions. Stoddard and colleagues used regional threshold values for multiple stressors to choose most disturbed sites, eliminating those that fell below the threshold value. T-tests were then used to compare the mean values of metrics between least- and most- disturbed sites within each ecoregion, with higher T-scores indicating the higher responsiveness and discriminatory power.

After passing filters for range and reproducibility, all candidate metrics were tested for redundancy, described by Stoddard et al. as being highly correlated to other metrics and those and providing similar biological information (i.e., EPT taxa count and % EPT taxa). However, some metrics may respond similarly to two different stressors that co-vary, or metrics that provide different biological data may co-vary to the same stressor. Therefore, responses to natural gradients are again evaluated within the reference data set to avoid eliminating metrics that fall into these two categories. In practice, correlations between two metrics in the least-disturbed sites having R^2 values greater than 0.5 or Pearson correlation values greater than 0.71 are considered too strongly correlated. Lougheed and coworkers used a similar approach, eliminating any metrics significantly correlated (R> 0.70) with another metric that was more highly correlated with their developed wetland disturbance axis (Lougheed et al. 2007). The final step is to score and calculate the final MMI values. Stoddard and coworkers chose to use a continuous scoring method because discrete scoring is subjective in nature. Scoring was performed by setting ceiling and floor values for each metric using the 95th percentile of the reference-site distribution values and the 5th percentile of the distribution values at all sites respectively. Good (score = 10) and poor (score = 0) biological condition were indicated by the ceiling and floor values found for each metric and all values in between were interpolated linearly. The final MMI score was calculated as the sum of all its scored metrics, and for convenience of interpretation, the values were rescaled to a range of 0 to 100. Only 21 of the over 250 metrics evaluated by Stoddard et al. passed their rigorous filtration technique, and only the single best metric from each of the six metric categories was used in calculating the final

MMI value. Stoddard et al. found the highest T-scores for the categories of taxonomic composition, taxonomic richness, habitat behavior, and pollution tolerance categories in the majority of ecoregions evaluated, and that diversity and feeding group metrics showed the poorest performance for macroinvertebrate data collected as part of the USEPA 2006 wadeable streams assessment. Final MMI scores were found to be more responsive than any individual metric and higher T-scores were associated with less disturbed ecoregions (Stoddard et al. 2008).

The United Stated Environmental Protection Agency recognized significant advantages for using benthic macroinvertebrates in the Rapid Bioassessment Protocols of Use in Streams and Wadeable River (Barbour et al. 1999). Many benthic macroinvertebrates have limited migration patterns and sessile lifestyles making them good indicators of localized conditions. Broad ranges of trophic levels and pollution tolerances within a macroinvertebrate community can indicate multiple stresses and cumulative effects; sensitive taxa can show rapid response to stress while overall community dynamics represent more long term effects. Sampling and identification macroinvertebrates is relatively easy and inexpensive. Identification of intolerant taxa that can be used to detect degraded conditions can be performed by an experienced taxonomist with only cursory examinations (Barbour et al. 1999). Today, many studies focused on assessing the biological integrity of wetlands or other water bodies, employ a ‘multi-metric’ approach using either a combined (abiotic and biotic components) index or separate water quality index (WQI) and Index of Biological Integrity (IBI) as evidence for disturbance affect on wetland condition (Spieles and Mitsch 2003,Chipps et al. 2006, Lougheed et al. 2007). The macroinvertebrates collected for the study can be used to identify water quality impairments not recognized by physical and chemical measures. The goal is to use previous metric development protocols to develop a multi-metric index (MMI) for the lower Missouri River floodplain wetland for the benthic macroinvertebrate samples. It is hypothesized that this MMI will show significant congruency with the results of other assessment methods in this project. In consideration of the underlying hydrogeomorphic characteristics associated with these wetlands it is also hypothesized that macroinvertebrate MMI scores will be higher for wetlands that are more highly connected to the Missouri River floodplain system and other wetlands.

2. Methods

2.1 Project Overview

From 2005 to 2009 a total of 64 wetland surveys have been conducted by the CPCB (Central Plains Center for BioAssessment) throughout the lower portion of the Missouri River (Figure 1). The final data set to be used for the macroinvertebrate multiple metric development study was drawn from 52 sites that contain human disturbance information, water quality data, floristic quality assessments, and macroinvertebrate samples. Data for all sampling seasons have been processed and are retained in an MS Access database. Four data tables were created to accommodate the final dataset, along with one complete table for all 64 sites used for analysis (Appendix A-E). The disturbance assessment and the floristic quality assessment are composed of metrics (values that represent qualitative aspects) and metrics within each assessment are combined to produce a score that is representative of the wetlands condition with respect to either the amount of disturbance or the quality of plant community found there. 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 consist 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, while water properties were measured with a Horiba U10 Water Quality Checker. One liter samples were collected along longitudinal transect at the three latitudinal transects intersection points 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 F for all analyte method and detection limit details). All water quality analyses were conducted in the Central Plains Center for BioAssessment (CPCB) chemical analysis lab except the herbicides analyses, which were performed at the University of Kansas's Chemistry Department laboratories housed in Mallott Hall.

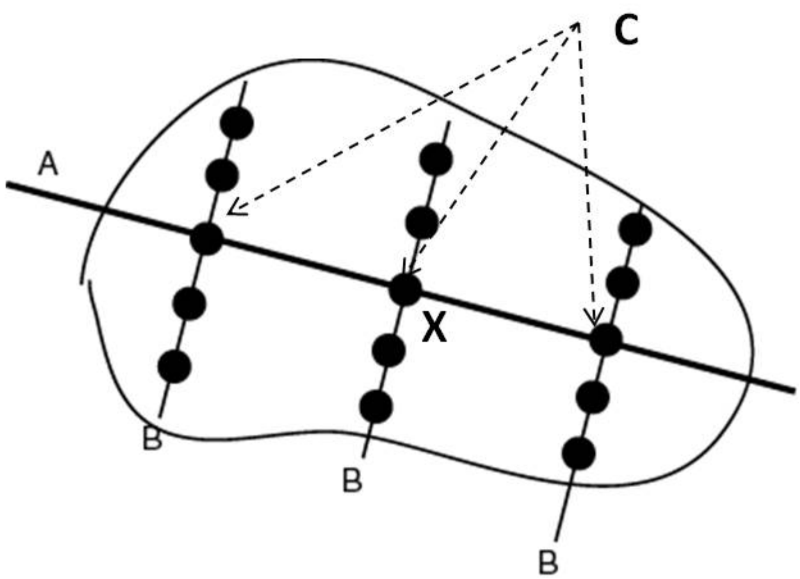


Figure 2: Illustration of wetland survey layout. A is the longitudinal transect line, B's are the latitudinal transect lines, and C represents the composite water sample, and mean in situ water quality measurement locations. X is the wetland centroid where GPS location was recorded.

In 2005, reference candidate (Phase One) sites were selected using GIS based land-use data and percentage of natural buffer area (Kriz et al. 2007). Using their best profession judgment, Kriz and colleagues identified 15 of the original 18 sites having floristic quality, disturbance, and water quality data as reference candidates. Macroinvertebrate samples from 2005 had not been processed at that time. In 2009, the concept of best professional judgment was tested by evaluating the distribution of the sites against all the parameters of the 2005 data, including cursory macroinvertebrate metrics. From this analysis one extreme outlier (site 7108) was found, confirming that is was distinctly not a reference candidate. The other Phase One sites grouped consistently with the reference sample population for many of the same water and floristic quality measures and were retained in the Phase One sample population. The goal of the next phase of this project was to assess the same population of wetlands identified in the National Wetlands Inventory (NWI) database that were 10 acres or greater in size, selected at random using EMAP methods (US EPA 2002). From the primary listing of random sites (Phase Two), 42 sites were visited and assessed for some or all parameters included in this study. However, only 37 wetlands retained the full spectrum of assessment parameters targeted for this study as several had no standing water. It was determined that all wetland sites would be used to develop the macroinvertebrate index since all sites were Lower Missouri floodplain wetlands that met the selection criteria of all our studies (i.e. wetlands $\geq$ than ten acres and were either non-woody palustrine or lacustrine with standing water areas). It was reasoned that the final sample population of 52 sites might better represent a biological condition gradient (BCG) that often exists in ecosystem populations (http://www.epa.gov/bioiweb1/html/bcg.html) and thus would provide a more useful population from which to develop a macroinvertebrate metric index.

2.2 Macroinvertebrate Collections

Macroinvertebrate collection was conducted in the littoral zone of four 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 with movement of the foot through the approximate first 10 centimeters of substrate then sweeping the net through the water column directly above the turbulence. This was repeated for the duration of 30 seconds. 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.

2.3 Processing and Enumeration

Macroinvertebrate samples were relinquished to the custody of the CPCB macroinvertebrate lab, then logged, rinsed of field fixative, and extracted according to the USEPA EMAP methods (USEPA 1995; USEPA 2004), explained in the Standard Operating Procedure (SOP) of the CPCB at the Kansas Biological Survey (KBS) (Blackwood 2007). Samples were processed according to EMAP methods using a 500 organism count with random subsamples (USEPA 2004). Specimens were counted and identified to the genus level for most taxonomic groups when possible by trained taxonomists (Blackwood 2007). Data were recorded on data sheets and entered into an electronic database (Microsoft Excel and Access 2003).

2.4 Data Organization and Analysis

Macroinvertebrate data containing taxonomic names and specimen counts were linked to an integrated taxonomic information system (ITIS) (http://www.itis.gov/index.html) data table and fields containing higher taxonomic groupings were created (Phylum, Class, Order, etc.). Errors in nomenclature were identified and corrected before further field creation and classification commenced. Final name, specimen, count, site ID, and date were entered into the ECOMEAS diversity measurement tool (Slater 1986). Total taxa richness, Shannon's diversity index, and other Diversity Indices were computed for each sample and included in the final macroinvertebrate data table. Fields for feeding guilds and habitat behavior identification and tolerance and sensitivity values were created and updated with available data bases constructed by CPCB for previous research endeavors (Table 1). Taxa missing data were updated from the aquatic insect identification and ecology literature (Smith 200, Dodds and Thorpe 2005, Merritt and Cummings 2008). Proportion calculations and ratios of the different taxonomic groupings were conducted and exported along with water quality, herbicide, floristic, and disturbance data (metrics) to the Number Cruncher Statistical System (NCSS) (Hintze 2004) for statistical analysis.

2.5 Establishing Degraded and Reference Groups

In order for metrics to delineate between least disturbed and degraded conditions, sites must be *a priori* selected for these conditions. Previous researchers have recognized that self-aligning groups are possible and that ecosystem conditions exist on an environmental condition gradient (Stoddard et al. 2008, Bouchard 1998). Many researchers establish water quality or environmental indices using multiple lines of evidence to score and separate sites into these groups based on water quality parameter benchmarks, land use, and habitat condition (Chipps et al 2006, Lougheed et al. 2007). Most of this work has been conducted on rivers and streams, which may not necessarily transfer ecologically to wetlands. Other researchers determined least disturbed and impacted sites based on land use as a surrogate for disturbance within wetlands (Chipps et al. 2006). Some have also chosen to combine multiple parameters of land use, water quality, and floristic quality toward a wetland disturbance axis toward selecting metrics in developing a wetland Index of Biological Integrity (Lougheed et al. 2007). This study was developed to include multiple lines of evidence, and it was important to determine which approach would be best for this study, so considerable investigation into each method was conducted. Initial evaluation of the water quality parameters among the reference (Phase One) and random (Phase Two) populations, with comparisons drawn between floristic quality analysis and the disturbance assessment, resulted in high variability in measured gradients. Assessment score gradients were most consistent between the floristic quality assessment and the disturbance assessment, with reference sites from 2005 maintaining higher mean and median values for both. Water quality parameters varied little between the two groups except for mean conductivity, total nitrogen, nitrate, TN:TP ratio (variance associated with total N) and chlorophyll *a*. In other systems such as rivers and streams, nitrogen gradients and increased productivity are associated with eutrophication and degraded ecosystems. However, the opposite was found, where reference sites maintained significantly higher mean values in FQAI metric scores and nutrient concentrations over the random population. It should be noted that for both groups there was significant overlap in range for most of these parameters. The significant overlap in parameter gradients among the two study phases provided further rationale for pooling the 52 sites with all assessment method data regardless of the time of collection (e.g. Phase One or Two of our study). Evaluation of the relationships between the disturbance assessment, floristic quality assessment, and water quality measures were not always consistent in identifying disturbance. Therefore, multiple stressors were identified and formed into individual a *priori* groups and tested against the macroinvertebrate metrics to determine the most significant responses.

2.6 Identifying Stressor-Response Relationships

Many macroinvertebrate population metrics have been identified that show significant response to various types of disturbance, both natural and anthropogenic. Invertebrates like amphipods and Ephemeroptera (mayflies) are quite sensitive to pollution and acidity which can indicate degraded systems (Adamus 2001, Applegate 2007). Gastropod abundance and invertebrate densities were found to be higher in wetlands where epiphytic algae were in association with submerged macrophytes than in emergent vegetation beds (Adamus 2001). Pollution tolerant species such as Oligochaeta exhibit increased abundance with increased nutrient pollution in streams and wetlands (Applegate 2007, Gallardo 2008). Larger numbers of crustaceans may be indicative of wetland stability because they are long lived and have less effective colonization strategies, unlike Dipteran species that have shorter life cycles and more highly effective dispersal methods (flight), which can colonize newly disturbed and isolated areas free of predators (Gallardo 2008). Therefore, in this study water quality parameters associated with increased pollution, such as increased nutrients, herbicide presence, pH, and conductivity were tested with metrics either selected objectively through filter processes or chosen based on ecological response in past studies.

Previous analysis of water quality measures in this wetland sample population revealed that increased nutrient concentrations and measures of productivity were significantly higher in

Phase One than in Phase Two populations. Water quality data were analyzed for normal distribution and log transformed to achieve *a priori* criteria for ANOVA means analysis. Kruskal-Wallace Non Parametric medians analyses were conducted where data did not undergo log transformations to achieve normal distribution. Non-normal distributions in these cases were not issues of scale, but were affects of skewness or curtosis as determined by NCSS normality tests and comparisons between the water quality parameters from the 'reference' (Phase One) and 'random' (Phase Two). Also, mean differences between the survey years (2008 and 2009) of the random wetland population were evaluated for all metrics to eliminate possible significant temporal variance due to climatic change. It was determined that no significant relationship existed between water quality values and the year of sample. However, some water quality parameters were found to be significantly different for the 2005 'reference' candidates and the random sample population.

2.7 Selection of *a priori* Stressor Groups

Relationships between macroinvertebrate metrics were evaluated with a Pearson correlation matrix. Those metrics that had significant (alpha < 0.05) autocorrelations with stressor parameters were identified and retained regardless of their R or R^2 value. Linear regression tests were performed for stressor response relationships that were surmised from examination of the correlation matrix. Groupings were created for water quality and plant community parameters using the 25^{th} and 75^{th} percentiles to delineate between 'low' and 'high' categories, with two-sample T-test performed among the sample population. The disturbance assessment can be considered as the site delineation model for least disturbed and degraded forms based on surrounding landscape, hydrology, and internal wetland structure. Furthermore, the ultimate goal of the larger project is to create rapid assessment tools that identify disturbance, which must also be validated. Median disturbance scores along with the 25th and 75th percentile benchmarks were determined. Those sites that scored at or below the 25th percentile were deemed "low" scores and considered the most degraded sites. Sites with scores at and above the 75th percentile benchmark were deemed "high" scores and considered as the "best attainable least disturbed" condition.

2.8 Metric Response

Stoddard et al. (2008) also recommended elimination of metrics showing limited range in the dataset. Metrics found to occur in less than 25% of the total number of samples were eliminated from the study because of their limited range.   Table 1 summarizes metrics and their hypothetical direction in response to the disturbance assessment score that were considered for development of the MMI. Metrics that pass the preliminary range filter were analyzed with two sampled T-tests as described by Stoddard et al. (2008) for the Disturbance Assessment and Stressor Response method. The top metric T-scores from each metric category were retained and further examined to eliminate possible redundancy by evaluating environmental response behavior.

2.8 Metric Redundancy

Finally linear relations between macroinvertebrate metrics were analyzed to identify redundancies. Linear relationships between stressor metrics, such as nutrient and water quality measures, floristic values, and disturbance assessment values were also performed to identify responses of macroinvertebrate communities. Where two macroinvertebrate response metrics were significantly correlated to known stressor values (alpha < 0.05) and also highly correlated (R^2 values $\geq$ 0 .90) with one another, the metric with the least significant p and lowest R^2 value that showed similar response to the same environmental stressor was eliminated from the final metric data set. Linear regression analyses were then used to quantify possible dependent (response variable) and independent factor(s) (stressor variable) relationships.

Elimination of metrics was performed according to the methods of Stoddard et al. (2008), except that MMI evaluation did not include randomly selecting a subset of the sample population because abundance was limited by a maximum count (500 in this study). Taxa proportions were calculated for each site using the specimen count of individual taxa divided by total abundance and multiplied by one hundred. Total abundance was not evaluated because it was essentially equal for all samples because of the upper limits imposed in the enumeration protocols. Furthermore, the signal to noise ratio due to sampling error described by Stoddard et al. (2008) could not be tested because there were no replicate samples collected at sites during the same visit. Seasonal variability is not considered in this study as all samples were collected only once during the summer season.

2.9 Scoring Individual Metrics and Final Index

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):

$M_s = M_r / M_{max} \times 10$

Where M_r is the raw metric score and M_{max} is the maximum score found in the sample population, and Ms 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.

2.10 Metric and MMI Validation

Validation was achieved by comparing the individual metrics response in the reference (Phase One) and random (Phase Two) population with the responses observed in the disturbance assessment scores, floristic quality assessment metrics, and water quality parameters. Perfect relationships were not expected from this exercise and only patterns of congruency were considered as evidence for "fitting" the most appropriate metrics to the study as the final assessment tool. Due to the high degree of variability found during the initial evaluation of the sample population, multiple lines of evidence were used to determine which metrics were best based on relationships with other assessment tools. After identifying variable correlations from Pearson's correlation matrix, selected robust regressions were run using the routines in NCSS (NCSS 1997). Robust regression was used to reduce the influence of possible outliers (Rousseeuw and Leroy 1987). This was followed by ANOVA and KW nonparametric analysis to evaluate the Phase One and Two sample populations. The metrics that show the highest congruency with the other wetland assessment components were selected and included in the additive multiple metric score to represent a macroinvertebrate index of biological integrity (IBI) for this study. Established relationships among the samples associated with ecoregions and wetland types that were found through analysis of variance (ANOVA) were also evaluated for the macroinvertebrate multiple metric index (MMI) created. Median box plot representations are used extensively throughout the text because range and distribution is readily visible. The box areas represent the inner quartile range (IQR), while "whiskers" represent the upper and lower observation.

3. Results

3.1 Metrics

There were a total of 44 metrics evaluated in the development of the MMI with only 18 found to be statistically significant 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 (Table 1) were unavailable because the specific family or group was not present in the samples. Substitutions were made and 44 metrics were used for wetland samples in this study (Table 2). Hydrophilidae, the superfamily of Helophoridae, was adopted because the Helophoridae taxonomic group was not present in any of the samples. Other notable additions were the various measures of intolerant species proposed by Huggins and Moffitt (1988). The count of intolerant taxa were derived by taking only those records that had values of tolerance that were less than three, based on the established scale of zero to five. Huggins and Moffitt 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). The Percent Less Than Mean RTV metric was calculated from the 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 2. Final metrics used in the development of the macroinvertebrate MMI. Metrics are grouped by richness and a diversity measures, taxa proportion, taxa count, tolerance, trophic guilds, and habitat behavior guilds.

Metrics Evaluated in MMI Development	
Richness and Diversity Measures	**Taxa Count**
Taxa Richness	Count Collembola Taxa
ChironomidaeTaxa Richness	Count Diptera Taxa
ChironomidaeTotal Abundance	Count Gastropoda Taxa
Percent Dominant 3 taxa	Count Leech Taxa
Percent Dominant Taxa	Count Odonata Taxa
Margalef's Index	Percent Less Than Mean RTV
Shannon's Index (H')	Count ETO Taxa
Chironomidae Margalef's Index	Count Intolerant Taxa AP
Chironomidae Shannon's Index (H')	Count Intolerant Taxa HM
	Count Intolerant Taxa NOD
Taxa Proportions	Count Intolerant Taxa POC
Percent Amphipoda	Count Intolerant Taxa SSS
Percent Chironomidae	**Feeding Guild Proportions and Counts**
Percent Coleoptera	Percent Collector-filterers
Percent Corixidae	Percent Omnivores
Percent Culicidae	Percent Predators
Percent Diptera	Percent Scrapers
Percent Hydrophilidae	Percent Shredders
Percent hydroptilidae	Count Parasitic Taxa
Percent Leeches	Count Scraper Taxa
Percent Libellulidae	**Habitat Behavior Proportions**

Metrics Evaluated in MMI Development	
Percent NonInsect taxa	Percent Burrowers
Percent Oligochaeta	Percent Clingers
	Percent Sprawlers
	Percent Swimmers

3.2 *a priori* Groups and Metric Selection

The stressor-response metrics were selected using a Pearson correlation matrix and linear regression test used by other researchers, except no ‘one’ reference or random groups were established *a priori*. In this study *a priori* ‘high’ and ‘low’ groups were established for parameters that showed consistent significant response to multiple macroinvertebrate metrics using the 25th and 75th percentile because significant variability in response existed between landscape, plant community, and water quality measures. Macroinvertebrate metrics were placed in a correlation matrix along with plant community floristic quality measures, water quality parameters, and surrogate spatial and temporal variables. All significant Pearson correlations with p values less than 0.05 were tested with linear regression and retained if significance was still found in their R^2 relationship. Numerous relationships existed between all the parameters evaluated and the 44 macroinvertebrate metrics evaluated. The relationships were commonly found between 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 and 75th percentile value, respectively. The metrics that responded in linear regression analyses to the parameter groups were then 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, and it was necessary to define each metric by its greatest T-score, which further eliminated many *a priori* groups.

There were 26 metrics retained by this process, with the greatest number of metric responses retained 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 lowest T-score between high and low a priori groupings were eliminated at this time 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 florist variability as well as anthropogenic disturbance, and the remaining 21 metrics were two sample T-tested in these high and low 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) (+), (see Table 3). 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 also found not to be significantly ($p<0.05$) auto-correlated with one another. The Disturbance Assessment (DA) was developed to characterize both internal and external hydrological and landscape features that would affect wetland condition. It had a range of 13 points, with a minimum and maximum value of two and 15, respectively. Sites in the median 25th percentile with scores of seven or less were deemed the 'low' group and sites with DA scores of 13 or more (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.

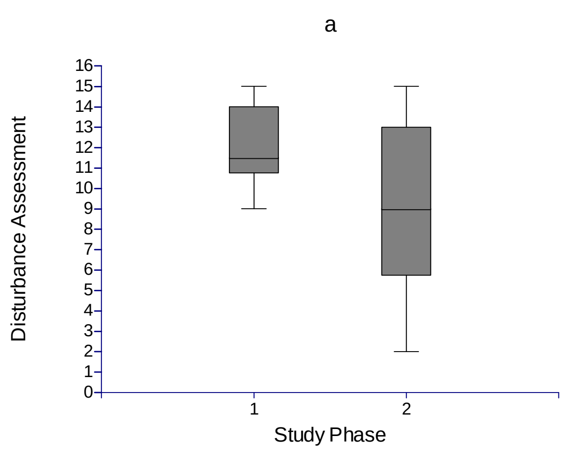
Table 3: Macroinvertebrate metrics determined to delineate between a priori groupings using two sample T-tests of high and low scores in the Disturbance Assessment (DA), Native plant richness values, maximum depth measures, and the number of herbicides detected.

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 (+)

3.3 Metric Testing

3.3.1 Differences Between Study Phases

Significant differences were found between study phases, years, regions, and wetland types in the disturbance assessment scores, FQA metrics, and water quality parameters from previous ANOVA tests of all 54 samples(see appendix A). 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 Disturbance Assessment scores were significantly higher (p=0.004) in the Phase One samples than in the Phase Two samples (Figure 3 a). Mean native plant richness was also found to be significantly higher (p =0.0008) for the Phase One sample population, though FQA values were not (Figure 3 b).



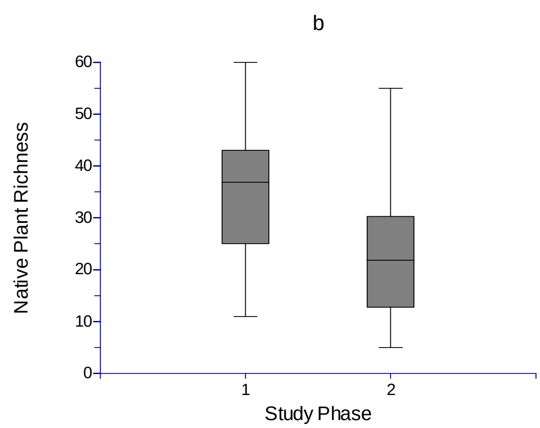


Figure 3: Median Box Plots showing the range and distribution of the Disturbance Assessment Scores (a) and Native Plant Richness (b). Box area represents inner quartile range (IQR), while "whiskers" represent the upper and lower observations.

To further illustrate the multiple levels of congruency among the assessment parameters mean differences among the sample Phase One (reference) (n=15) and Phase Two (random) populations also remained significant for this sample subset (n=37). Log transformed total nitrogen mg/L, chlorophyll-*a*, log transformed mean conductivity, and number of herbicides detected were significantly different between the phases, (all p values< 0.05) (Figure 4).

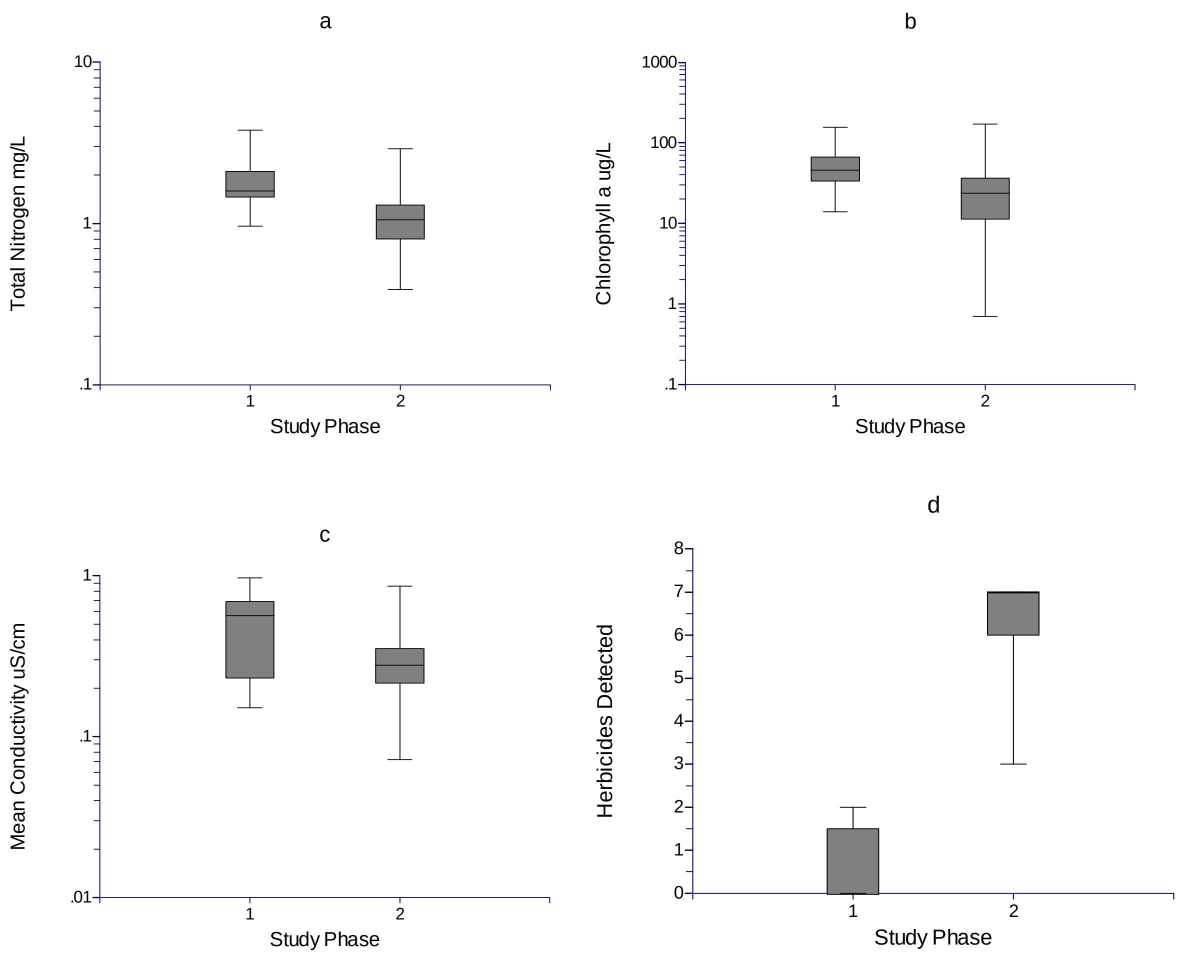


Figure 4: Median box plots of water quality parameters that were found to be significantly different between study Phase One and Two: Log Total Nitrogen mg/L (a), Log Chlorophyll-a (b), Log Mean Conductivity mS/cm (c), and the number of herbicides detected (d). Median Box plots of water quality parameters that were found to be significantly different between study Phase One and two: Log Total Nitrogen mg/L (a), Log Chlorophyll-a (b), Log Mean Conductivity mS/cm (c), and the number of herbicides detected (d). Box area represents inner quartile range (IQR), while "whiskers" represent the upper and lower observations.

All metrics found to delineate between the established *a priori* groups metric selection were tested for congruency with other wetland assessment tools and water quality parameters using ANOVA or KW analysis. KW analysis was performed when metrics failed to meet the normal distribution assumption of ANOVA after log transformations of the data. Metric scores for percent burrowers, and 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 statistically significantly different between the two study groups (Figure 5). Log transformed mean percent Hydroptilidae was significantly different (p =0.0043) between phases (i.e. reference and random populations) having a mean value of 0.34 percent for Phase One and 0.05 percent for Phase Two Log transformed mean count of suspended solids and sediment intolerant taxa were significantly higher (p= 0.009) for Phase One than for Phase Two, having mean percentage values of 5.27and 2.97, respectively. Counts of intolerant taxa to heavy metals and percent burrowers did not need log transformations and were statistically different between phases. With a Phase One mean value of 17.4 (STERR=1.01) and a Phase Two mean value of 13.32 (STERR=0.64), the Phase One population was significantly (p= 0.0013) higher than Phase Two. Mean percent burrowers (38.72, STERR=5.04) was significantly lower (p= 0.012in Phase One sample than the Phase Two samples (mean of 54.35, STERR= 3.21).

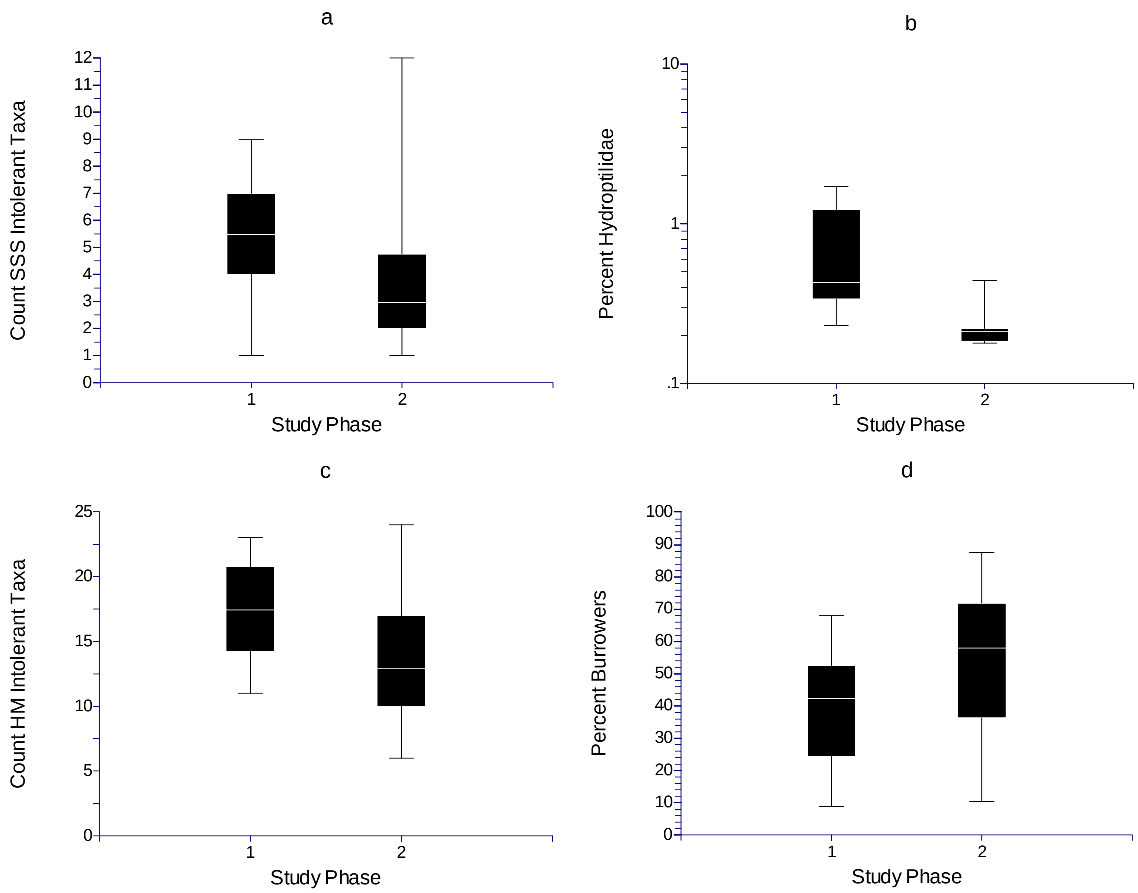


Figure 5: Median box plots of macroinvertebrate metrics having significant differences in means between Phase One and Phase Two sample populations determined through ANOVA: (a) Count of Taxa Intolerant to Suspended Solids and Sediments (SSS),), (b) Percent Hydroptilidae,(c) Count of Taxa Intolerant to Heavy Metals (HM) and (d) Percent Burrowers. Box area represents inner quartile range (IQR), while "whiskers" represent the upper and lower observations.

3.3.2 Metric Correlations

The metrics selected after ANOVA testing were found to have significant relationships to many wetland water quality parameters and floristic quality values. Though it is understood that correlation does not beget causation, most of the variability in the metrics is thought to be the result of biological responses associated with these water quality and floristic factors. Many important water quality measures were correlated with multiple macroinvertebrate metrics, which may indicate widespread ecological effect by certain stressors in wetland ecosystems.

The metric Percent Hydroptilidae was significantly correlated to depth to flood (DTF), mean specific conductivity (mS/cm), total organic carbon (TOC) (mg/L), dissolved organic carbon (DOC) (mg/L), and atrazine metabolite desisopropylatrazine (DIA) (ug/L), and desethylatrazine (DEA) (µg/L) see Table 4). However, for many samples collected during Phases One and Two of this study, 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 was also found that explained about 41 percent of the variation in Percent Hydroptilidae (adjusted R^2=0.41). The Percent Hydroptilidae regression equation

$$=1.463766 + 0.5988605 \times \text{MeanCond(mS/cm)} - 0.1281936 * \text{TOC (mg/L)} - 2.909601 \times \text{Desisopropylatrazine } \mu\text{g/L}$$

Table 4: Pearson product moment correlations for macroinvertebrate metrics and stressors. Significant r relationships having $p< 0.05$ indicated by * and those having $p<=0.001$ indicated by †. Abbreviations: (SSS) - Taxa Intolerant to Suspended Solids and Sediments and Sediments and (HM) – Taxa Intolerant to Heavy metals.

Stressor	Macroinvertebrate Metric Response			
	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 ug-/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 ug/L	-0.32*	0.37*	-0.30*	
DEA ug/L	-0.30*	0.32*	-0.29*	-0.30*
Metribuzin ug/L		0.29*	-0.37*	-0.36*
Alachlor ug/L		0.32*	-0.40*	
Cyanazine ug/L			-0.39*	-0.30*
Number of Herbicides Detected		0.35*	-0.44†	-0.36*

The metric Percent Burrowers correlated with little less than one half of water quality and plant variables listed in Table 4. Two of the listed stressors were retained in a significant robust regression equation (adjusted R^2=0.33)

= 79.74749 - 0.677929 × Native plant richness -10.21359 × Maximum Depth (m).

The metric Count Intolerant Heavy Metal Taxa was significantly correlated to total nitrogen (mgN/L)*, available N:P ratio, DIA (ug/L), DEA (µg/L), metribuzin (ug/L), alachlor (ug/L), cyanazine(µg/L), and Number of Herbicides Detected. In addition, a significant robust regression model was produced having a single independent variable, Number of Herbicides Detected, and a low adjusted R^2 value of 0.16*. This Count Heavy Metal Intolerant Taxa robust regression equation

= 36.04802 + 3.058258 × Number of Herbicides Detected.

The metric 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 (mS/cm), ammonia-NH_3 (ug/L), total N (mgN/L), dissolved N (mg/L), available N:P ratio, atrazine metabolite desethylatrazine (DEA) (ug/L), metribuzine (ug/L), cyanazine (ug/L), and Number of Herbicides Detected. Robust regression analysis of Count SSS Intolerant Taxa and the stressor variables in Table 4 showed that NH_3 and Number of Herbicides Detected as the only significantly correlated variables. The robust regression equation

= 4.284377+ 12.98026 × NH_3 (µg/L) – 384267 × Number of Herbicides Detected

The equation explained about 36 percent of the observed variance in the Count SSS Intolerant Taxa metric (adjusted R^2=0.36).

3.4 The Macroinvertebrate Multiple Metric Index (MMI)

The metrics stated above were determined to be useful for assessing the biological integrity of the lower Missouri River floodplain wetland sample population and were combined in a multiple metric index (MMI). The MMI's were scored using the following equations, where M_r is the raw metric score, M_{max} is the maximum score found in the sample population, and Ms is the resulting individual metric score (Table 5). Refer to methods section 2.9 for metric score and MMI calculations.

Table 5: Descriptive statistics for the lower Missouri River floodplain wetlands (n=53) individual metric scores. Table includes means, standard deviations (STDEV), Standard Error (STDERR), range of values and median measures.

Metric	Mean	STDEV	STDERR	Minimum	Maximum	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

The final MMI had a fairly broad range of 75.61 points from 14.45 to 86.02, the distribution was slightly skewed toward the lower end, indicating a large number of sites had lower index values (Table 6 and Figure 6).

Table 6: The Final MMI Score descriptive statistics showing mean, median and range of values over the sample population.

Count	Mean	Standard Deviation	Standard Error	Median	Minimum	Maximum	Range
53	35.36	14.45	2	33.69	10.42	86.02	75.61

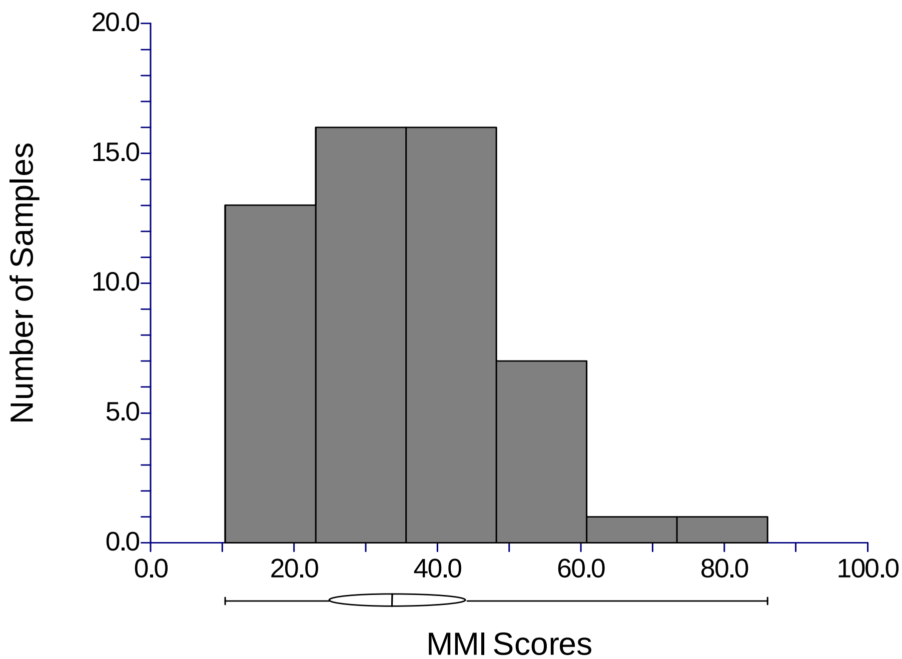


Figure 6: Distribution of values within the entire sample population (n=53, includes site 7107), with median value, IQR, and upper and lower observations.

The MMI assumes a normal distribution due to metric scoring and when study phase differences were evaluated with ANOVA, a statistically significant (p= 0.000015*) higher mean value was observed in the Phase One (reference) population than in the Phase Two (random) population. KW non-parametric medians analysis found similar results with a p value equal to 0.000072 (Figure 7). One outlier (SITE 7111) was identified having a significantly higher MMI score than all other sites among the study Phase One samples and the Inner quartile ranges of the 25th and 75th percentile overlap when comparing Phase One to Phase Two. Site 7107 of the Phase One sample population is also part of the population represented in Figure 6 and part of the calculated values found in the descriptive statistics in Table 5 and Table 7. Site 7107 was removed earlier because disturbance assessment data were not available, and I wanted to limit any bias that this would impose in the metric development process. Though site 7108 has always been excluded from this project, it was scored and found to have a significantly low MMI score in comparison to both sample populations.

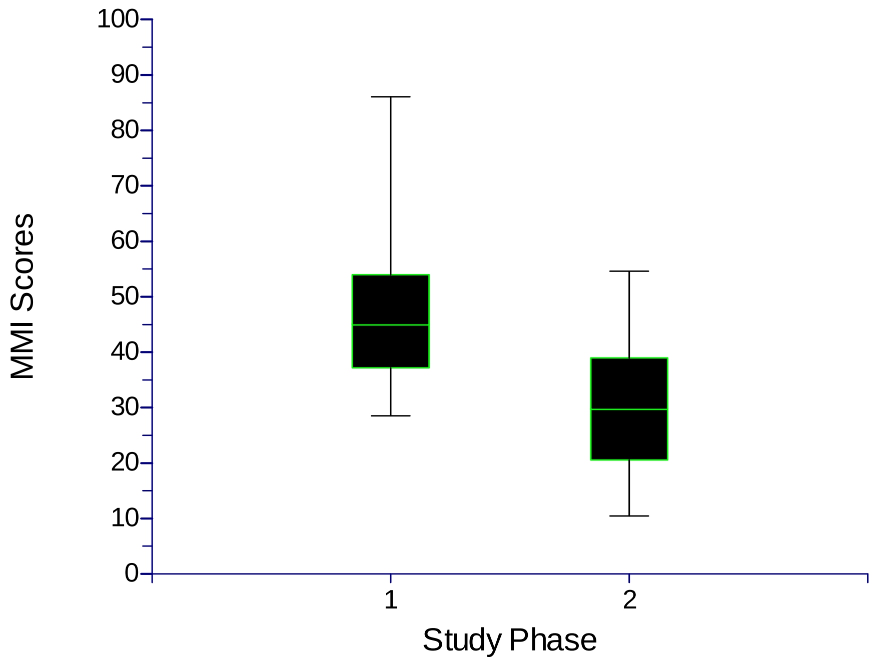


Figure 7: Median Box plots of MMI scores for Phase One and Two samples. Box area represents inner quartile range (IQR), while "whiskers" represent the upper and lower observations.

3.5 Index Range

The final MMI selected for this data set had a range of 75.61 points ranging from 10.42 to 86.02 for all samples (Table 6). A median box plot representation revealed one outlier at the top of the range (site 7111 in Wilson Island State Park). It is indicated by the index as being extremely pristine in macroinvertebrate community structure. Despite site number 7111 having a very high MMI score, it had a medium FQA score, low dissolved oxygen concentration, and a low disturbance assessment score. All constituent metrics scores were high for this site though its nearest neighbor site 7107, located within the same conservation area, had a moderate MMI score of 43.15. The most distinguishing difference between these two sites was their types (i.e. classification group). Site 7111 was identified as being an unconsolidated bed type while 7107 was an emergent macrophyte bed. Other significant differences were that site 7111 had higher ammonia, total nitrogen, orthophosphates, total phosphorus, mean conductivity, and lower turbidity than site 7107. Given that site 7111 had a relatively low depth to flood and is near other wetlands in a managed conservation area, dispersion and surface flow recruitment of various invertebrate fauna may explain the highly diverse and healthy macroinvertebrate community at this site. Though macroinvertebrate data were not available at the conclusion of Phase One of this project both sites were considered high quality wetlands and retained as primary reference candidates.

Table 7: Descriptive statistics of Phase One and two sample populations with median, 25th and 75th percentile values. Scores for sites 7107 and 7108, which were not part of the development process are also shown in the table

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

3.6 MMI in Relation to Other Measures

3.6.1 Responses to Ecoregion

Many wetland assessment values showed responses to ecoregion position along the Missouri River Channel from Sioux City Iowa to St. Louis Missouri, though only a few parameters were found to differ significantly between the river floodplain portions of the ecoregions of the Western Corn Belt Plains, Central Irregular Plains. Regional differences are due to land-use activities and geomorphologic differences in the landscapes. The floodplain throughout the Central Irregular Plains 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. From the floodplain model created to identify our sample population members of the geospatial group KARS were also able to estimate the average flood depth for each site. 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 (Kastens 2008). Significant mean differences between samples within each Ecoregion (p=0.0063) in the depth to flood measure were observed, with the greatest mean depth to flood value found in the Central Irregular Plains region being significantly different than that foundin the Western Corn Belt Plains, based on a KW nonparametric test (Figure 8).

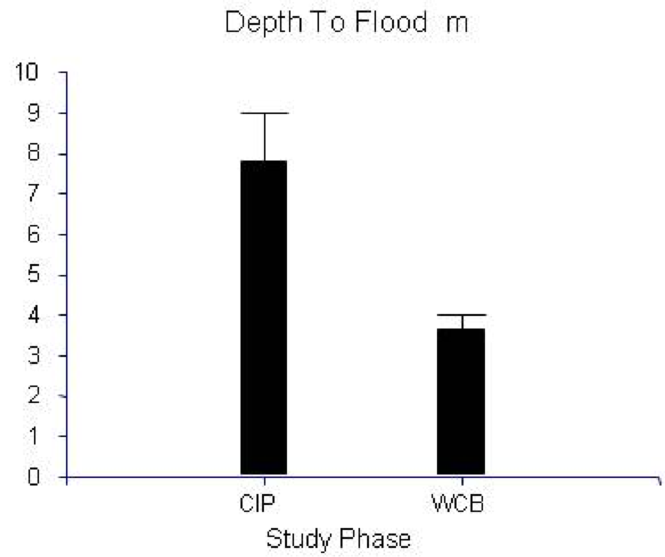


Figure 8: Error-bar plot of the mean depth to flood (DTF) values for the Central Irregular Plains and Western Corn Belt Plains (WCB). Error bars represent standard error.

Only the mean conservatism measures for all the plants and native plants (p=0.00045) was found to be significantly different among the FQA metrics. Mean conservatism was lower in the Western Corn Belt Plains than the Central Irregular plains. Consequently this was also found to be the case between the study phases with mean conservatism measures being lower in the Western Corn Belt Plains. However, almost all the 2005 sites are located in this region and the differences in mean conservatism may be inherent differences between the ecoregions, influenced by temperature, precipitation, or even differing land use practices. Mean conservatism is measured on a very small scale, though greater values indicate positive responses. Log mean conductivity mS/cm means were (different among ecoregions, with the Central Irregular Plains having a statistically significant (p <0.001) lower mean value than the other two ecoregions. Mean pH was also found to be significantly different (p=0.030) between the Western Corn Belt plains and the Central Irregular Plains. Mean pH among the wetland sites in the Central Irregular Plains was approximately 0.5 pH lower than the Western Corn Belt Plains (mean pH value of 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 phase and ecoregion factors (Figure 9)

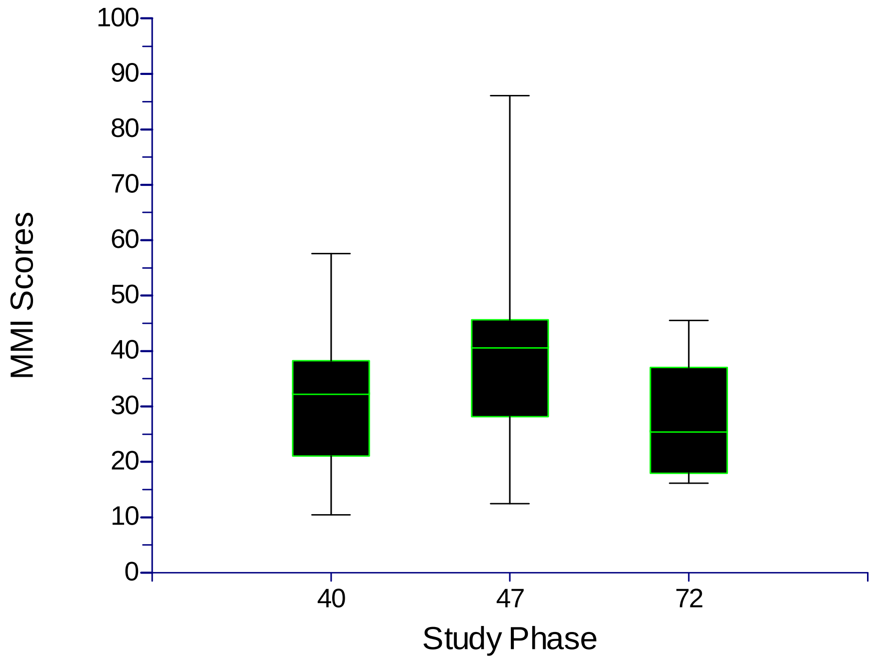


Figure 9: Median Box plots of the MMI scores for the entire sample population (n=53) by ecoregions: 40=Central Irregular Plains, 47=Western Corn Belt Plains, and 72=Interior River Valley. Box area represents inner quartile range (IQR), while "whiskers" represent the upper and lower observations.

3.6.2 Differences in Wetland Types

Wetlands within the study population 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).

Many significant differences were found between the wetland types for many of the FQA metrics, Disturbance Assessment scores, and a few water quality parameters. Total organic carbon concentrations (TOC), log Secchi depths (m), and log total nitrogen concentrations (TN) also showed similar significant separations between the wetland types. ANOVA and KW nonparametric 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 FQAI scores. Between classes and types differences were observed, but not all were statistically significant. Though the ANOVA results for means comparisons of Phase One and Phase Two unconsolidated bed types were statistically significant, only four samples made up the population in the Phase One population. Observations of the means and distributions expressed by the MIX type in water quality, FQA, and Macroinvertebrate MMI, suggest that it is influenced considerably by the UB structural component. Study phase differences in FQA Index means and mean native plant richness were not observed in the EM type. Significant differences in native plant richness were observed between phases when lacustrine and palustrine sites were evaluated separately (Figure 10 c and d). Only one Riverine type was observed in the Phase One samples and ANOVA could not be performed for this group.

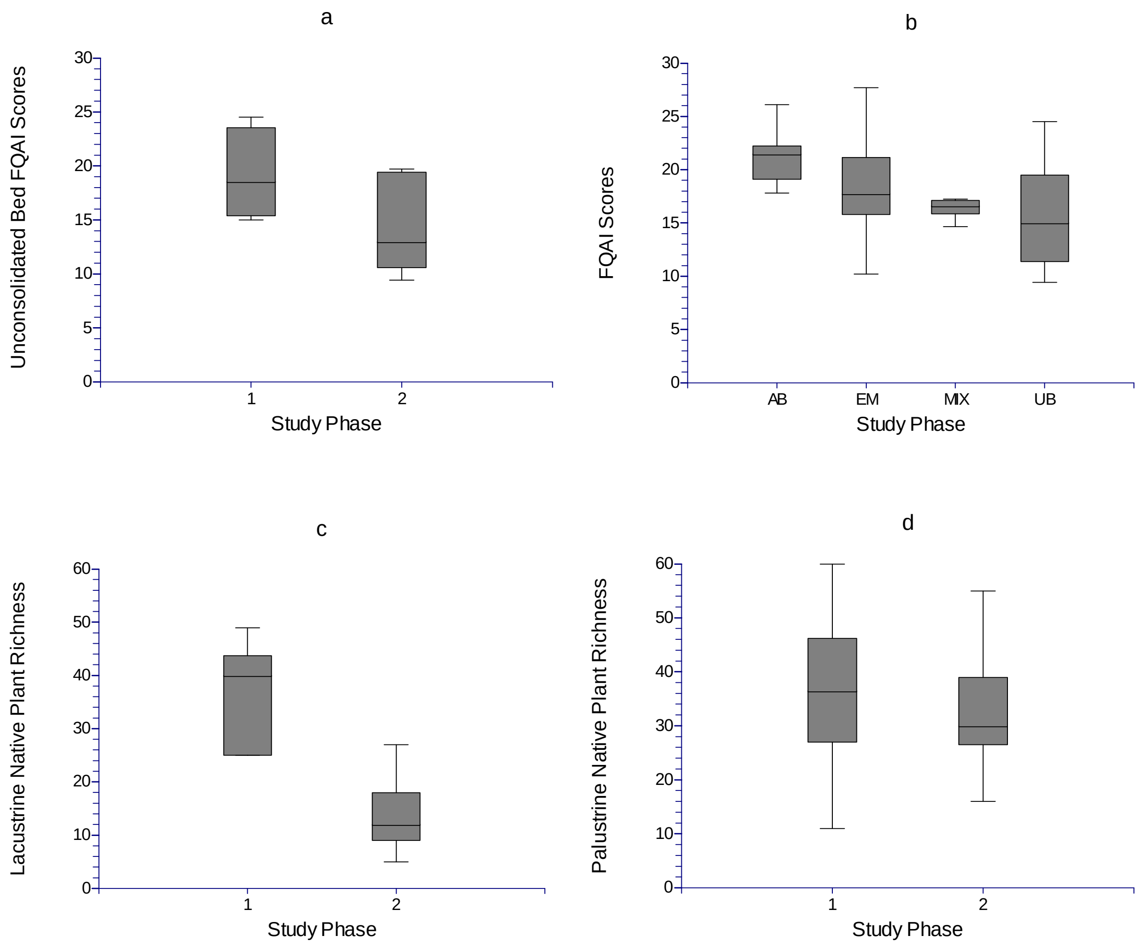


Figure 10: Graph (a) shows median box plots of floristic quality index scores for unconsolidated bed wetlands and (b) graph shows all wetland types among the entire study population (n=53). Median Box plots in graph (c) and (d) show differences in native plant represent the upper and lower observations.

3.6.3 Wetland Types and MMI scores

The macroinvertebrate MMI was evaluated with ANOVA, and no significant differences were found between wetland types or major classes (i.e., palustrine, lacustrine, or riverine). However, when samples were evaluated within these groups for the EM and UB types, significant differences were seen between the two phases which had p values = 0.0006 and 0.004, respectively (Figure 11). Others lacked sufficient representation between study phases, with only one aquatic bed (AB) and type identified in Phase One and Phase Two having only three MIX groups. ANOVA tests of differences between study phases within the major classes palustrine and lacustrine were also significantly different, with a p value of 0.0044 for palustrine sites and a p value of 0.0077 for lacustrine sites (Figure 12).

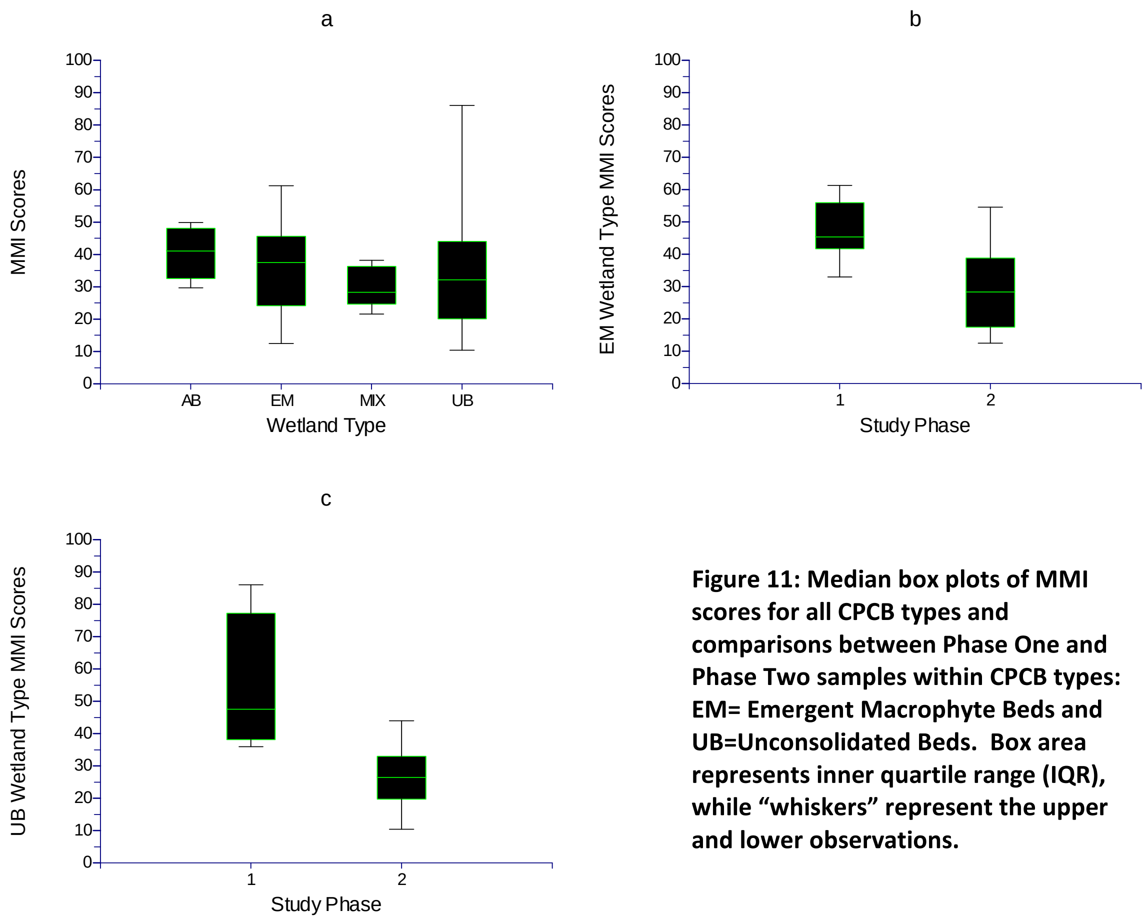


Figure 11: Median box plots of MMI scores for all CPCB types and comparisons between Phase One and Phase Two samples within CPCB types: EM= Emergent Macrophyte Beds and UB=Unconsolidated Beds. Box area represents inner quartile range (IQR), while “whiskers” represent the upper and lower observations.

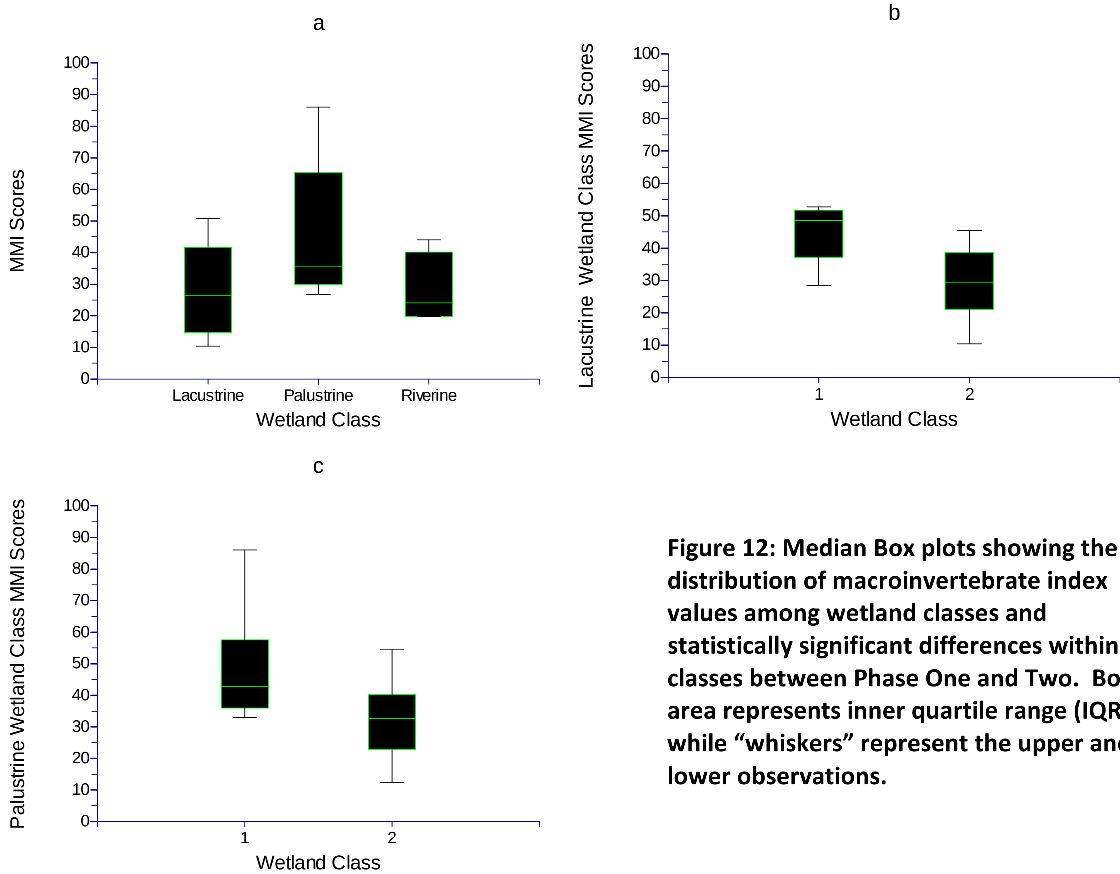


Figure 12: Median Box plots showing the distribution of macroinvertebrate index values among wetland classes and statistically significant differences within classes between Phase One and Two. Box area represents inner quartile range (IQR), while "whiskers" represent the upper and lower observations.

3.7 Result Conclusions

Tests of the MMI's response to measures of floodplain connectivity including the model depth to flood (DTF) value, measured distance from the Missouri River Channel, and measured distances between the sample wetlands did not support the hypothesis that the developed macroinvertebrate MMI could indicate floodplain 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 measure. Evidence to support this relationship was not determined in this study and it was concluded that the MMI could not identify hydrological connectivity effects in macroinvertebrate community structure. However, the macroinvertebrate MMI did show consistent congruency with the other wetland assessment indices and water chemistry metrics providing supportive evidence that the Phase One reference sample population overall has greater wetland quality. The strongest feature of this MMI is that does not significantly respond to the ecoregion, class, or type, yet can delineate 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. Examination of how individual metrics contribute to the assessment of wetland condition will be addressed in the discussion.

4. Discussion

4.1 Individual Metric Response

Some metrics were found to be more definitive than others when evaluating their individual performance in ANOVA analysis and multiple comparison tests. The final MMI is composed of many metrics that were determined useful by other researchers in developing indices of biological integrity or identifying wetland impairment. Percent burrowers and measures of intolerant taxa were also found in the macroinvertebrate MMI developed for the National Streams Assessment, USEPA 2006 (Stoddard et al. 2008). In the study of the upper portion of the Missouri River Flood Plain wetlands, different macroinvertebrate metrics were found useful in delineating low impact from high impact sites in the development of a wetland condition index by Chipps et al. (2008). Chironomidae typically comprise the greatest proportion of the insect taxa in wetlands and respond to hydroperiods and floodplain connectivity (Galat et al. 1998) Although Percent Chironomidae was found responsive in the metric development for assessing biological integrity of the wetlands in the upper Missouri River floodplain (Chipps et al. 2006), it was not responsive in the metric evaluation for this study.

4.2 Metric Statistical Response

Metrics responded differently to individual stressor groups that were used during T-test analysis. When T-tests were performed for the Shannon's Diversity metric with the groupings for high (n= 14) and low (n=13) native plant richness, the response was still significant (T value 2.40, p=0.024). This was also found to be true when herbicide groups (high, n=20: low, n=15) were evaluated with the T-test, except the response was negative (T value -2.36, p=0.024). However, when comparing the mean Shannon's diversity index value between the Phase One reference population and the Phase Two random sample population, the two sample populations were not statistically different from one another. This was also found in the study of riparian wetlands along the Ebro River in Northeast Spain, where individual taxa were more telling of the hydrological connectivity differences between sites than Shannon's diversity, which assumed a uni-model response (Gallardo et al. 2008).

4.3 Methods Affect Metrics

It was recognized early on that some sites in the random population assumed wetland quality or condition much like that of the reference sites and it was also understood that these two groups overlapped in range of assessment values. Therefore, statistically significant differences would not always be apparent. The resolution of the metric measure itself must be taken into consideration, especially diversity indices. Differences in collection and enumeration may contribute a significant amount of variation between study findings as taxonomic resolution can greatly affect the measures of overall invertebrate community structure. Wetland evaluation in the upper Missouri River was conducted with multiple visits, different sampling techniques, and gear which may have influenced the results as much as the selection of *a priori* reference and degraded sites. The macroinvertebrate collection in this study consisted of a significantly large proportion of Oligochaetes (aquatic worms) that were only identified to order. Many of the other non-insect groups also lacked higher taxonomic identification (i.e. gastropods, hydra, and annelids). The aquatic insects had more refined degree of taxonomic resolution and often family, genus, and even species identification was possible. It must also be considered that unlike many other studies, only benthic macroinvertebrates were collected in this study and only during one season. Both Chipps et al. and Stoddard et al. reported that time of collection greatly affects the variability associated with metric data and indices development (Chipps et al.

2006, Stoddard et al. 2008). It may be helpful to exclude groups with low taxonomic resolution in future analysis of wetland macroinvertebrate diversity. Compared to findings by Chipps et al. 2006, invertebrate diversity in the Phase One and Phase Two study population was moderate to low (Table 8).

Table 8: Comparison of mean Shannon diversity index scores from this study with that found for the upper Missouri River, Chipps et al. 2006.

Lower Missouri River		Upper Missouri River		
Phase One-reference	Phase Two- random	Random	High Impact	Low Impact
1.07	0.95	1.71	88	1.57

4.4 Final MMI metrics

4.4.1 Percent Hydroptilidae

Hydroptilidae are a family of caddis flies (order Trichoptera), that are reported as having a narrow feeding niche and are expected to decline in numbers with increased disturbance; this metric was included in the development of the Ohio River Macroinvertebrate IBI (Applegate et al. 2007). In this study, the percent Hydroptilidae metric passed all selective tests and responded to many stressors determined to be important in assessing wetland condition. The overall numbers of this taxa were very low for all sites, however differences between *a priori* groups (i.e. plants, herbicides, maximum depth, and phase groups) were significant based on T-tests. Among the entire population, only three genera of Hydroptilidae were collected: *Oxyethira sp.*, *Orthotrichia sp.*, and *Hydroptila sp.*, being present in only 17 sites. The order Trichoptera made up a small proportion of the sample populations and was represented by only two families and six genera in only 24 sites including those with Hydroptilidae. The three genera of

Hydroptilidae were sensitive to more than one impairment, including agricultural pesticides (AP), persistent organic carbons (POC), heavy metals (HM), suspended solids and sediments (SSS), and nutrient and oxygen demanding compounds (NOD).

Species richness of Trichoptera in the lower Missouri River floodplain is considerably low in comparison to other wetland studies. A survey of caddis flies in the Tomah Stream wetland of Maine reported 46 to 100 species of which 88 had identifiable larval habitats available (Huryn and Harris 2000). Twenty percent of habitat specialists (n=35) were reported from temporary pools and streams and 17 percent were reported from permanent pools and lakes. All Hydroptilidae genera in our study were indentified in Merrittand Cummins' Aquatic Insects of North America (2008) as having lotic habitat preference, with *Oxyethira* also having associations with lentic habitats, particularly with filamentous algae. The Tomah wetland survey was a relatively long term study conducted over the summer of 1997 from June to September, with emerging adults being collected in light traps. When Huryn and Harris compared their results to a three year study of Ohio bogs, marshes, and fens they found that their reported Trichoptera richness was greater than in the Ohio study, which reported 25 to 85 different species. Most Trichoptera species are univoltine, reproducing only once per season (Merritt and Cummins 2008). The low numbers of Trichoptera observed in this study correspond to the limited sampling that was performed in the littoral benthos and only at one time during the summer season. However, the goal of this study was not to identify all species within each system, but to conduct a survey that could draw rapid qualitative and quantitative measures of Missouri River floodplain wetlands' conditions. The multiple metric development process identified the percent Hydroptilidae metric as being highly responsive toward stressors such as atrazine metabolites, total and dissolved organic carbon, and the floodplain model variable, depth to flood. It was also observed to be significantly different among *a priori* groups of maximum depth, herbicides detected, and native plant richness. Comparison of log mean percent

Hydroptilidae revealed significant differences between the reference and random populations (Figure 13).

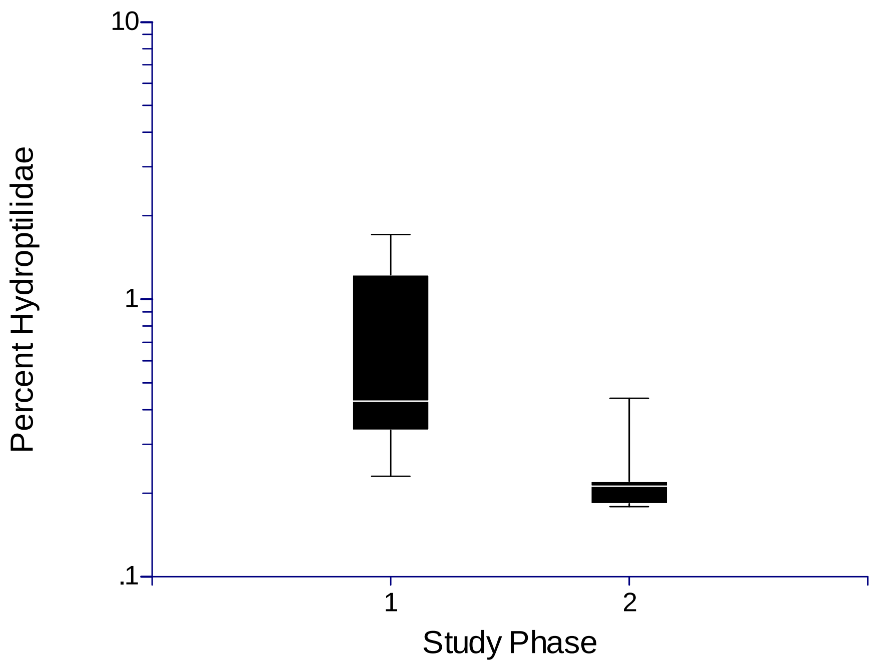


Figure 13: Median box plot of percent Hydroptilidae from Phase One (reference) and Two (random) sample populations. Box area represents inner quartile range (IQR), while "whiskers" represent the upper and lower observations.

In a study of how wetland macroinvertebrate communities respond to hydrological connectivity it was found that Trichoptera abundance was higher in permanent and semi-permanent connected wetlands than in isolated and limited connected wetlands (Gallardo et al. 2008). Most Trichoptera are adapted to fluctuations water permanence and low dissolved oxygen conditions associated wetland habitats because of evolved strategies in lifestyle and reproduction (Smith 2001). Their presence in wetland ecosystems is expected, and highly sensitive taxa such as Hydroptilidae provide evidence of impairments caused by elevated concentrations in heavy metals, persistent organic carbons, and agricultural herbicides associated with the floodplain landscape. As a metric, the percent Hydroptilidae reveal considerable information about wetland condition.

4.4.2 Percent Burrowers

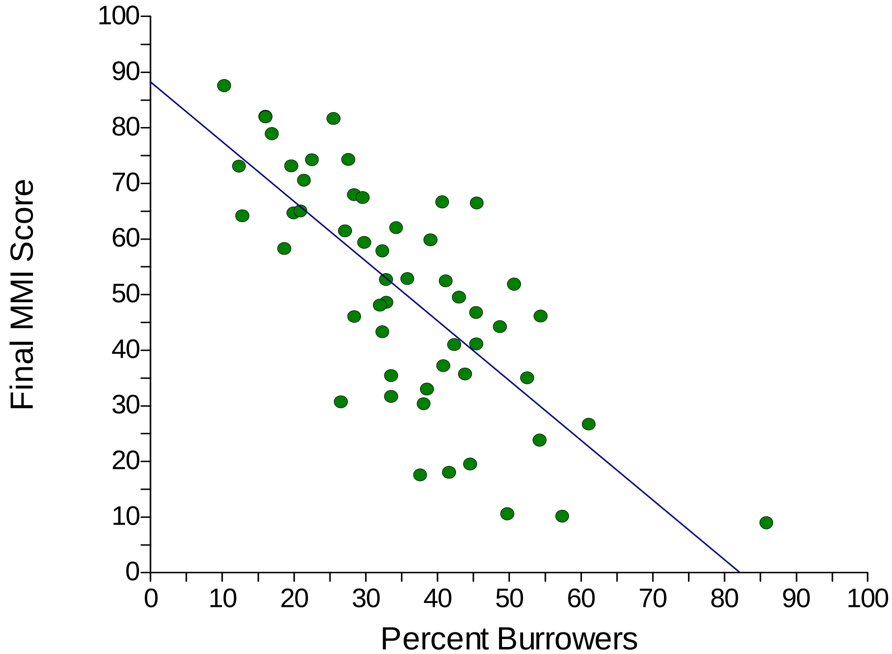


Figure 14: Scatter plot of Final MMI Scores over the percent burrowers value for all samples (n=53). Best Fit Line is least squares regression.

The metric, Percent Burrowers, was the strongest component of the final MMI explaining approximately 60 percent of the variation in the MMI score (Figure 14). About 70 percent of the burrower populations were aquatic worms (Oligochaetes) and 84 % were gatherer collectors, a metric that was not evaluated in this study. Though a significant component of the burrower population, Oligochaetes were not found to be significantly different between the reference and random populations, ecoregions, or wetland classes or types. Oligochaetes are very common among wetland habitats of varying quality and hydrological conditions. Aquatic worms can dominate river sites, have higher abundance in farmland sites, or remain indifferent to vegetative conditions or herbicide treatments (Gallardo et al. 2008, Davis and Bidwell 2008, Kulesza et al. 2008). They serve as good indicators of pollution in streams in rivers as their numbers tend to increase with pollution (Applegate et al. 2007). Oligochaetes showed a slightly significant response to the *a priori* low and high grouping of sites based on Native plant richness in T-test analysis, but were eliminated due to significant correlations with percent burrowers and Shannon's diversity index. ANOVA analysis confirmed that Oligochaetes were commonly abundant in all samples and were not useful in determining wetland quality in this study.

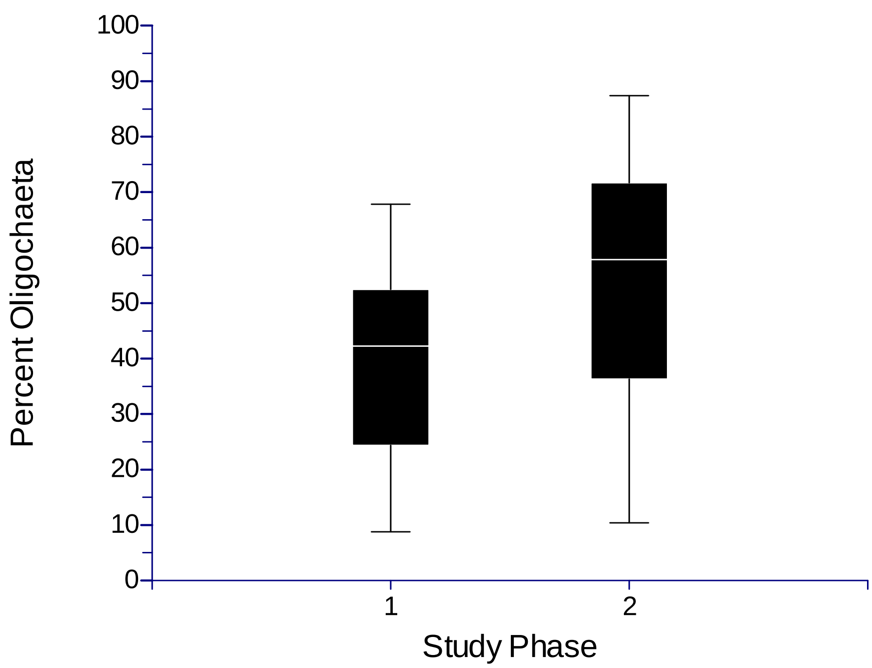


Figure 15: Medians Box plot illustrating that Oligochaeta populations are a significant component of all wetlands among the lower Missouri River Floodplain wetlands. Box area represents inner quartile range (IQR), while "whiskers" represent the upper and lower observations.

Gatherer-collectors were reported as having dominant abundance in both natural and restored wetlands, but they can experience shifts in assemblages with variations in hydroperiods (Meyer and Whiles, 2008). Gatherer-collectors, as a macroinvertebrate community structural component can reveal greater differences between sedimentation of sand or organic detritus than the measures of sand or organic detritus alone (Cooper et al. 2007). Gatherer-collectors may offer some indication of condition and quality in future wetlands studies using benthic macroinvertebrate collections to assess biological integrity.

Sixty six percent of the gatherer-collectors not Oligochaetes were Chironomidae, which is 20% of the total burrower population. Larvae of the genera *Glyptotendipes* and *Chironomus* were the most abundant of midges among the entire population of Chironomidae. Although, the proportion of Chironomidae was found to be greater in low impact wetlands in the upper Missouri River floodplain (Chipps et al. 2006), it was not found statistically determinate in our metric development process. Many researchers have reported that Chironomidae are quite tolerant of eutrophic conditions and pollution and may increase in abundance and overall biomass when wetlands are notably impaired by invasive plants, surrounding land-use practices, or isolation (Hartzell et al. 2007, Davis and Bidwell 2008, Gallardo et al. 2008, and Kulesza 2008). Furthermore, macroinvertebrate community homogeneity can indicate widespread degradation of wetlands associated with sustained agricultural development (Lougheed et al. 2008).

Despite the lack of response other metrics that contributed to the composition of this metric, percent burrowers, was demonstratively successful in delineating wetlands of high and low quality. The burrowing population responded positively to increasing measures of disturbance and negatively to increasing measures indicating improved water quality. Specifically, when maximum depth values and plant richness values rose, the burrower populations represented smaller proportions of the population. Increases in water depth can impose oxygen stress in the benthos but also flood emergent macrophyte dominated zones allowing more plant species to become established. This effect opens up more available niche habitats to more invertebrate taxa. Observations made concerning the burrower population dynamics also provide support for the positive relationships with increased herbicide concentrations and number of herbicides detected. The dominant taxa components are Oligochaetes and Chironomidae, which are both reported as being very tolerant to pollution and surrounding landscape disturbances. This metric appears to be a fundamental component for assessing wetland quality and identification of impairments.

4.4.3 Count Heavy Metal (HM) Intolerant Taxa

Approximately 53 % of the HM intolerant taxa were gatherer-collectors. About 35 % were predators and 11 % were shredders, leaving about 1 % comprised of omnivores (OM), piercers (PI) and scrapers (SC). The habit guild, sprawlers was dominant in all groups identified as HM intolerant at 58 % the total HM intolerant taxa population. Zavereliella, a genus of Chironomidae was the most sensitive taxa (sensitivity value 0) observed in this study and was found in 32 of the total 54 samples collected. The most abundant taxon *Caenis sp.*, (Ephemeroptera) was found in 50 of the 54 samples with the highest abundances (298 specimens) at sites 7476. Ephemeroptera (mayflies) are generally considered sensitive taxa and are among the first orders to disappear when waters become polluted (Applegate et al. 2007). The rarest taxa collected in this study was in the order Neuroptera and occurred in our highest scoring wetland (number 7111). A single specimen of the spongilla fly genus *Sisyra* (Sisyridae) was found at this site. Larvae (i.e. caterpillars) of the order Lepidoptera were another rare taxa group and were found only at reference sites. Aquatic caterpillars belong in the feeding guild of shredders and are considered intolerant to nutrient oxygen demanding chemicals, as well as acidic and saline conditions. High levels of iron and other heavy metals such as cadmium, copper, lead, mercury, and zinc can be directly toxic to aquatic invertebrates and though little is known about sub-lethal concentrations, it is assumed that long term exposure to trace metals can inhibit growth, reproduction, and larval development (Adamus 2001). Wetlands can store and concentrate heavy metals which can also bioaccumulate in aquatic invertebrates. Mobilization of heavy metals and invertebrate toxicity can be influenced by the acidity of a wetland and increased mortality has been observed in amphipods and mayflies exposed to high acidity and aluminum concentrations (Adamus 2001). Some wetland studies comment on the possible effects of heavy metal toxicity in wetlands and its speculative relationship to specific conductivity, but direct measures of macroinvertebrate community effects are rare (Cooper et al. 2007 and Davis and Bidwell 2008). In this study taxa intolerant to heavy metals were a significantly responsive metric and could indicate heavy metal retention among the Missouri River floodplain wetland population. Heavy metal retention by wetlands could provide ecosystem services beneficial to the greater floodplain region. Evaluation of invertebrate tolerances to elevated concentrations in conjunction with hydrogeomorphic characterization of wetlands could assist in restorations toward maximizing this function. However, internal wetland quality and ecosystem functions that provide habitat and resources for a diversity of wildlife are more common criteria for wetland assessments. Assessing wetland health on the basis of ecological benefits was the impetus for this study, and this macroinvertebrate metric responded indicates conditions of wetland quality. Heavy metal concentrations were not measured in this study and the relationship between this metric and wetland condition is unclear. Indications of wetland quality provided by this metric may have resulted from the overlap in responses of the taxa within this metric to other wetland conditions. Regardless, Heavy metal intolerant taxa were responsive in the metric evaluation process and were found in significantly higher proportions among the Phase One population than Phase Two (Figure 16).

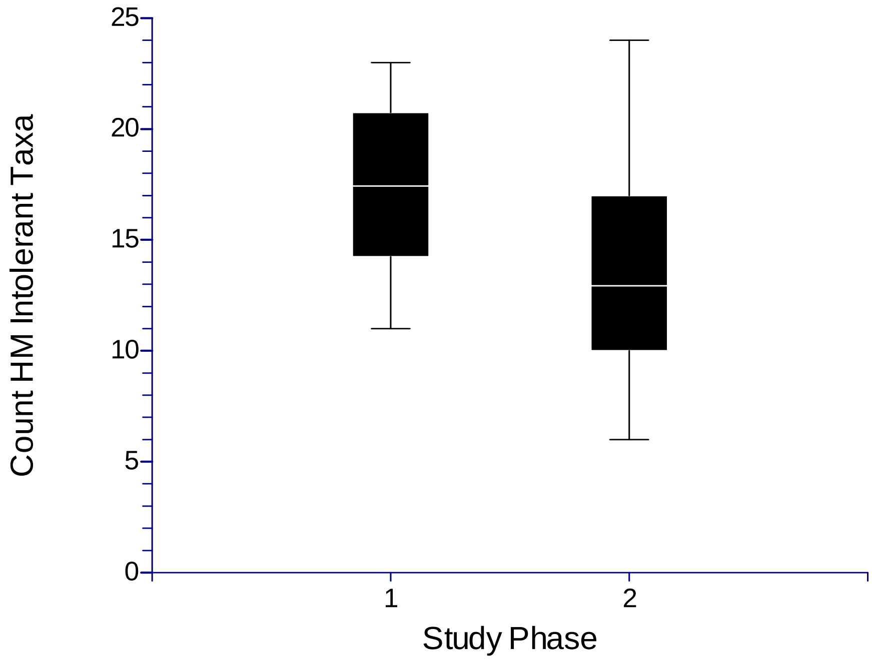


Figure 16: Median box plots showing the distribution of the count of heavy metal intolerant taxa in study Phase One and Two. Box area represents inner quartile range (IQR), while "whiskers" represent the upper and lower observations.

4.4.4 Count Suspended Solids and Sediments (SSS) Intolerant Taxa

Approximately 50% of the total suspended solids and sediments (SSS) intolerant taxa population (n=707 individuals) were mayfly larvae of the genus *Callibaetus* (n=357) that were also sensitive to agricultural pesticides and persistent organic carbons. Thirty nine of the 54 samples contained this taxon, but at relatively low numbers, the largest abundance was 66 specimen found at site 7103, followed closely by three other reference sites, 7116, 7112, 7114. Most of the SSS intolerant taxa were of the functional feeding guild gatherer-collectors (GC) followed by predators (PR), and then filterer collectors (FC). The FC guild would be expected to be the most affected group, given their food source is largely made up of suspended particulate matter in the water column. This group was comprised of three planktonic genera of mosquitoes (Culicidae). *Anopheles, Culex*,and *Uranotaenia* mosquito larvae were collected at only 11 sites, often together, but in very low numbers (1 to 8 specimens total per site). Their being planktonic in nature may have contributed to the low numbers that were collected considering that benthic areas were the target habitat in this study. Six sites having these taxa were reference sites that were considered to be of high quality. The FC taxa observed were not intolerant to any other stressors, but they were the only filterer collectors found in this study, which was a very rare guild. Overall, Culicidae were found at 21 sites with the largest total being *Culex sp.* The MMI development process did not find the percent Culicidae metric as a determinate factor between *a priori* groups.

Some insect taxa that were sensitive to suspended solids and sediments were also sensitive to other anthropogenic stressors (Table 9). Members of the mayfly family, Baetidae are among the most sensitive taxa found in this study and have a very low tolerance to anthropogenic compounds, nutrient enrichment, and acidity. As an individual indicator taxa Ephemeroptera were not found to have significant power as a metric for indicating biological integrity in other studies (Chipps 2007 and Stoddard et al. 2008). Spieles and Mitsch (2000) retained another Ephemeroptera family in the analysis between a high impacted site and low impacted sites and reporting only presence and absence data, showed that it was only found in the middle portion of the wetland. This particular genus is sensitive to NOD, AP, and HM by the criteria we established in this study but is not sensitive to SSS. Ephemeroptera and Odonata were reported in high numbers for isolated wetlands not connected to the river floodplain in a study of invertebrate community response to variable wetland hydrological connectivity (Gallardo et al. 2008). The percent ETO (Ephemeroptera, Trichoptera, and Odonata) taxa metric was able to differentiate between wetlands based on maximum depth, but not other indicators of reference conditions (i.e. mean conductivity, native plant richness, and herbicides). This particular metric is a modified version of the EPT metric commonly used for flowing waters and appears to be respond conditions of water permanence but also may indicate isolation from floodplain system. We, also we observed that Hydroptilidae are also sensitive to a wide variety of water quality stressors and contribute to many of the metrics found to delineate between the reference and random samples. The rarest observation in the Count of Intolerant Taxa to SSS metric was the Corixidae taxa (water boatmen), which can be found in deeper wetland pools in association with hydrophytes such as water lilies (*Nelumbo sp.)* or in natural sites having substantial emergent vegetation (Kulesza et al.2008, Hartzell et al. 2007). However the general consensus has been that water boatmen are considered indicators of degradation as they can occur in high numbers at sites that have been recently disturbed. As an individual metric the percent Corixidae was not retained in the final MMI, because it lacked the statistical power to separate *a priori* groups of reference and non reference condition.

Table 9: Rare taxa intolerant to suspended solids and sediments and also sensitive to other toxic compounds and water quality parameters. Scores 3 and below indicate intolerance in this study except for RTV scores. Abbreviations are FG=feeding guild, OM= omnivore, PI=piercer, HB=habitat behavior, sw=swimmer, cb=climber, SSS=suspended Solids and Sediments, RTV=regional tolerance value, NOD= nutrient and oxygen demanding compounds, AP=agricultural pesticides, HM=heavy metals, POC=persistent organic carbons, and SA=salinity and acidity.

IDCPCB	order	family	Count	FG	HB	SSS	RTV	NOD	AP	HM	POC	SA
7110	Ephemeroptera	Baetidae	2	OM	sw	2	3.5	2	1	2	1	2
7116	Trichoptera	Hydroptilidae	1	PI	cb	1	5.2	2	3	1	2	3

4.5 Interrelated Stressor Effects

4.5.1 Spatial and Temporal Effects

Many of the macroinvertebrate metrics showed response to variables that were found to have significant differences between ecoregions, which showed significant interactions with the study phase. Mean depth to flood (used as a surrogate for return period and hydrological connectivity) and mean conductivity values were statistically different between ecoregions ($p=0.00072$ and $p<0.001$, respectively) and the two variables were also found significantly related to one another having a R^2 value of 0.34 ($p=0.00002$). From previous analysis it was determined that many plant community metrics also appeared to respond to mean conductivity mS/cm, with varying degrees of response within the Western Corn Belt Plains and Central Irregular Plains. Mean conservatism for all plant species and native plant species was significantly negatively correlated (linear R^2 values of 0.32 and 0.35) to mean conductivity when the samples population was analyzed with robust regression analysis. Mean conservatism is calculated as the sum of coefficients of conservatism for each plant divided by the total number of plant taxa in a sample. Coefficients of conservatism values range from 0 to 10 as an estimated probability that a plant is likely to occur in a relatively unaltered landscape (Minc and Albert 2004). Two-factor General Linear Model (GLM) ANOVA was performed on mean plant conservatism values, and mean conductivity with ecoregion and wetland type. Both factors were found significantly different for mean plant conservatism values, but only ecoregional differences were found for mean conductivity. However further evaluation of the relationship between these parameters indicated that they were not directly related. When palustrine and lacustrine classes were evaluated the same was true of the response variables. All three variables were also significantly correlated to depth to flood with mean conductivity showing greater response in study Phase One samples, $R^2 > 0.50$. The relationship appears to be exponential and increasing distance results in less conductivity. Samples from the CIP ecoregion show significantly lower stressor-response relationship because the population of sites is further from the main channel and has higher DTF values (Figure 17). The stronger relationship observed in the study Phase One population between mean conductivity and DTF is supported by the observed significant interactions found between ecoregion and study phase in two a factor GLM ANOVA. Although a significant relationship was found between the DTF model treatment variable, mean conductivity may also be associated by vegetation density causing increased evapotransporation and plant detritus within the emergent macrophyte dominated wetlands (Mitsch and Gosselink, 2000).

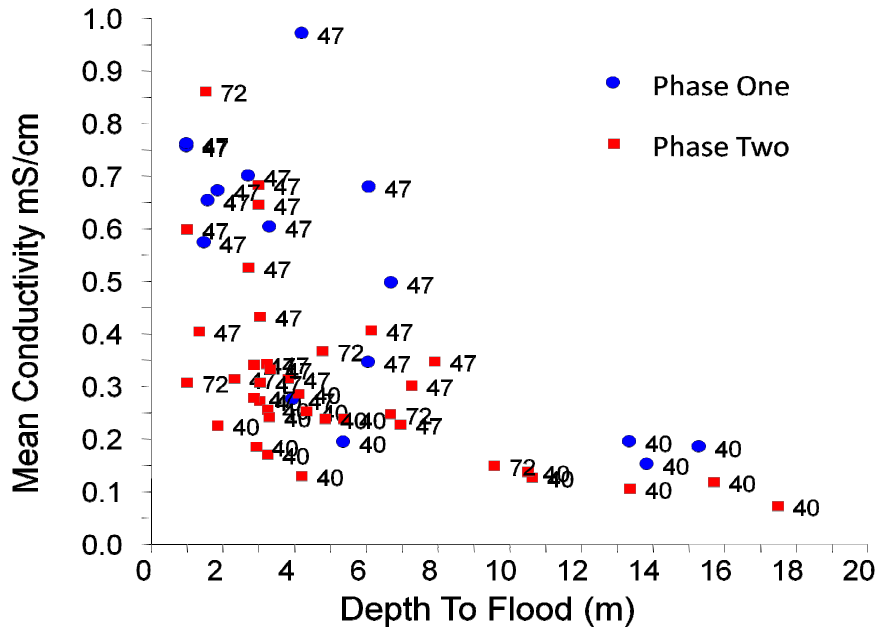


Figure 17: Scatter plot showing the relationship between mean conductivity and depth to flood; the best fit regression was determined to be exponential, expressed by the following equation: $y = 0.5324e-0.099x, R^2 = 0.4574$. The number labels represent the three different ecoregions: 40= Central Irregular Plains, 47= Western Corn Belt Plains, and 72=Interior River Valleys and Hills.

4.5.2 Effects of Wetland Types

A disproportionate number of emergent macrophyte beds (EM) are found in the Western Corn belt plain ecoregion for both the Phase One and Two sample population. Most of Phase One sites were distributed in the WCB Ecoregion with a significant proportion of them being palustrine emergent macrophyte beds. Eleven of the Phase One samples were in the Western Corn Belt Plains (WCB) and four in the Central Irregular Plains. Of those eleven seven were emergent macrophyte beds (EM), two were MIX types (combination of all three recognized types) and two were unconsolidated beds (UB). The Phase One sites in the Central Irregular Plains (CIP) Ecoregion consisted of one AB and EM, two UB's and four MIX. Though no interactions between study phases or ecoregion and type were indicated in the ANOVA, differences observed in mean conservatism measures may mostly be due to the overwhelming number of EM types observed in Phase One. The Phase Two sample population had a more even distribution of types overall: two MIX, three AB, three UB, and 13 EM types in the Western Corn belt region and one MIX, four AB, five EM, and six UB in the CIP. The differences in types of wetlands is important when we consider that many macroinvertebrate metrics were found to have statistically significant auto-correlations with plant community metrics including native species richness and mean conservatism, as well as mean conductivity. Though plant density measures were not assessed in this project, it became apparent through analysis of plant community components and water chemistry that significant differences in productivity and function of wetland responded to internal localized variability associated with wetland structure (Beury et al. 2009).

4.5.3 Spatial Distribution Effects on Metrics

When macroinvertebrate metrics and the final MMI were evaluated within each Ecoregion, Phase One samples were significantly higher for the percent Hydroptilidae metric and the Final MMI ($p = 0.016$and ($p=0.011$) in the WCB ecoregion. Other individual metrics were observed as having higher mean values and elevated distributions (opposite relation for mean percent burrowers) for Phase One samples within each ecoregion, but no significant differences in means were found. Hydroptilidae occurred at only one Phase Two site in the CIP ecoregion and could not be evaluated. It was suspected that this taxa may be restricted to the northern portion of the Lower Missouri River Floodplain, but considering that the site maintained consistent reference-like conditions based on all the assessments and measure water quality parameters, the regional effect may be arbitrary. Despite the regional variability, its occurrence in the sample population was evenly distributed between both phases and it remains clear that reference candidates have higher mean percentage of Hydroptilidae. When the CIP ecoregion was evaluated mean count HM intolerant taxa ($p=0.00067$), mean count SSS intolerant taxa ($p= 0.0061$), and the final MMI ($p=0.0011$) were all significantly higher in the Phase One population than in Phase Two. Though the KW medians tests also shows the relationships as significant, a true test of ANOVA means was not completely statistically valid here because only four Phase One sites were located in the Central irregular plains. Percent Burrowers held a lower distribution of values within the Phase One samples than the Phase Two samples but means were not statistically different. Phase One and Two differences within the ecoregion boundaries confirm that differences in individual metric and MMI scores between reference and random population are not the direct result of spatial distribution differences among wetlands.

4.5.4 Metric Response to Interrelated Stressors

The analysis of relationships between individual macroinvertebrate metrics revealed that count SSS intolerant taxa and percent Hydroptilidae significantly positively correlated (R=0.35 and 0.39 respectively) with mean conductivity having R^2 values of 0.35 and 0.39. respectively. Percent Burrowers was significantly negatively correlated (R=0.34) with mean conductivity. Mean conductivity was described by Lougheed et al. (2007) as a trophic indicator, but also a as a measure of salinity. It was used along with nutrient concentrations, land use, and hydrological impairments in their development of the wetland disturbance axis (WDA) for identifying shift points in plant, phytoplankton, and zooplankton communities of riparian wetlands in Michigan. Significant shifts in the WDA , a wetland index developed from six biological response metrics (i.e., plants, phytoplankton, etc.), were seen at above and below 350 µS/cm, with greater values indicating the site was impacted. No significant mean conductivity benchmarks were obvious in this study and the relationship of mean conductivity to many of the macroinvertebrate metrics was observed as being positive. Initial assessment showed that percent amphipods had a slightly larger R^2 relationship to mean conductivity than percent Hydroptilidae. However, the percent Hydroptilidae metric had a more significant p value, responded to the model variable DTF, and was found significant in T-test evaluation, unlike percent amphipods.

Given that individual macroinvertebrate metrics responded to stressors like mean conductivity, mean Secchi depth, and depth to flood, we have some indication that the various stressor are not only linked to one another but also cumulatively affect the macroinvertebrate population. However, the most common significant stressors for all metrics were measures of the concentration of one or more herbicides analyzed from the water samples or the number of herbicides detected in each sample. Herbicide detection remains one of our most significant measures of anthropogenic disturbance associated with agricultural practices in the surrounding landscape.

4.5.5 Metric Response to Wetland Type

When individual and MMI scores were evaluated by wetland type only count HM intolerant taxa was found to be statistically higher in aquatic bed types than emergent macrophyte beds for the Phase Two population (Figure 18). Though not statistically significant, percent burrower scores were higher in the aquatic beds than all other types while count SSS intolerant taxa scores were lower in both the aquatic bed and unconsolidated bed types for the Phase Two population. The differences reflect the structure and density of the dominant plant communities found within each wetland type. Emergent macrophyte beds consist of dense stands of cattails (*Typha sp*.), bulrush (*Shoenoplectus sp.),* and reed canary grass (*Phragmites sp.).* These plant types have been found to be tolerant to high levels of heavy metals (Baldantoni et al. 2009). Dense emergent macrophyte stands also contribute large amounts of organic matter to the benthos substrate. The observed significant response by heavy metal intolerant taxa to wetland type may be associated with higher amounts of wetland detritus containing high concentrations of heavy metals.

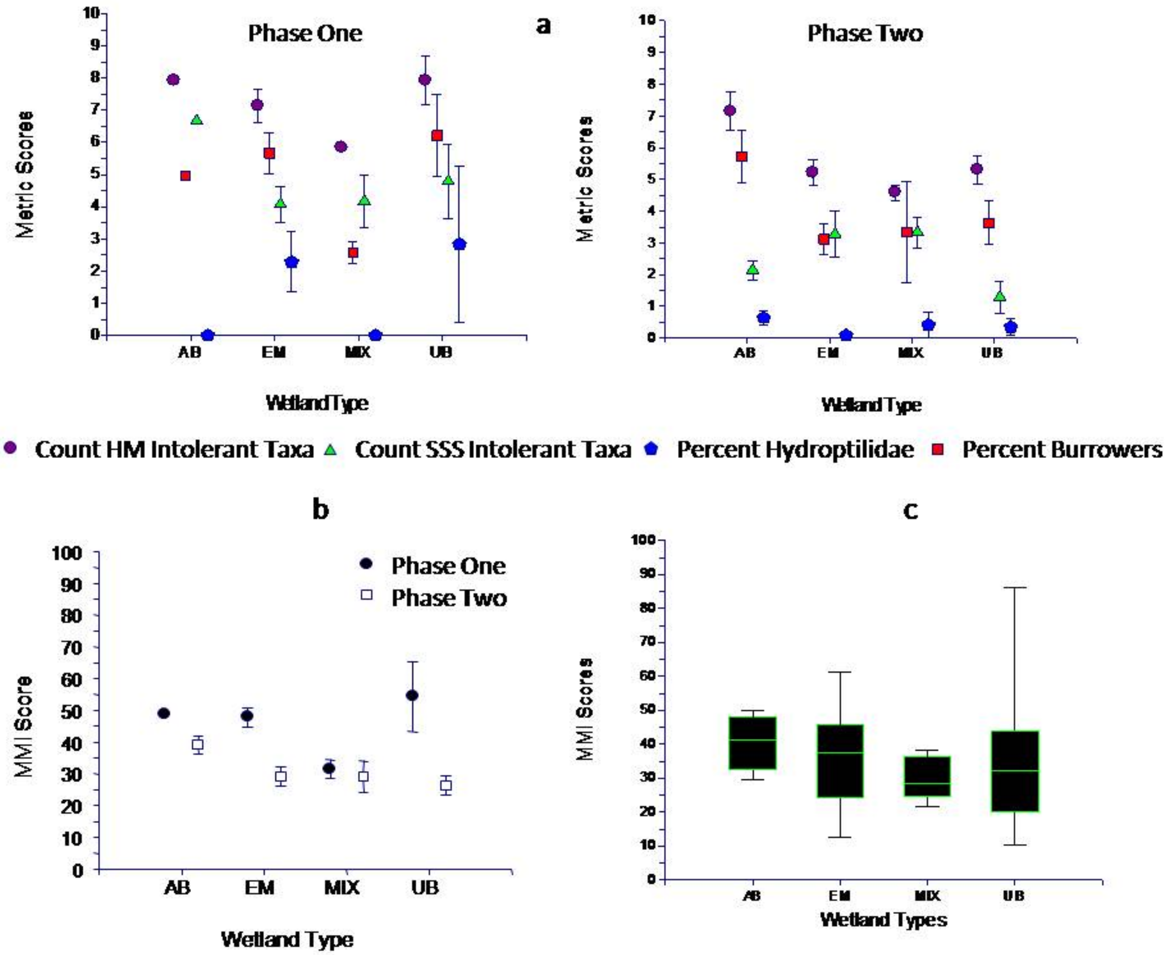


Figure 18: Graph (a) is two error-bar plots of individual metric scores for the different study phases by wetland type. Graph (b) is an error-bar plot of the final MMI scores for Phase One and two by wetland type and graph (c) is median box plot of final MMI scores for all samples by wetland type. Error bars represent standard error and box area represents inner quartile range (IQR), while "whiskers" represent the upper and lower observations.

Aquatic beds and unconsolidated beds were observed as having lower counts of intolerant SSS taxa than emergent macrophyte beds and MIX types in the Phase Two samples. Once again these differences can be associated with wetland structure as AB and UB types have larger open water zones that are more susceptible to wind shear and wave action than EM or MIX types that have vertical vegetation cover to serve as a buffer to these abiotic factors. However, Cooley Lake was the only aquatic bed type identified in Phase One showing higher values for this metric. While having a large aquatic bed habitat, it has a significant portion of emergent fringe along with a significant portion of wetland buffer.   Furthermore, it is also bermed and has a steep shore relief that also contributes to the reduced amount of disturbance to the water's surface.

In general, Phase One sites had higher individual metric scores and MMI scores than the Phase Two population. When combined, the populations indicate that aquatic bed types have the highest median score of all types, followed by EM, UB, and MIX. However, the EM and UB types had wide ranges of scores illustrating that wetland quality is not directly controlled by type. Interestingly, it was observed that the MIX type consistently scored low, not only in metric and MMI scores, but also in floristic and water quality. It is suspected that because the MIX type consisted of multiple dominant habitats and was quantifiably low in many assessment measures, showing that these wetlands are in a state of transition or significant structural disturbance.

4.5.6 Hydrological variability

Although responses to herbicides were consistent among metrics, significant differences in herbicide detection were observed between the two study phases drawing into question the validity of using the number of herbicides detected as an appropriate anthropogenic stressor indicator. Given that 2005 was reported to be a warmer dryer season, based on field crew observations and localized community feedback, it was suspected that some bias may occur when using this information (Figure 19).

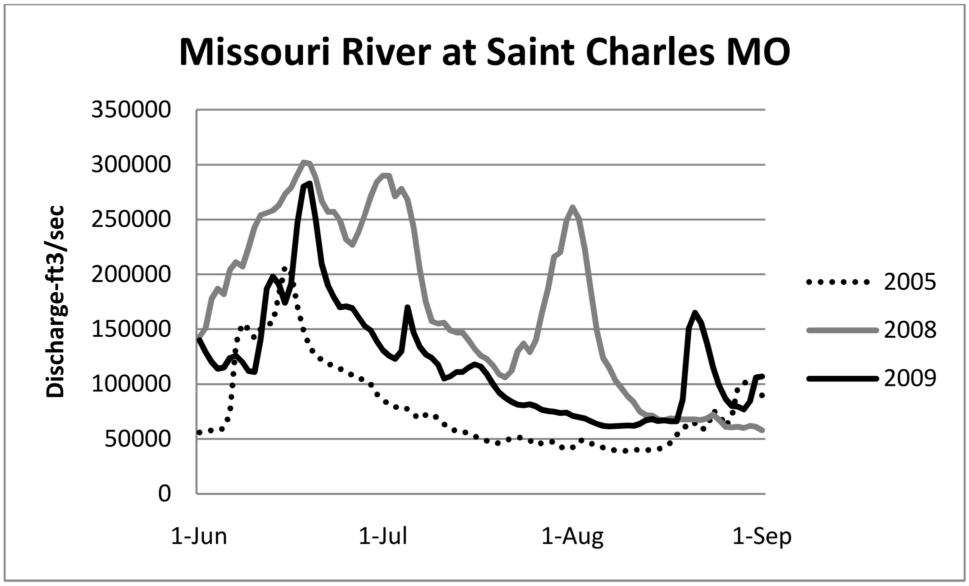


Figure 19: USGS discharge data at Saint Charles Missouri River gauging station for 2005, 2008, and 2009.

Among the random sample population four sites surveyed were visited in the preliminary reference phase of this study. There were three sites that were considered identical based on the wetland polygons surveyed. Water and macroinvertebrate samples were taken at Cooley Lake and Swan Lake for both study phases, but samples were taken only in Phase Two for Forney Lake, as water demands of the surrounding agricultural community did not permit the filling of the wetland basin. Only FQA and Disturbance Assessments were performed at this site. Significant shifts in plant communities were observed in all three wetlands and in the two where water and macroinvertebrate samples were permitted by adequate inundation, distinct differences in water depths, nutrient concentrations, and detected herbicides were observed. While water depths and detectable herbicides were higher in the Phase Two sampling season, nutrient concentrations were lower, as observed for the sample population at large. The differences in nutrient concentrations and herbicides can be attributed to hydrological effects and the variable amounts of runoff received over the survey period, which in turn caused shifts in plant communities and macroinvertebrates. Plant richness and macroinvertebrate diversity were higher at Cooley Lake in 2005 and Floristic Quality was scored slightly lower than in 2008 because of increased terrestrial succession observed in the plant community during 2005 (Table 10 and Figure 20). Despite the difference in assessment scores, macroinvertebrate community shifted considerably in many metrics that respond to hydrological differences and anthropogenic influences, such as counts of heavy metal and suspended sediment intolerant invertebrates. Given that plants and macroinvertebrates may respond to changes in hydrology and influent numbers and concentrations of pesticides, the shifts in communities may be seasonal conditions that have relatively short response times to these disturbances. A GLM ANOVA was performed on the entire sample population using factors of year, depth to flood groups (high, medium, and low), major classes and wetland types (separately) on all *in situ*, nutrient, and herbicide data. This revealed no significant differences or interactions between the factors except for the response variable atrazine concentration, with differences in both DTF groups and major classes. Yearly differences were not found but differences in concentrations among the DTF groups and the classes were observed. It was concluded that temporal shifts in weather patterns did not cause significant effect on wetland water quality or biota and that some degree of inherent variability is expected within all wetland samples.

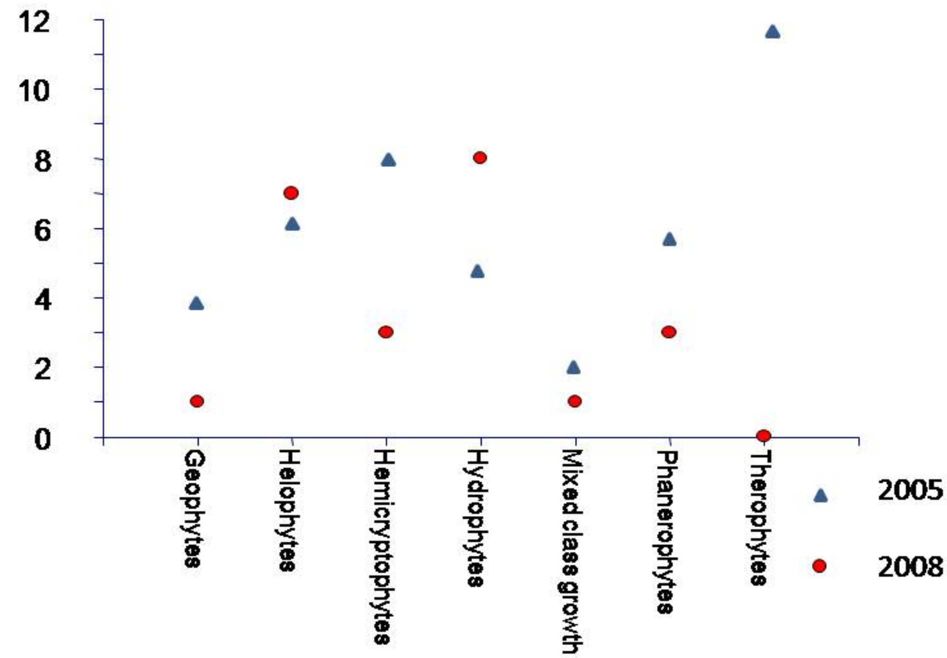


Figure 20: Scatter plot showing plant community shifts at Cooley Lake for the 2005 and 2008 survey seasons. Geophytes are plants with rhizomes, tubers, or bulbs located well below the surface of the soil; Helophytes are water or swamp plants protruding above the water surface but with submerged winter buds; Hemicryptophytes are perennial and biennial herbs and graminoids with buds located at or near surface of soil; Hydrophytes are submerged or floating aquatic plants with winter buds at the bottom; Phanerophytes are trees and tall shrubs with buds >0.25 m above ground; Therophytes are annual plants that survive unfavorable periods as seeds.

Table 10: Water quality and plant community shifts at Cooley Lake site (7117 and 7439) from 2005 reference study and 2008 random study.

Cooley Lake Data 2005 and 2008						
Parameter	2005	2008		Parameter	2005	2008
Maximum Depth (m)	1.2	1.56		Chlorophyll-a µg/L	74.7	34.37
Mean Secchi (m)	0.3	0.83		TOC mg/L	9.5	10.8
Mean Conductivity mS/cm	0.19	0.24		DOC mg/L	7.3	10.8
Mean Turbidity NTU	30.3	8		Organic C mg/L	2.2	0
Mean Dissolved Oxygen mg/L	1.13	6.55		DIA µg/L	0	0.1
NO3 + NO2 mg N/L	0.04	0		DEA µg/L	0	0.16
NH3 mg N/L	44.2	166		Simazine ug/L	0	0
NO3/NH3	0.9	0		DAR	0	0.3
Total N mg/L	2.69	0.8		Atrazine µg/L	0.66	0.61
Organic N mg/L	2.61	0.63		Metribuzin µg/L	0	0.2
Dissolved N mg/L	0.084	0.17		Alachlor µg/L	0	0.12
PO4 µgP/L	51.6	264		Metalochlor µg/L	0	0.33
Total P mg/L	554	397		Cyanazine µg/L	0	0.16
Available N:P ratio	1.63	0.65		Number of Herbicides Detected	1	7
TN:TP	4.86	2.02		DA Total	12	15
Total Plant Richness	43	23		Native Plant Richness	39	20
Metric- Count Intolerant HM taxa	7.92	5.83		MMI Score	48.86	32.13

4.6 MMI Development Process

Many researchers have reported that the ability of multiple metric indices developed to quantify biological integrity based on common selection approaches represented in this study, greatly depend on the accuracy in establishing groups (e.g. Bouchard et al. 1998, Stoddard et al. 2008). Researchers use a wide variety of approaches to characterize wetland quality in wetland condition indices, formulated from wetland features that are considered to be important stressor parameters. A number of statistical methods are used by several researchers, though many warn that index results may or may not be true measures of wetland quality because of the interdependence in classification. In this study, complex mathematical models, or multiple nonlinear approaches were not used in conjunction with water quality, landscape, or hydrological parameters to establish *a priori* wetland condition gradients. Instead, stressor-response variability was scrutinized thoroughly to identify the strongest relationships that existed between abiotic and biotic factors that influenced the macroinvertebrate component of wetland ecosystems in this study area. Furthermore, considering that varying results between studies were discovered in the literature review; it seemed appropriate that a complete investigation to determine the most indicative response metrics was needed for this population of wetlands. Observation of the overwhelming amount of variation in wetlands across this large study area, and recognition that the project purpose was to develop wetland assessment tools prompted the modification to the MMI approach to ensure that the macroinvertebrate MMI was the best indication of biological integrity for this trophic guild. Despite the variability in study designs, metrics determined to be responsive in this study were similarly reported by others as significant indicators of aquatic biological integrity, though a few novel measures of toxicity intolerance were discovered(Table 11).

Table 11: Index, metrics, and range of multiple metric indices from wetland and river quality assessment studies. EPT is Ephemeroptera, Plecoptera, and Trichoptera.

Study	Spieles and Mitsch 2000 Macroinvertebrates in Constructed Wetlands	Chipps et al. 2006 BioAssessment of Flood Plain Wetlands	J.M. Applegate et al.2007 Ohio River Macroinvertebrate Index			Stoddard et al. 2008 National Wadeable Stream Assessment	Koontz 2010 Lower Missouri River Floodplain Wetlands
Index	Invertebrate Community Index (ICI)	Wetland Condition Index (WCI)	Percent Metric Index	Panel Metric Index		National Macro-invertebrate MMI	Macro-invertebrate MMI
Metric	Percent Ephemeroptera	Chironomidae (proportion of total invertebrate abundance)	Percent Hydroptilidae	Total Number of Taxa	Percent EPT Individuals	Percent EPT Taxa	Percent Hydroptilidae
	Taxa Richness	Invertebrate Diversity (Shannon Index)	Percent Cricotopus	Total Number of individuals	Number of Ephemeroptera	Shannon Diversity Index	Percent Burrowers
	EPT Taxa	Culicidae (proportion of total invertebrate biomass)	Percent Trichoptera	Number of Diptera Taxa	Percent Hydroptilidae	Scraper Richness	Count Intolerant Taxa To Heavy Metals
	Chandler Biotic Index	Exotic Plant Species (proportion of total number of species)	Number of Diptera Taxa	Percent Diptera	Ratio of Ephemeroptera and Trichoptera to Chironomidae	Percent Burrower Taxa	Count Intolerant Taxa to Suspended Solids and Sediments
	Percent Tolerant Organisms	Total Number of Plant Species	scrapers/ (scraper+gatherer collectors)	Percent Tanypodinae	Percent Amphipoda	EPT Taxonomic Richness	
	Percent Diptera and Non-insects	Number of Sensitive Diatom Taxa		Number of EPT Taxa	Percent Tolerant Individuals	Intolerant Richness	
					Percent Oligochaeta		
Range	0-20	18-74	0-36	0-72		0-100	10-86

4.7 Macroinvertebrates as Indicators of Wetland Quality

The dynamics of aquatic insect communities within wetlands are linked to direct and indirect hydrological and plant community influences, and macroinvertebrate communities respond to floodplain connectivity and the particulate organic matter inputs (Galat et al 1998). Aquatic insects also respond to a wide variety of natural and human disturbances, such as hydrological isolation, siltation, eutrophication, acidification, anthropogenic compounds, and heavy metal toxicity. In an agricultural landscape such as the Midwestern United States, wetlands can succumb to overwhelming amounts of nutrient enrichment and herbicide contamination. Large efforts have been made to measure the long term effects of invertebrate exposure to herbicides, eutrophication, and other toxic compounds. A vast amount of information about invertebrate physiology, behavior, and toxicity has been gathered for aquatic systems. The United States Environmental Protection Agency reports extensive profiles on the fate of atrazine in our nation's surface and groundwater, as well as wetland ecosystem degradations that have been linked to elevated exposure to atrazine (USEPA 1990 and 2003). In the lower Missouri River Floodplain system we observed multiple levels of toxicity exposures and ecological impairments measured in the numbers and concentrations of herbicides and nutrient enrichments that undoubtedly have degraded the wetlands, lakes, rivers, and streams in this area. The significant responses found in the metrics used to develop the macroinvertebrate MMI also indicated other aquatic impairments that were not measured in this survey (i.e. suspended sediments and heavy metals). Traditional physical and chemical monitoring does not always capture ecosystem anthropogenic affects that could be deleterious to higher trophic orders such as fish, amphibians, birds, and mammals that consume invertebrates as a substantial portion of their diet (Cooper et al. 2007).

Understanding the chemical and energy flow throughout a watershed is essential for establishing water quality criteria, defining surface water recovery goals, and implementing best management practices. Assessing a wetland ecosystems aquatic invertebrate population can provide information of biological response to the quality of systems and identify disturbances causing impairments. Macroinvertebrate multiple metric indices are also useful in assessing mitigation efforts that require continuous monitoring to evaluate the progress of created, restored, or preserved wetlands. Given that many of the individual metric components respond to various chemical and physical factors, conditional assessments can be focused to answer specific questions about ecosystems. In this study we focused on creating an index of biological integrity that gave us the best response to multiple factors that showed significant congruency with the other assessment components. The overall goal of this project was to develop and calibrate assessment methods that can determine overall wetland health among the lower Missouri River Floodplain and quantify wetland disturbances in this region. The substantial weight of evidence found and presented here strongly indicates that the Macroinvertebrate MMI can serve as an effective tool in supporting this endeavor.

5. Conclusions

The metrics Percent Burrowers, Percent Hydroptilidae, Count Intolerant Taxa to Heavy Metals, and Count Intolerant Taxa to Suspended Solids and Sediments were found to significantly respond to *a priori* reference and nonreference groups in the development of the macroinvertebrate MMI and showed consistent congruencies with the other parameters and assessments delineating significant differences between the reference and random sample populations in this study. The final MMI developed produced scores that also reflected the relationships observed between study phase populations, ecoregions, and wetland classifications that were found in the Disturbance Assessment and plant Floristic Quality Assessment metrics. The MMI appears to be very robust, indicating gradients of wetland quality across a spectrum of conditions. The usefulness of the Percent Burrowers and Percent Hydroptilidae metrics reflected previous observations made by other researchers developing indices for biological integrity. Macroinvertebrate metrics indicating low tolerances to heavy metals and suspended solids and sediments are unique to this study and offer valuable information toward identifying wetland impairments. The use of the stressor-response method identified many significant water quality and plant community factors that contribute to overall macroinvertebrate composition. It was determined that depth to flood was not a significant factor contributing to the variation in macroinvertebrate community structure among the greater sample population, indicating that the lower Missouri River wetlands may be significantly impacted by extensive hydrological disturbance. Metrics such as Shannon's diversity index and Chironomidae richness that were not significantly responsive in the MMI development process may indicate invertebrate community homogenization caused by land development degradation.

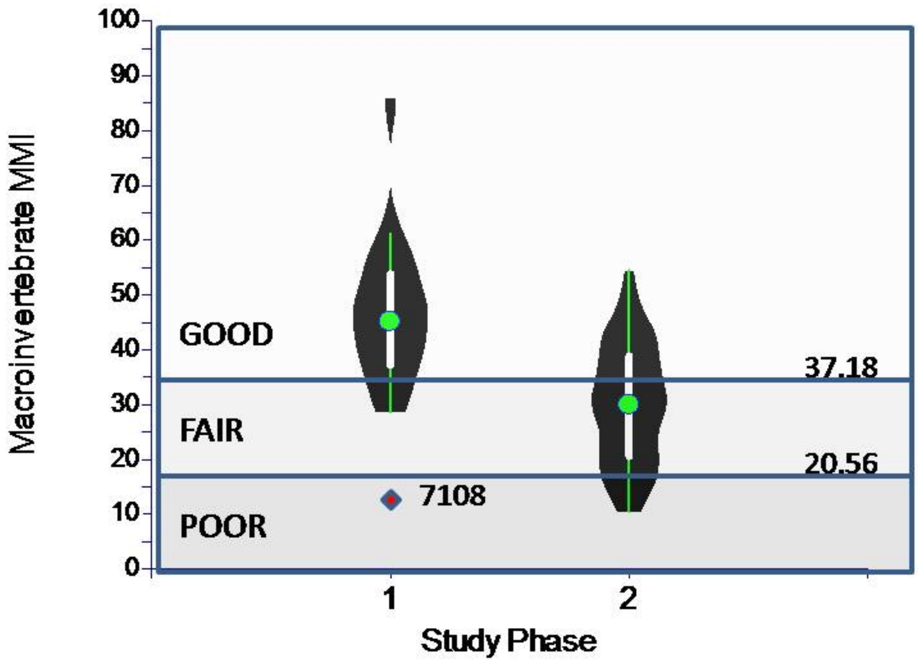


Figure 21: Violin plots of reference and random sample populations with benchmark values identifying wetland quality categories based on macroinvertebrate MMI 25th and 75th percentile values.

There is significant evidence that the reference study sample population is higher in quality than the random sample population based on the multiple assessment tools. The MMI reference and random population range overlap and can be used to indentify categories of good, fair, and poor condition using the 25th and 75th percentile ranges as benchmark values(Figure 21). All samples with macroinvertebrate MMI scores that are $\geq$ 37.18 (25th percentile of the reference population) will be regarded as wetlands in good ecological condition. A wetland in good condition is highly productive, rich in native flora, and able to support diverse macroinvertebrate communities. Samples that have scores within the 25th percentile IQR (20.56<>37.18) will be considered wetlands of fair quality and samples that are $\leq$ 20.56 will be considered wetlands of poor quality. Levels of condition can also be viewed as priority grades where wetlands of poor quality will require intensive restoration effort; whereas fair quality wetlands need only a few system adjustments to achieve good wetland quality. The

Macroinvertebrate MMI may be used to assess wetland quality of river floodplain wetlands in future studies and be regarded as a robust measure of condition because metrics were developed from intensive evaluation of the relationships between the macroinvertebrate community and all possible wetland stresses.

6. Future Implications

The results of this study support the efforts toward understanding the dynamic interplay between abiotic and biotic components in wetlands, and developing assessment methods that provide a robust measure of wetland quality. Understanding macroinvertebrate responses to various stressors, in coordination with multiple measures of wetland condition, was determined to be the best approach for metric development and validation. By focusing on metrics and an MMI that characterized wetland quality over a broad range of spatial and structural conditions, metrics found in this study are unique and offer valuable information about the lower Missouri River floodplain wetlands. The response of sensitive taxa to heavy metals and suspended sediments may indicate a necessity for quantifying these chemical parameters in future studies. The index development process and the metrics determined in this study may also be useful in other floodplain systems that support a variety of wetland types.

Energy flow and physiochemical transformation of nutrients in an ecosystem can be further explained by combining information gathered from multiple trophic groups. The measure of movement of carbon, nitrogen, and phosphorus through a wetland system is important to capture. This information allows engineers to identify conditions that encourage increased rates of productivity and storage of nutrients in the system, while sustaining a variety of higher trophic organisms. Small mammals, waterfowl, and amphibians rely on the plant and macroinvertebrate communities as a major part of their diets. Accumulation of excessive nutrients, pesticides, and heavy metals can impart toxicity toward sensitive organisms and significantly reduce the establishment of many plants, insects, mammals and birds in a wetland habitat. The presence and number of higher trophic organisms along with concentration measures of carbon, nitrogen, phosphorus, and heavy metals in wetland soils, water, and vegetation are important for identifying ecosystems as sources or sinks of toxins and excess nutrients. The success of higher trophic organisms, such as macroinvertebrates, is useful indication of overall wetland function and performance.

The relatively weak macroinvertebrate community response to the floodplain connectivity measures coupled observations that macroinvertebrate communities responded to internal structural components of these wetlands, indicates that hydrological connectivity is not a significant factor in the overall wetland structure in this region or that nearly or all study wetlands were severely disconnected and thus no differences could be observed. Isolated cases, where wetlands revealed significant positive responses to hydrological connectivity, supported the overwhelming consensus that this region is severely impacted by land-use practices and that floodplain connectivity improves wetland function and health. Efforts toward restoring the lower Missouri River watershed to historical hydrological connectedness would support natural processes of flooding, and nutrient and contaminant attenuation by floodplain wetlands.

7. References

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APPENDIX A: SITE INFORMATION (PHASE ONE AND TWO)

IDCPCB	Study Phase	Longitude	Latitude	Site Name	Date	Ecoregion Number	Ecoregion Name	County	State
7100	1	-95.02899	39.50008	Little Bean Marsh	11-Jul-05	47	Western Corn Belt Plains	Platte	MO
7101	1	-95.23602	40.0962	Squaw Creek	12-Jul-05	47	Western Corn Belt Plains	Holt	MO
7102	1	-95.26411	40.0698	Squaw Creek	12-Jul-05	47	Western Corn Belt Plains	Holt	MO
7103	1	-93.203	39.61183	Swan Lake	14-Jul-05	40	Central Irregular Plains	Chariton	MO
7104	1	-93.15128	39.60701	Swan Lake	14-Jul-05	40	Central Irregular Plains	Chariton	MO
7105	1	-93.23465	39.62194	Swan Lake	14-Jul-05	40	Central Irregular Plains	Chariton	MO
7106	1	-96.03905	41.52168	Desoto Sand Chute	21-Jul-05	47	Western Corn Belt Plains	Harrison	IA
7107	1	-96.00577	41.49416	Desoto Sand Chute	21-Jul-05	47	Western Corn Belt Plains	Pottawatt amie	IA
7108	1	-95.86308	41.29599	Big Lake	20-Jul-05	47	Western Corn Belt Plains	Pottawatt amie	IA
7109	1	-96.33112	42.30553	Browns Lake	27-Jul-05	47	Western Corn Belt Plains	Woodbur y	IA
7110	1	-96.33191	42.27663	Snyder Bend Lake	29-Jul-05	47	Western Corn Belt Plains	Woodbur y	IA
7111	1	-96.00095	41.4814	Wilson Island	26-Jul-05	47	Western Corn Belt Plains	Pottawatt amie	IA
7112	1	-96.17571	42.04803	Blue Lake	27-Jul-05	47	Western Corn Belt Plains	Monona	IA
7113	1	-96.19015	42.00844	Middle Decatur Bend	27-Jul-05	47	Western Corn Belt Plains	Monona	IA
7114	1	-96.03114	41.74194	Round Lake	26-Jul-05	47	Western Corn Belt Plains	Harrison	IA

IDCPCB	Study Phase	Longitude	Latitude	Site Name	Date	Ecoregion Number	Ecoregion Name	County	State
7115	1	-96.23383	42.00829	Tieville-Decatur Bend	28-Jul-05	47	Western Corn Belt Plains	Monona	IA
7116	1	-95.8053	40.98954	Keg Lake	04-Aug-05	47	Western Corn Belt Plains	Mills	IA
7117	1	-94.23274	39.25611	Cooley Lake	26-Aug-05	40	Central Irregular Plains	Clay	MO
7118	1	-95.24734	40.09355	Squaw creek	12-Jul-05	47	Western Corn Belt Plains	Holt	MO
7119	1	-96.11201	41.61032	Tyson Bend WMA	05-Aug-05	47	Western Corn Belt Plains	Harrison	IA
7120	1	-95.78052	40.85327	Forney Lake	20-Jul-05	47	Western Corn Belt Plains	Fremont	IA
7121	1	-96.17746	42.03449	Blue Lake	27-Jul-05	47	Western Corn Belt Plains	Monona	IA
7433	2	-95.84749	40.82027	FRW	28-Jul-08	47	Western Corn Belt Plains	Cass	NE
7434	2	-92.93709	39.0842	Big Muddy NWR	23-Jul-08	72	Interior River Valleys and Hills	Saline	MO
7435	2	-93.24189	39.57662	Bosworth Hunt Club	11-Aug-08	40	Central Irregular Plains	Chariton	MO
7436	2	-94.90613	39.75889	Browning Lake	25-Jul-08	47	Western Corn Belt Plains	Doniphan	KS
7437	2	-96.32427	42.31215	Browns Lake	30-Jul-08	47	Western Corn Belt Plains	Woodbury	IA
7438	2	-95.68838	40.3287	Bullfrog Bend	31-Jul-08	47	Western Corn Belt Plains	Nemaha	NE
7439	2	-94.23274	39.25611	Cooley Lake CA	07-Jul-08	40	Central Irregular Plains	Clay	MO
7440	2	-94.23288	39.24842	Cooley Lake CA	24-Jul-08	40	Central Irregular Plains	Clay	MO

IDCPCB	Study Phase	Longitude	Latitude	Site Name	Date	Ecoregion Number	Ecoregion Name	County	State
7441	2	-96.05734	41.57493	Cornfield NRCS	29-Jul-08	47	Western Corn Belt Plains	Harrison	IA
7442	2	-90.4699	38.73339	Crystal Springs GC	14-Aug-08	72	Interior River Valleys and Hills	Saint Louis	MO
7443	2	-93.02812	39.36448	Cut-off Lake	23-Jul-08	40	Central Irregular Plains	Chariton	MO
7444	2	-93.03012	39.37474	Cut-off Lake	23-Jul-08	40	Central Irregular Plains	Chariton	MO
7445	2	-93.03266	39.35659	Cut-off Lake	07-Jul-08	40	Central Irregular Plains	Chariton	MO
7446	2	-93.04834	39.32547	Forest Green	11-Aug-08	40	Central Irregular Plains	Chariton	MO
7447	2	-95.78646	40.85321	Forney Lake	28-Jul-08	47	Western Corn Belt Plains	Fremont	IA
7448	2	-93.25825	39.58086	Grassy Lake	12-Aug-08	40	Central Irregular Plains	Chariton	MO
7449	2	-96.13304	41.95692	Louisville Bend	29-Jul-08	47	Western Corn Belt Plains	Monona	IA
7450	2	-96.13594	41.97426	Louisville Bend	29-Jul-08	47	Western Corn Belt Plains	Monona	IA
7451	2	-92.75496	39.02148	MKT Lake	11-Aug-08	72	Interior River Valleys and Hills	Howard	MO
7452	2	-91.75686	38.70043	Mollie Dozier Chute	15-Aug-08	72	Interior River Valleys and Hills	Callaway	MO
7453	2	-95.81085	40.68384	NRCS	28-Jul-08	47	Western Corn Belt Plains	Fremont	IA
7454	2	-95.81622	40.69553	NRCS	28-Jul-08	47	Western Corn Belt Plains	Fremont	IA

IDCPCB	Study Phase	Longitude	Latitude	Site Name	Date	Ecoregion Number	Ecoregion Name	County	State
7455	2	-95.28514	40.13354	Old Channel	24-Jun-08	47	Western Corn Belt Plains	Holt	MO
7456	2	-96.21407	42.05731	casino	30-Jul-08	47	Western Corn Belt Plains	Monona	IA
7457	2	-96.43845	42.4351	S. Sioux City	30-Jul-08	47	Western Corn Belt Plains	Dakota	NE
7458	2	-93.15744	39.62371	Silver Lake	12-Aug-08	40	Central Irregular Plains	Chariton	MO
7459	2	-95.22478	40.10962	Squaw Creek NWR	24-Jun-08	47	Western Corn Belt Plains	Holt	MO
7460	2	-95.23213	40.07662	Squaw Creek NWR	24-Jun-08	47	Western Corn Belt Plains	Holt	MO
7461	2	-95.27962	40.10469	Squaw Creek NWR	23-Jun-08	47	Western Corn Belt Plains	Holt	MO
7462	2	-95.27493	40.0939	Squaw Creek NWR	23-Jun-08	47	Western Corn Belt Plains	Holt	MO
7463	2	-93.14423	39.6398	Swan Lake NWR	12-Aug-08	40	Central Irregular Plains	Chariton	MO
7464	2	-93.23518	39.62242	Swan Lake NWR	12-Aug-08	40	Central Irregular Plains	Chariton	MO
7467	2	-93.97916	39.20817	Sunshine Lake	07-Jul-09	40	Central Irregular Plains	Ray	MO
7468	2	-93.78772	39.18867	Kerr Orchard	23-Jul-09	40	Central Irregular Plains	Lafayette	MO
7469	2	-94.97184	39.4546	Lewis and Clark Wetland Reserve	22-Jul-09	47	Western Corn Belt Plains	Platte	MO
7470	2	-95.82191	41.07535	Folsom Lake	21-Jul-09	47	Western Corn Belt Plains	Mills	IA

IDCPCB	Study Phase	Longitude	Latitude	Site Name	Date	Ecoregion Number	Ecoregion Name	County	State
7471	2	-92.68753	38.98735	Franklin Island	06-Jul-09	72	Interior River V and H	Howard	MO
7472	2	-93.10271	39.40514	Trophy Room	06-Jul-09	40	Central Irregular Plains	Chariton	MO
7473	2	-93.9696	39.18112	Sunshine Lake	07-Jul-09	40	Central Irregular Plains	Ray	MO
7474	2	-94.87099	39.33801	Mud Lake	22-Jul-09	40	Central Irregular Plains	Platte	MO
7475	2	-94.88828	39.79213	French Bottoms	07-Jul-09	47	Western Corn Belt Plains	Buchanan	MO
7476	2	-95.82133	41.08235	Folsom Wetland	21-Jul-09	47	Western Corn Belt Plains	Mills	IA

APPENDIX A: SITE INFORMATION (CONTINUED)

IDCPCB	NWI Classification	Wetland Type	Wetland Class	Area Acres	Distance From Missouri River m	Distance To Nearest Wetland Site m	Nearest Site ID	Depth To Flood	Reference Class Fraction
7100	Freshwater Emergent Wetland	MIX	Palustrine	48.34	1688	7080	7469	3.97	0.54
7101	Lake	EM	Palustrine	49.34	11771	972	7118	6.72	1.00
7102	Lake	MIX	Palustrine	577.35	7844	2703	7460	6.08	0.92
7103	Freshwater Emergent Wetland	EM	Palustrine	28.65	23529	2819	7105	13.85	1.00
7104	Freshwater Emergent Wetland	UB	Palustrine	20.21	25145	2041	7458	15.32	1.00
7105	Lake	UB	Lacustrine	911.86	25145	2800	7103	13.37	0.83
7106	Freshwater Emergent Wetland	EM	Palustrine	37.45	782	4173	7107	1.50	0.94

IDCPCB	NWI Classification	Wetland Type	Wetland Class	Area Acres	Distance From Missouri River m	Distance To Nearest Wetland Site m	Nearest Site ID	Depth To Flood	Reference Class Fraction
7107	Freshwater Emergent Wetland	EM	Palustrine	9.04	1024	541	7111		
7108	Lake	MIX	Lacustrine	38.68	642	24577	7111	5.98	0.93
7109	Freshwater Emergent Wetland	MIX	Lacustrine	53.23	2814	934	7437	2.73	0.90
7110	Freshwater Emergent Wetland	EM	Palustrine	218.08	1073	3398	7109	1.00	0.91
7111	Freshwater Pond	UB	Palustrine	19.27	897	1541	7107	1.88	0.59
7112	Lake	EM	Lacustrine	184.61	5570	1602	7121	4.23	0.76
7113	Lake	EM	Lacustrine	181.77	1631	3214	7121	1.00	0.64
7114	Freshwater Emergent Wetland	EM	Palustrine	149.11	5243	16149	7119	6.10	0.63
7115	Freshwater Emergent Wetland	UB	Palustrine	202.60	475	3412	7113	1.60	0.80
7116	Freshwater Emergent Wetland	EM	Palustrine	66.91	2235	10218	7470	3.33	0.51
7117	Lake	AB	Lacustrine	152.87	2660	846	7440	5.39	0.75
7118	Freshwater Emergent Wetland	EM	Palustrine	161.73	10992	946	7101		1.00
7119		EM	Riverine	27.81	279	5983	7441		0.90
7120	Freshwater Emergent Wetland	EM	Palustrine	585.96	4050	6632	7433		0.96
7121	Lake	EM	Lacustrine	126.44	4830	1609	7112		0.75
7433	Lake	EM	Riverine	17.49	234	6223	7447	2.70	0.57
7434	Freshwater Emergent Wetland	UB	Riverine	17.40	1524	16640	7451	4.79	0.40
7435	Freshwater Emergent	UB	Palustrine	47.03	19805	1422	7448	10.64	0.92

IDCPCB	NWI Classification	Wetland Type	Wetland Class	Area Acres	Distance From Missouri River m	Distance To Nearest Wetland Site m	Nearest Site ID	Depth To Flood	Reference Class Fraction
	Wetland								
7436	Lake	MIX	Lacustrine	253.16	3180	4159	7475	2.88	0.34
7437	Lake	EM	Lacustrine	24.79	3424	960	7109	2.73	0.59
7438	Lake	AB	Lacustrine	184.75	2614	39451	7455	3.33	0.44
7439	Lake	AB	Lacustrine	152.87	2660	846	7440	5.39	0.75
7440	Lake	AB	Lacustrine	35.25	1900	846	7439	4.34	0.66
7441	Freshwater Emergent Wetland	EM	Palustrine	10.75	1781	5988	7119	3.43	0.18
7442	Freshwater Emergent Wetland	EM	Palustrine	23.56	2614	104828	7452	6.69	0.50
7443	Lake	UB	Lacustrine	22.51	3818	1002	7445	3.25	0.76
7444	Lake	EM	Lacustrine	29.07	3819	1224	7443	3.30	0.96
7445	Lake	UB	Lacustrine	66.81	3377	1002	7443	3.25	0.80
7446	Freshwater Emergent Wetland	UB	Riverine	18.52	458	3840	7445	2.95	0.46
7447	Lake	MIX	Lacustrine	585.96	4050	6632	7433	3.24	0.91
7448	Lake	UB	Lacustrine	32.76	20779	1414	7435	10.52	1.00
7449	Lake	UB	Riverine	14.46	441	2073	7450	1.00	0.71
7450	Freshwater Emergent Wetland	EM	Palustrine	6.61	449	2074	7449	2.01	0.55
7451	Lake	AB	Lacustrine	32.55	4832	6793	7471	9.58	0.97
7452	Lake	UB	Riverine	51.91	904	83863	7471	1.00	0.58
7453	Freshwater Emergent	EM	Palustrine	21.03	2050	1451	7454	3.00	0.37

IDCPCB	NWI Classification	Wetland Type	Wetland Class	Area Acres	Distance From Missouri River m	Distance To Nearest Wetland Site m	Nearest Site ID	Depth To Flood	Reference Class Fraction
	Wetland								
7454	Freshwater Emergent Wetland	EM	Palustrine	23.29	2950	1451	7453	3.00	0.57
7455	Freshwater Pond	EM	Palustrine	15.28	8808	3424	7461	7.92	0.58
7456	Freshwater Emergent Wetland	EM	Palustrine	20.83	3718	3162	7112	2.33	0.52
7457	Freshwater Pond	AB	Lacustrine	16.18	3142	16973	7437	1.34	0.47
7458	Lake	MIX	Lacustrine	53.66	25026	2014	7104	15.71	0.96
7459	Lake	EM	Palustrine	78.43	13400	1820	7101	7.92	0.87
7460	Lake	EM	Palustrine	649.24	10000	2344	7118	6.14	1.00
7461	Freshwater Emergent Wetland	EM	Palustrine	139.44	10414	1330	7462	7.27	0.86
7462	Freshwater Emergent Wetland	EM	Palustrine	17.69	9614	1330	7461	6.97	0.98
7463	Freshwater Emergent Wetland	EM	Palustrine	52.66	26926	2197	7458	17.50	0.94
7464	Lake	UB	Lacustrine	911.86	25145	2800	7103	13.37	0.83
7467	Lake	AB	Palustrine	58.14	5905	3325	7473	3.04	0.48
7468	Lake	AB	Palustrine	12.10	1242	14904	7473	4.87	0.93
7469	Lake	UB	Palustrine	16.04	1574	7123	7100	3.84	0.28
7470	Lake	UB	Lacustrine	45.14	3360	831	7476	3.05	0.66
7471	Freshwater Emergent Wetland	EM	Riverine	20.85	1208	6826	7451	1.52	0.52
7472	Freshwater Emergent	EM	Palustrine	27.01	2358	6849	7444	4.21	0.56

IDCPCB	NWI Classification	Wetland Type	Wetland Class	Area Acres	Distance From Missouri River m	Distance To Nearest Wetland Site m	Nearest Site ID	Depth To Flood	Reference Class Fraction
	Wetland								
7473	Freshwater Emergent Wetland	EM	Palustrine	56.71	3328	3325	7567	4.12	0.52
7474	Freshwater Emergent Wetland	EM	Palustrine	52.50	2992	16119	7469	1.86	0.59
7475	Freshwater Emergent Wetland	EM	Palustrine	45.14	460	4189	7473	2.88	0.47
7476	Freshwater Emergent Wetland	AB	Palustrine	105.20	3218	831	7470	3.05	0.57

APPENDIX B: PLANT COMMUNITY METRICS (FQA)

IDCPCB	Study Phase	FQI All	Richness All	FQI Natives	Richness Native	Percent Adventive	Mean Conservatism All	Mean Conservatism Natives
7100	1	17.24	21	17.66	20	4.76	3.76	3.95
7101	1	17.76	28	19.19	24	14.29	3.36	3.92
7102	1	16.88	11	16.88	11	0.00	5.09	5.09
7103	1	22.96	45	24.35	40	11.11	3.42	3.85
7104	1	24.53	42	25.46	39	7.14	3.79	4.08
7105	1	20.60	28	21.80	25	10.71	3.89	4.36
7106	1	22.12	46	22.61	44	4.35	3.26	3.41
7107	1	21.14	42	22.52	37	11.90	3.26	3.70
7108	1	15.33	36	16.80	30	16.67	2.56	3.07
7109	1	17.08	29	18.40	25	13.79	3.17	3.68
7110	1	27.73	67	29.31	60	10.45	3.39	3.78
7111	1	16.54	33	17.95	28	15.15	2.88	3.39
7112	1	19.01	55	20.14	49	10.91	2.56	2.88
7113	1	18.14	49	19.60	42	14.29	2.59	3.02
7114	1	19.78	63	21.57	53	15.87	2.49	2.96
7115	1	15.02	66	16.60	54	18.18	1.85	2.26
7116	1	16.05	42	17.58	35	16.67	2.48	2.97

IDCPCB	Study Phase	FQI All	Richness All	FQI Natives	Richness Native	Percent Adventive	Mean Conservatism All	Mean Conservatism Natives
7117	1	21.50	43	22.58	39	9.30	3.28	3.62
7118	1	22.55	38	23.17	36	5.26	3.66	3.86
7119	1	10.21	28	11.02	24	14.29	1.93	2.25
7120	1	12.02	40	13.44	32	20.00	1.90	2.38
7121	1	16.96	46	17.96	41	10.87	2.50	2.80
7433	2	10.87	22	11.70	19	13.64	2.32	2.68
7434	2	19.45	32	20.43	29	9.38	3.44	3.79
7435	2	19.72	30	20.78	27	10.00	3.60	4.00
7436	2	16.36	13	17.03	12	7.69	4.54	4.92
7437	2	14.23	10	15.00	9	10.00	4.50	5.00
7438	2	19.75	16	19.75	16	0.00	4.94	4.94
7439	2	22.10	23	23.70	20	13.04	4.61	5.30
7440	2	22.39	22	23.48	20	9.09	4.77	5.25
7441	2	12.33	38	14.36	28	26.32	2.00	2.71
7442	2	24.74	59	25.62	55	6.78	3.22	3.45
7443	2	13.00	9	13.79	8	11.11	4.33	4.88
7444	2	16.36	13	17.03	12	7.69	4.54	4.92
7445	2	12.06	11	13.33	9	18.18	3.64	4.44
7446	2	10.22	23	12.25	16	30.43	2.13	3.06
7447	2	16.26	18	16.26	18	0.00	3.83	3.83
7448	2	13.42	5	13.42	5	0.00	6.00	6.00
7449	2	9.43	18	11.09	13	27.78	2.22	3.08
7450	2	11.67	47	13.15	37	21.28	1.70	2.16
7451	2	18.48	12	18.48	12	0.00	5.33	5.33
7452	2	10.58	7	11.43	6	14.29	4.00	4.67
7453	2	15.74	38	16.89	33	13.16	2.55	2.94
7454	2	16.55	45	17.34	41	8.89	2.47	2.71
7455	2	12.41	48	14.54	35	27.08	1.79	2.46
7456	2	20.88	58	22.26	51	12.07	2.74	3.12
7457	2	21.36	27	21.36	27	0.00	4.11	4.11
7458	2	14.67	9	15.56	8	11.11	4.89	5.50
7459	2	16.49	17	17.00	16	5.88	4.00	4.25
7460	2	19.72	30	20.78	27	10.00	3.60	4.00
7461	2	16.58	21	17.91	18	14.29	3.62	4.22
7462	2	18.57	29	20.41	24	17.24	3.45	4.17
7463	2	21.17	36	21.17	36	0.00	3.53	3.53
7464	2	17.49	11	17.49	11	0.00	5.27	5.27
7467	2	21.91	31	23.93	26	16.13	3.94	4.69
7468	2	26.11	33	26.94	31	6.06	4.55	4.84
7469	2	19.50	16	19.50	16	0.00	4.88	4.88
7470	2	11.40	17	12.56	14	17.65	2.76	3.36
7471	2	15.89	35	17.16	30	14.29	2.69	3.13
7472	2	23.58	29	24.44	27	6.90	4.38	4.70

IDCPCB	Study Phase	FQI All	Richness All	FQI Natives	Richness Native	Percent Adventive	Mean Conservatism All	Mean Conservatism Natives
7473	2	18.46	66	20.23	55	16.67	2.27	2.73
7474	2	25.98	48	27.14	44	8.33	3.75	4.09
7475	2	15.90	38	17.89	30	21.05	2.58	3.27
7476	2	17.83	29	18.48	27	6.90	3.31	3.56

APPENDIX B: PLANT COMMUNITY (CONTINUED)

IDCPCB	Geo-phytes	Helo-phytes	Hemi-cryptophytes	Hydro-phytes	Phanero-phytes	Thero-phytes	Mixed class growth habit
7100	0	3	4	5	2	5	2
7101	2	8	2	2	4	8	2
7102	0	3	1	5	1	0	1
7103	3	15	6	1	1	12	7
7104	2	11	7	5	7	7	3
7105	1	6	3	5	1	10	2
7106	11	10	12	3	5	2	3
7107	4	10	12	6	3	4	3
7108	4	10	5	3	3	9	2
7109	4	7	4	5	1	6	2
7110	7	15	11	7	6	17	4
7111	6	6	7	9	0	4	1
7112	13	10	13	0	7	9	3
7113	10	12	9	4	2	7	5
7114	12	10	13	5	7	12	4
7115	13	6	12	4	5	20	6
7116	5	8	7	1	3	15	3
7117	4	6	8	5	6	12	2
7118	8	8	11	0	1	7	3
7119	4	3	3	0	3	12	3
7120	2	9	3	1	1	20	4
7121	10	12	12	0	6	5	1
7433	2	3	3	0	4	8	2
7434	4	4	5	0	15	4	0
7435	5	7	5	1	5	6	1
7436	0	2	1	8	0	0	2
7437	0	1	0	8	0	1	0
7438	0	6	1	7	0	1	1
7439	1	7	3	8	3	0	1
7440	0	5	4	9	1	2	1

IDCPCB	Geo-phytes	Helo-phytes	Hemi-cryptophytes	Hydro-phytes	Phanero-phytes	Thero-phytes	Mixed class growth habit
7441	4	6	9	0	1	17	1
7442	7	11	13	4	11	10	3
7443	0	1	3	2	2	0	1
7444	0	1	3	5	2	0	2
7445	0	1	4	2	3	0	1
7446	2	1	6	0	6	8	0
7447	1	6	2	6	2	1	0
7448	0	0	2	3	0	0	0
7449	1	5	2	1	0	7	2
7450	10	4	13	0	4	11	5
7451	0	4	0	7	0	0	1
7452	0	1	1	2	3	0	0
7453	7	6	5	0	8	11	1
7454	5	8	11	2	9	8	2
7455	4	4	4	0	3	28	5
7456	13	10	14	2	3	11	5
7457	4	3	6	4	4	6	0
7458	0	2	1	4	1	0	1
7459	2	2	5	3	1	3	1
7460	2	7	3	3	4	9	2
7461	2	6	1	3	5	2	2
7462	2	5	2	3	4	11	2
7463	7	10	8	2	2	5	2
7464	0	3	3	3	1	0	1
7467	3	6	4	10	2	4	2
7468	6	11	6	5	3	1	1
7469	3	4	4	3	1	1	0
7470	1	4	1	3	1	6	1
7471	3	2	5	0	6	15	4
7472	3	9	4	3	6	3	1
7473	8	9	13	1	3	26	6
7474	3	7	8	8	9	10	3
7475	3	6	8	0	6	11	4
7476	2	3	4	8	3	8	1

APPENDIX C: DISTURBANCE ASSESSMENT SCORES AND FORM

IDCPCB	Study Phase	Attributes	Reference	Disturbance	Total
7100	1	11	5	1	15
7101	1	12	3	4	11
7102	1	12	4	3	13
7103	1	9	4	2	11
7104	1	9	3	2	10
7105	1	11	3	2	12
7106	1	9	3	1	11
7107	1				
7108	1	13	0	2	11
7109	1	10	3	2	11
7110	1	11	3	0	14
7111	1	10	2	2	10
7112	1	11	2	2	11
7113	1	12	3	1	14
7114	1	11	2	3	10
7115	1	13	4	2	15
7116	1	9	3	3	9
7117	1	11	3	2	12
7118	1	12	4	1	15
7119	1				
7120	1	12	3	3	12
7121	1				
7433	2	9	3	3	9
7434	2	9	2	2	9
7435	2	10	0	3	7
7436	2	9	1	1	9
7437	2	11	1	2	10
7438	2	7	1	4	4
7439	2	14	4	3	15
7440	2	12	4	2	14
7441	2	6	0	3	3
7442	2	10	4	1	13
7443	2	8	0	5	3
7444	2	10	0	3	7
7445	2	9	0	3	6
7446	2	9	1	3	7
7447	2	13	4	4	13
7448	2	11	0	4	7
7449	2	9	2	4	7
7450	2	8	1	0	9
7451	2	13	3	2	14
7452	2	6	1	5	2

IDCPCB	Study Phase	Attributes	Reference	Disturbance	Total
7453	2	9	0	2	7
7454	2	7	0	3	4
7455	2	7	0	3	4
7456	2	7	0	2	5
7457	2	7	0	2	5
7458	2	12	4	1	15
7459	2	12	2	3	11
7460	2	12	2	4	10
7461	2	13	4	2	15
7462	2	9	4	1	12
7463	2	10	4	2	12
7464	2	13	4	3	14
7467	2	11	3	3	11
7468	2	11	3	1	13
7469	2	8	0	3	5
7470	2	7	1	1	7
7471	2	9	3	1	11
7472	2	11	3	2	12
7473	2	11	3	1	13
7474	2	12	3	5	10
7475	2	11	0	6	5
7476	2	12	2	0	14

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)=	

APPENDIX D: WATER QUALITY PARAMETERS
(IN SITU)

IDCPCB	Study Phase	Mean Depth m	Maximum Depth m	Mean Secchi m	Mean pH	Mean Cond mS/cm	Mean Turbidity NTU	Mean Dissolved Oxygen mg/L	Mean Temp C
7100	1	0.48	0.51	0.13	7.07	0.274	200.3	1	26.3
7101	1	0.3	0.38	0.04	8.3	0.496	315	10.9	31.1
7102	1	0.67	0.73	0.31	7.24	0.345	145.3	4.11	29.1
7103	1	0.37	0.44	0.31	7.25	0.151	217	6.58	31.3
7104	1	0.57	0.72	0.14	7.41	0.184	165	4.03	27.6
7105	1	0.32	0.4	0.11	7.22	0.194	241	5.51	29.5
7106	1	0.71	0.87	0.29	8.29	0.572	72.7	4.63	28.6
7107	1	0.5	0.58	0.5	7.98	0.539	54	2.71	28.1
7108	1	0.27	0.3	0.16	7.9	0.6	799	4.73	30.1
7109	1	1.41	2.1	0.28	8.84	0.699	63.3	11.22	26.3
7110	1	1.52	1.95	0.76	8.31	0.76	22.7	6.9	24.4
7111	1	0.91	1.01	0.67	8.28	0.671	7	1.88	24.9
7112	1	0.44	0.55	0.27	7.79	0.97	53	5.17	18.2
7113	1	0.49	0.63	0.33	8.35	0.755	57.7	6.72	23.6
7114	1	0.45	0.5	0.45	7.99	0.678	60.7	9.75	26.7
7115	1	1.64	2.46	0.33	9.31	0.652	64	10.63	25.5
7116	1	0.7	1.4	0.34	8.38	0.602	47	6.81	27.9
7117	1	1.2	1.2	0.3	7.14	0.193	30.3	1.13	23.5
7434	2	0.36	0.44	0.14	8.11	0.367	121	6.46	26.6
7435	2	0.48	0.79	0.09	7.67	0.126	223	9.07	25.5
7436	2	1.36	1.99	0.55	7.73	0.341	31	3.51	25.1
7437	2	0.5	0.58	0.58	7.6	0.525	24	3.14	27.8
7438	2	0.94	2.02	0.8	7.36	0.332	10	3.32	25.7
7439	2	0.95	1.56	0.83	7.59	0.238	8	6.55	27.4
7440	2	0.8	1.12	0.67	7.04	0.252	8	1.53	26.1
7442	2	0.24	0.3	0.3	7.13	0.247	9	2.19	25.1
7443	2	0.56	0.85	0.14	8.24	0.255	126	9.69	29.7
7444	2	0.53	0.65	0.13	7.45	0.241	107	4.38	28.4
7445	2	0.68	1.05	0.23	8.38	0.17	93	10.75	30.3
7446	2	0.6	1	0.24	7.85	0.185	132	8.11	27.7
7447	2	1.05	1.15	0.76	7.33	0.342	13	4.93	29
7448	2	0.65	1.05	0.09	7.65	0.137	161	8.29	26.5
7449	2	0.4	0.68	0.33	8.39	0.598	67	9	29.6
7451	2	0.85	2	0.33	6.96	0.149	59	5.9	26.8
7452	2	1.06	1.78	0.33	7.44	0.307	73	5.06	26.8
7453	2	0.34	0.62	0.27	7.61	0.645	109	5.95	27.3
7454	2	0.26	0.42	0.1	7.44	0.683	113	2.63	25.4
7456	2	0.35	0.51	0.26	7.15	0.314	28	2.67	27.1
7457	2	1.73	2.98	2.82	9.31	0.404	3	9.63	28.5
7458	2	0.79	1.02	0.08	7.34	0.118	242	5.24	24.6

IDCPCB	Study Phase	Mean Depth m	Maximum Depth m	Mean Secchi m	Mean pH	Mean Cond mS/cm	Mean Turbidity NTU	Mean Dissolved Oxygen mg/L	Mean Temp C
7459	2	0.52	0.7	0.18	6.99	0.347	150	0.38	22.2
7460	2	0.2	0.3	0.21	7.32	0.406	18	3.69	21.8
7461	2	0.36	1.2	0.36	8.3	0.301	8	10.76	31.9
7462	2	0.28	0.5	0.32	9.25	0.227	21	9.21	27.8
7463	2	0.3	0.3	0.12	5.59	0.072	56	1.11	26
7464	2	1.22	1.75	0.27	6.88	0.105	54	5.9	25.6
7467	2	0.42	0.6	0.26	8.01	0.272	78	6.39	32.7
7468	2	0.49	0.98	0.72	8.11	0.238	12	5.17	23.5
7469	2	0.26	0.37	0.34	8.62	0.314	68	11.9	29.8
7470	2	2.08	4.2	0.81	8.5	0.432	27	5.95	24.5
7471	2	0.13	0.28	0.18	7.54	0.86	45	8.69	27
7472	2	0.37	0.45	0.38	7.29	0.129	75	7.1	29.8
7473	2	0.7	1.2	0.75	8.04	0.285	17	4.52	26.9
7474	2	0.37	0.65	0.35	9.23	0.225	36	10.86	27.7
7475	2	0.11	0.2	0.16	9.53	0.278	122	12	33.5
7476	2	0.71	2	0.69	7.43	0.307	25	3.4	20.4

APPENDIX D: WATER QUALITY CONTINUED (NUTRIENTS)

IDCPCB	NO3+NO2 mg-N/L	NH3 ug-N/L	TOTAL N mg-N/L	PO4 ug-P/L	TOTAL P ug-P/L	Available N:P	TN:TP	Chloro-phyll a ug/L	TOC mg/L	DOC mg/L
7100	0.05	164	3.79	614	1185	0.35	3.20	71.00	20.90	19.30
7101	0.04	36.6	3.71	82.5	2030	0.93	1.83	156.90	15.50	14.40
7102	< 0.01	26.8	1.61	29.2	271	0.92	5.94	43.60	15.40	6.60
7103	0.02	23.5	1.97	12.2	238	3.57	8.28	61.30	19.90	16.60
7104	0.01	47.8	1.18	9.2	186	6.28	6.34	47.10	8.60	8.00
7105	0.03	72.3	1.67	8.6	496	11.90	3.37	61.00	8.90	8.40
7106	0.11	71.2	1.53	27.7	130	6.54	11.77	47.10	10.40	7.80
7107	< 0.01	160	1.5	19.8	156	13.13	9.62	31.40	10.90	8.20
7108	0.59	195	3.66	75	435	10.47	8.41	291.40	13.70	13.60
7109	0.07	37.5	1.44	18.8	123	5.72	11.71	53.80	9.30	6.10
7110	0.13	49.1	0.96	36.1	83.1	4.96	11.55	16.40	9.30	6.10
7111	0.04	193	2.01	428	672	0.54	2.99	29.90	5.10	3.80
7112	0.1	425	1.48	13.5	127	38.89	11.65	37.40	20.30	20.20
7113	0.03	68.6	1.66	16.4	114	6.01	14.56	36.60	7.30	6.00
7114	0.03	77.2	0.96	154	242	0.70	3.97	14.00	7.10	6.30
7115	0.09	67.5	1.61	33.9	98.6	4.65	16.33	82.20	9.70	4.50
7116	0.03	40	2.19	30.2	132	2.32	16.59	63.50	8.10	6.90

IDCPCB	NO3+NO2 mg-N/L	NH3 ug-N/L	TOTAL N mg-N/L	PO4 ug-P/L	TOTAL P ug-P/L	Available N:P	TN:TP	Chloro-phyll a ug/L	TOC mg/L	DOC mg/L
7117	0.04	44.2	2.69	51.6	554	1.63	4.86	74.70	9.50	7.30
7434	0.02	50.6	1.2	65.7	356	1.07	3.37	67.24	8.20	6.40
7435	< 0.01	24.8	0.8	31.8	267	0.94	3.00	62.01	10.10	8.70
7436	0.02	42.3	1.05	50.2	197	1.24	5.33	42.33	6.72	6.63
7437	< 0.01	39.2	1	13	86.7	3.40	11.53	32.87	8.10	7.90
7438	0.01	42.7	0.69	593	842	0.09	0.82	17.18	8.90	7.20
7439	< 0.01	166	0.8	264	397	0.65	2.02	34.37	10.80	10.80
7440	< 0.01	30.6	0.91	115	396	0.31	2.30	31.00	10.00	9.20
7442	< 0.01	79.6	1.3	72.3	384	1.17	3.39	15.69	14.20	12.10
7443	< 0.01	44.2	1.25	28.7	262	1.71	4.77	84.42	10.50	10.00
7444	0.13	116	1.38	33.9	330	7.26	4.18	28.02	12.00	10.10
7445	< 0.01	48	1.26	30.7	249	1.73	5.06	62.01	9.60	8.10
7446	< 0.01	22.8	0.61	34.2	145	0.81	4.21	27.64	8.40	7.10
7447	< 0.01	44.2	0.86	251	270	0.20	3.19	38.10	11.00	10.00
7448	0.05	58.9	1.08	57.1	316	1.91	3.42	68.48	11.00	8.80
7449	0.03	49.9	0.96	48.4	209	1.65	4.59	28.39	7.80	7.00
7451	< 0.01	52.4	1.17	36.6	153	1.57	7.65	44.08	9.40	7.40
7452	0.04	55.9	0.75	35.7	136	2.69	5.51	24.28	7.40	6.80
7453	< 0.01	536	1.53	45.7	224	11.84	6.83	36.23	11.80	11.10
7454	< 0.01	555	1.38	18.9	129	29.63	10.70	17.18	7.90	7.10
7456	0.01	52.5	1.13	52.6	269	1.19	4.20	24.65	16.40	14.50
7457	0.02	42.5	0.39	6.9	16.3	9.06	23.93	2.99	5.60	5.40
7458	< 0.01	63.4	0.84	63.5	442	1.08	1.90	28.02	10.40	8.80
7459	0.1	84.7	1.31	137	413	1.35	3.17	13.07	10.00	9.90
7460	0.05	74.5	1.78	2630	3710	0.05	0.48	19.42	20.56	16.66
7461	0.05	65.8	1.87	391	1120	0.30	1.67	10.46	20.74	17.93
7462	0.03	36.6	1.37	218	588	0.31	2.33	13.82	15.90	13.11
7463	< 0.01	45.1	2.38	241	1200	0.21	1.98	171.83	14.50	11.60
7464	< 0.01	18.9	0.74	44.5	149	0.54	4.97	20.54	7.60	7.30
7467	0.1	35.6	0.74	54.5	203	2.49	3.65	10.50	6.60	6.30
7468	0.02	38.3	0.67	12.3	85.2	4.74	7.86	0.70	6.80	5.90
7469	0.01	35.4	1.16	269	425	0.17	2.73	21.30	11.00	10.00
7470	0.01	85.2	1.1	6.9	53.5	13.80	20.56	25.80	8.20	8.10
7471	0.06	67.7	1.03	167	256	0.76	4.02	4.10	9.00	8.20
7472	0.03	32.2	0.66	135	391	0.46	1.69	8.60	6.80	6.00
7473	0.06	196	0.82	90	185	2.84	4.43	7.80	6.50	5.60
7474	< 0.01	35.4	1	31	97.5	1.30	10.26	7.00	12.00	9.80
7475	0.02	44.3	2.91	106	590	0.61	4.93	11.60	19.00	14.00
7476	0.02	117	1.28	31.3	204	4.38	6.27	2.20	9.20	9.20

APPENDIX D: WATER QUALITY CONTINUED (HERBICIDES)

IDCPCB	Desiso-propyl-atrazine ug/L	Desethyl-atrazine ug/L	Sima-zine ug/L	DAR	Atra-zine ug/L	Metri-buzin ug/L	Alach-lor ug/L	Metol-achlor ug/L	Cyana-zine ug/L	Herbi-cides Detected
7100	0	3.38	0	1.33	2.93	0	0	0	0	2
7101	0	0	0	0.00	0	0	0	0	0	0
7102	0	0	0	0.00	0	0	0	0	0	0
7103	0	0	0	0.00	0	0	0	0	0	0
7104	0	0	0	0.00	0	0	0	0.23	0	1
7105	0	0	0	0.00	6.11	0	0	0.08	0	2
7106	0	0	0	0.00	0	0	0	0.5	0	1
7107	0	0	0	0.00	0	0	0	0.45	0	0
7108	0	0	0	0.00	0	0	0	0	0	0
7109	0	0	0	0.00	0	0	0	0	0	0
7110	0	0	0	0.00	0	0	0	0	0	0
7111	0	0	0	0.00	0	0	0	0	0	0
7112	0	0	0	0.00	0	0	0	0	0	0
7113	0	0	0	0.00	0.29	0	0	0	0	1
7114	0	0	0	0.00	1.35	0	0	0.02	0	2
7115	0	0	0	0.00	0	0	0	0	0	0
7116	0	0	0	0.00	0.82	0	0	0.04	0	2
7117	0	0	0	0.00	0.66	0	0	0	0	1
7434	0.09	0.2	0	0.28	0.813	0.188	0.109	0.437	0.139	7
7435	0.12	0.17	0	0.78	0.25	0.46	0.19	0.29	0.16	7
7436	0.07	0.15	0	0.34	0.514	0.186	0.468	0.154	0.135	7
7437	0.09	0.18	0	0.35	0.595	0.19	0.1	0.077	0	6
7438	0.09	0.22	0	0.22	1.128	0.188	0.104	0.718	0.142	7
7439	0.1	0.16	0	0.30	0.605	0.195	0.118	0.328	0.158	7
7440	0.08	0.15	0	0.51	0.335	0.203	0.103	0.15	0.148	7
7442	0.3	0.12	0	1.42	0.097	0.193	0.102	0.075	0	6
7443	0.09	0.21	0	0.41	0.59	0.189	0.105	0.137	0.157	7
7444	0.09	0.16	0	0.48	0.386	0.194	0.11	0.148	0.164	7
7445	0.08	0.19	0	0.25	0.872	0.205	0.132	0.188	0.17	7
7446	0.24	0.15	0	0.42	0.414	0.197	0.103	0.173	0	6
7447	0.09	0.14	0	0.51	0.313	0.188	0.098	0.104	0	6
7448	0.08	0.17	0	0.75	0.26	0.202	0.109	0.107	0.137	7
7449	0.08	0.11	0	1.13	0.112	0.185	0.109	0.075	0	6
7451	0.09	0.2	0	0.96	0.239	0.207	0.116	0.08	0	6
7452	0.15	0.2	0	0.90	0.255	0.202	0.106	0.182	0	6
7453	0.2	0.14	0	0.74	0.217	0.203	0.115	0.091	0.118	7
7454	0.1	0.17	0	0.76	0.256	0.192	0.113	0.094	0.152	7
7456	0.18	0.4	0	0.15	3.075	0.188	0.104	0.082	0.199	7
7457	0.09	0.13	0	0.97	0.154	0.206	0.11	0.075	0.302	7
7458	0.11	0.15	0	0.72	0.24	0.203	0.107	0.135	0.171	7
7459	0.08	0.27	0	0.14	2.151	0.18	0.127	0.104	0.148	7

IDCPCB	Desiso-propyl-atrazine ug/L	Desethyl-atrazine ug/L	Sima-zine ug/L	DAR	Atra-zine ug/L	Metri-buzin ug/L	Alach-lor ug/L	Metol-achlor ug/L	Cyana-zine ug/L	Herbi-cides Detected
7460	0.1	0.33	0	0.28	1.333	0.18	0.106	0.121	0.146	7
7461	0.15	0.13	0	0.51	0.295	0.19	0.098	0.093	0.154	7
7462	0.09	0.17	0	0.35	0.555	0.44	0.192	0.235	0.181	7
7463	0.29	0.17	0	1.14	0.172	0.201	0.101	0.131	0	6
7464	0.13	0.23	0	0.27	0.984	0.201	0.102	0.337	0.147	7
7467	0.054	0.104	0	0.23	0.522	0.048	0.006	0.006	0.04	3
7468	0.054	0.1	0.066	0.47	0.244	0.07	0.024	0.01	0.064	5
7469	0.064	0.074	0	0.99	0.086	0.104	0	0.016	0.062	5
7470	0.124	0.268	0	0.44	0.702	0.1	0.016	0.026	0.05	5
7471	0.034	0.02	0	0.61	0.038	0.052	0	0.01	0.064	3
7472	0.204	0.418	0.222	0.19	2.498	0	0	0.246	0.1	6
7473	0.056	0.096	0	0.11	0.97	0.054	0.092	0	0.17	6
7474	0	0.07	0.014	0.28	0.284	0.076	0.014	0	0.038	3
7475	0.194	0.516	0	0.34	1.75	0.054	0.002	0.114	0	5
7476	0.072	0.13	0.042	0.38	0.394	0.08	0	0.016	0.048	4

APPENDIX E: MACROINVERTEBRATE METRICS AND MMI SCORES

IDCPCB	Study Phase	Taxa Richness	Chiro-nomidae Taxa Richness	Chiro-nomidae Total Abundance	Margalef's Index	Shannon's Index (H')	Chiro-nomidae Margalef's Index	Chiro-nomidae Shannon's Index (H')
7100	1							
7101	1	36	12	108	5.56	0.87	2.35	0.50
7102	1	38	11	36	6.40	0.86	2.79	0.82
7103	1	50	11	51	7.93	1.30	2.54	0.85
7104	1	40	18	75	6.17	0.92	3.94	1.01
7105	1	56	14	60	8.84	1.14	3.18	0.92
7106	1	33	17	158	5.11	0.95	3.16	0.94
7107	1	41	14	171	6.41	1.05	2.53	0.95
7108	1	27	7	64	4.12	0.56	1.44	0.61
7109	1	36	9	23	5.47	0.70	2.55	0.84
7110	1	40	12	49	6.11	1.10	2.83	0.95
7111	1	49	15	92	7.81	1.24	3.10	0.88
7112	1	48	15	95	7.85	1.30	3.07	0.96
7113	1	31	11	116	4.73	1.01	2.10	0.74
7114	1	54	14	46	8.56	1.10	3.40	0.94
7115	1	38	16	143	6.09	1.12	3.02	0.88
7116	1	47	18	146	7.50	1.20	3.41	0.91

IDCPCB	Study Phase	Taxa Richness	Chiro-nomidae Taxa Richness	Chiro-nomidae Total Abundance	Margalef's Index	Shannon's Index (H')	Chiro-nomidae Margalef's Index	Chiro-nomidae Shannon's Index (H')
7117	1	46	11	134	7.25	1.24	2.04	0.74
7434	2	38	16	356	5.87	1.03	2.55	0.77
7435	2	33	16	34	5.07	0.73	4.25	1.10
7436	2	41	15	317	6.36	0.93	2.43	0.37
7437	2	33	13	49	5.23	0.72	3.08	0.84
7438	2	44	13	93	6.80	1.06	2.65	0.57
7439	2	47	17	138	7.25	1.07	3.25	0.93
7440	2	40	13	44	6.17	0.78	3.17	0.98
7442	2	23	7	45	3.72	0.51	1.58	0.44
7443	2	27	13	79	4.36	0.61	2.75	0.74
7444	2	30	13	148	4.68	0.83	2.40	0.60
7445	2	25	10	41	3.90	0.46	2.42	0.70
7446	2	34	18	220	5.22	0.96	3.15	0.96
7447	2	35	17	185	5.52	0.89	3.06	0.84
7448	2	50	22	137	7.81	1.29	4.27	1.13
7449	2	42	14	114	6.61	1.28	2.74	0.90
7451	2	48	20	114	7.43	1.15	4.01	1.12
7452	2	30	14	89	4.86	0.78	2.90	0.98
7453	2	48	12	79	7.45	0.96	2.52	0.79
7454	2	53	11	65	8.74	1.26	2.40	0.48
7456	2	43	16	195	6.76	1.14	2.84	0.78
7457	2	43	15	272	6.57	1.08	2.50	0.56
7458	2	40	11	33	6.41	0.91	2.86	0.90
7459	2	20	4	9	3.07	0.53	1.37	0.57
7460	2	35	13	97	5.71	0.83	2.62	0.82
7461	2	36	11	62	5.69	0.89	2.42	0.76
7462	2	44	17	82	6.88	0.90	3.63	0.93
7463	2	58	16	129	9.22	1.39	3.09	1.01
7464	2	33	13	120	5.13	0.87	2.51	0.88
7468	2	42	14	49	6.68	1.07	3.34	0.98
7469	2	40	21	101	6.37	0.90	4.33	1.07
7470	2	33	18	61	5.23	0.88	4.14	1.14
7471	2	33	11	77	5.29	0.88	2.30	0.70
7472	2	40	12	106	6.41	1.17	2.36	0.89
7473	2	38	10	60	6.09	1.20	2.20	0.63
7474	2	40	16	110	6.33	1.19	3.19	1.04
7475	2	44	17	120	7.02	1.24	3.34	0.98
7476	2	36	17	40	5.61	0.77	4.34	1.03

APPENDIX E: METRICS (CONTINUED)

IDCPCB	Percent dominant taxa	percent dominant 3 taxa	percent Amphi-poda	percent chiro-nomidae	percent coleop-tera	percent Cori-xidae	percent Culi-cidae	percent diptera
7101	46.75	47.50	14.29	20.04	0.93	0.56	0.00	20.59
7102	58.46	60.92	6.46	11.08	1.23	1.54	0.31	13.23
7103	4.37	23.49	0.21	10.60	7.07	0.21	0.00	13.72
7104	48.03	57.89	0.18	13.44	1.25	0.00	0.00	17.38
7105	41.58	46.93	1.78	11.88	2.77	0.00	1.19	18.61
7106	37.52	57.71	0.19	30.10	0.00	1.52	0.00	30.67
7107	42.69	43.66	0.58	33.33	1.17	0.19	0.19	41.33
7108	73.24	73.24	1.99	11.57	1.63	3.98	0.00	13.02
7109	62.94	75.13	1.00	3.84	2.34	0.50	0.00	7.51
7110	16.39	36.66	22.47	8.28	1.01	0.00	0.00	15.03
7111	1.07	11.59	22.53	19.74	0.64	0.00	0.00	24.46
7112	22.86	31.16	7.54	23.87	2.01	1.26	0.00	35.18
7113	22.07	44.31	19.26	20.32	1.05	0.53	0.00	21.89
7114	37.55	38.98	16.73	9.39	2.24	0.20	0.20	13.06
7115	4.61	39.17	1.15	32.95	0.23	0.00	0.46	45.16
7116	3.70	23.26	0.22	31.74	0.87	0.22	0.43	35.65
7117	30.91	33.54	4.44	27.07	4.65	0.20	1.21	34.75
7434	5.32	6.42	0.00	65.32	1.28	22.39	0.00	66.61
7435	23.27	29.09	0.55	6.18	0.00	54.00	0.00	9.82
7436	14.07	16.48	0.19	58.70	2.41	0.93	0.00	59.44
7437	64.24	68.43	5.30	10.82	0.88	0.00	0.00	17.00
7438	26.48	51.34	3.40	16.64	0.89	0.00	0.00	23.08
7439	25.17	26.57	10.31	24.13	0.70	0.00	0.52	26.05
7440	57.37	57.55	0.18	7.91	15.83	0.00	0.18	15.29
7442	74.80	74.80	0.00	12.20	0.81	0.00	0.81	18.16
7443	68.64	68.89	0.00	20.31	0.00	0.00	0.00	22.62
7444	46.83	46.83	0.00	30.27	0.20	6.95	0.00	41.51
7445	79.44	79.87	0.64	8.78	0.21	1.71	0.00	9.42
7446	44.68	45.59	0.00	39.64	0.54	3.60	0.00	43.06
7447	46.50	52.65	0.00	39.28	0.42	0.00	1.06	44.37
7448	15.41	33.83	0.00	25.75	0.19	7.52	0.00	38.72
7449	18.99	25.45	10.10	23.03	8.28	0.20	0.00	35.15
7451	32.97	52.87	2.33	20.43	1.08	0.00	0.00	23.12
7452	62.50	64.54	0.00	22.70	0.00	0.00	0.00	23.72
7453	52.28	52.46	0.73	14.39	3.83	2.55	1.64	23.50
7454	24.02	24.02	2.87	16.97	13.05	11.75	6.79	30.03
7456	22.55	22.95	0.00	38.92	0.20	0.00	0.20	63.87
7457	6.83	10.67	20.50	45.33	0.00	0.00	0.17	49.00
7458	26.09	67.73	2.52	7.55	3.89	5.49	0.23	10.30
7459	62.81	63.02	24.79	1.86	1.45	0.00	0.00	3.51

IDCPCB	Percent dominant taxa	percent dominant 3 taxa	percent Amphi-poda	percent chiro-nomidae	percent coleop-tera	percent Cori-xidae	percent Culi-cidae	percent diptera
7460	55.32	58.96	0.26	25.19	2.34	1.30	0.00	34.03
7461	45.53	46.17	2.13	13.19	1.49	0.43	0.00	14.89
7462	52.03	54.93	0.00	15.86	5.80	0.39	0.00	17.21
7463	20.21	24.33	0.00	26.60	1.65	1.44	1.86	48.45
7464	51.67	57.56	0.20	23.58	0.79	8.64	0.00	26.13
7468	24.46	55.84	6.71	10.61	3.90	0.22	0.00	15.80
7469	42.64	67.69	0.00	22.20	1.54	0.22	0.00	25.27
7470	25.77	65.64	10.79	13.44	0.00	0.22	0.00	16.30
7471	48.12	48.36	0.00	18.08	5.16	0.00	2.58	44.37
7472	36.16	36.84	0.00	24.26	2.52	1.37	0.00	33.87
7473	7.09	32.49	0.00	13.73	1.37	4.58	0.00	24.71
7474	24.95	33.62	17.34	23.26	1.48	0.00	0.00	25.16
7475	17.11	32.24	0.00	26.32	2.63	0.00	0.22	55.92
7476	7.45	65.88	7.25	7.84	0.39	0.59	0.00	9.22

APPENDIX E: METRICS (CONTINUED)

IDCPCB	percent Hydro-philidae	Percent Hydrop-tilidae	Percent leeches	percent Libel-lulidae	Percent Non Insect taxa	Percent Oligo-chaeta	Percent less than mean RTV
7101	0.56	0.00	0.00	0.00	16.67	46.75	14.88
7102	0.31	0.00	0.00	3.08	23.68	58.46	56.25
7103	3.53	0.00	0.00	4.16	16.00	4.37	61.72
7104	0.00	0.00	0.00	10.39	12.50	48.03	77.54
7105	0.59	0.00	0.00	8.51	17.86	41.58	72.29
7106	0.00	1.14	0.00	0.00	15.15	37.52	75.97
7107	0.39	0.39	0.00	0.97	14.63	42.69	72.00
7108	0.00	0.00	0.72	0.00	22.22	73.24	50.00
7109	0.50	0.00	0.00	0.17	16.67	62.94	83.84
7110	0.51	0.34	0.00	0.00	15.00	16.39	80.21
7111	0.21	1.72	0.00	0.00	16.33	1.07	60.31
7112	0.50	0.00	0.00	1.01	10.42	22.86	68.24
7113	0.00	1.23	0.00	0.00	19.35	22.07	78.21
7114	0.20	0.00	0.20	0.00	20.37	37.55	47.06
7115	0.00	0.23	0.00	0.23	10.53	4.61	85.48
7116	0.22	0.43	0.00	0.22	8.51	3.70	68.85
7117	1.21	0.00	0.00	4.85	15.22	30.91	86.93
7434	0.00	0.00	0.00	0.00	18.42	5.32	24.92
7435	0.00	0.00	0.36	0.00	24.24	23.27	71.83

IDCPCB	percent Hydro-philidae	Percent Hydrop-tilidae	Percent leeches	percent Libel-lulidae	Percent Non Insect taxa	Percent Oligo-chaeta	Percent less than mean RTV
7436	0.19	0.00	0.56	0.74	26.83	14.07	90.71
7437	0.00	0.22	0.44	0.00	18.18	64.24	44.83
7438	0.36	0.18	0.00	4.83	20.45	26.48	83.98
7439	0.17	0.00	0.00	28.85	17.02	25.17	57.64
7440	0.00	0.00	0.00	3.24	12.50	57.37	47.06
7442	0.00	0.00	0.00	1.08	17.39	74.80	10.20
7443	0.00	0.00	0.00	0.26	18.52	68.64	84.62
7444	0.20	0.00	0.00	0.61	16.67	46.83	54.79
7445	0.21	0.00	0.43	0.00	36.00	79.44	89.19
7446	0.36	0.00	0.18	0.00	11.76	44.68	60.20
7447	0.00	0.21	0.00	0.42	8.57	46.50	35.32
7448	0.19	0.00	0.00	0.19	20.00	15.41	75.74
7449	0.61	0.00	0.00	0.40	21.43	18.99	44.44
7451	0.00	0.18	0.18	3.41	14.58	32.97	80.69
7452	0.00	0.00	0.00	0.26	20.00	62.50	34.23
7453	2.37	0.00	0.18	0.18	14.58	52.28	42.72
7454	11.23	0.00	0.00	0.78	13.21	24.02	11.39
7456	0.20	0.00	0.00	2.99	20.93	22.55	20.71
7457	0.00	0.00	0.00	4.33	16.28	6.83	61.03
7458	0.69	0.00	0.00	0.92	20.00	26.09	89.40
7459	0.00	0.00	0.62	0.00	35.00	62.81	35.29
7460	0.52	0.00	0.00	0.00	22.86	55.32	40.95
7461	0.64	0.00	0.21	1.70	22.22	45.53	30.56
7462	0.19	0.00	0.58	2.13	15.91	52.03	39.22
7463	0.62	0.00	0.41	1.65	20.69	20.21	48.20
7464	0.39	0.00	0.00	0.00	18.18	51.67	61.97
7468	0.00	0.22	0.22	1.52	19.05	24.46	75.81
7469	0.44	0.22	0.00	0.22	5.00	42.64	77.10
7470	0.00	0.44	0.00	0.66	12.12	25.77	91.21
7471	2.35	0.00	0.00	0.94	6.06	48.12	39.78
7472	0.23	0.00	0.00	7.78	17.50	36.16	48.78
7473	0.23	0.00	0.00	2.97	23.68	7.09	61.65
7474	0.00	0.00	0.42	1.90	22.50	24.95	67.57
7475	1.97	0.00	0.00	3.29	6.82	17.11	57.75
7476	0.00	0.20	0.00	4.12	13.89	7.45	93.70

APPENDIX E: METRICS (CONTINUED)

IDCPCB	percent collector filterers	percent omni-vores	percent predators	percent scrapers	percent shred-ders	count parasitic taxa
7101	0.74	1.30	24.68	5.01	2.41	0
7102	0.62	0.31	14.46	5.54	4.00	0
7103	0.42	8.33	31.88	11.25	7.71	0
7104	2.33	5.20	28.49	0.36	1.97	0
7105	1.39	5.15	23.76	5.74	5.94	0
7106	6.48	0.00	13.90	0.76	13.52	0
7107	1.36	0.00	25.93	1.56	15.01	0
7108	0.00	0.00	19.53	0.72	3.62	1
7109	0.50	0.00	12.69	1.84	1.17	0
7110	0.34	0.34	28.21	3.72	3.04	0
7111	0.86	0.00	20.60	24.89	10.30	0
7112	0.25	0.00	39.95	5.03	2.26	0
7113	0.18	0.00	12.78	3.85	12.78	0
7114	0.61	0.82	24.90	3.47	4.69	1
7115	2.53	0.00	25.12	0.69	12.44	0
7116	0.65	0.00	40.65	0.22	17.17	0
7117	1.21	0.00	32.53	1.21	15.96	0
7434	8.99	0.18	44.40	0.73	3.12	0
7435	0.18	1.27	62.55	3.27	1.27	0
7436	0.19	3.52	11.11	9.07	50.56	1
7437	0.00	0.00	20.18	0.22	0.89	1
7438	0.18	0.18	30.41	5.37	0.72	0
7439	4.72	8.04	39.69	1.57	1.92	0
7440	0.00	0.36	19.78	0.36	16.73	0
7442	1.08	0.00	17.89	1.36	1.08	0
7443	0.00	2.57	10.54	4.11	10.28	0
7444	0.20	0.82	20.86	0.00	14.52	0
7445	0.43	3.43	5.14	3.64	4.50	1
7446	1.98	0.00	12.61	0.36	11.53	1
7447	0.42	0.00	14.65	1.27	2.55	0
7448	0.38	2.64	44.26	1.88	7.34	0
7449	3.03	0.00	37.37	8.89	4.24	0
7451	2.51	0.00	21.15	3.94	6.45	0
7452	0.26	0.00	15.05	4.08	9.18	0
7453	0.36	0.91	15.48	8.01	1.64	1
7454	2.61	4.18	32.38	4.70	0.78	0
7456	1.40	0.00	32.34	5.99	3.99	0
7457	1.00	0.00	29.33	0.67	1.17	0
7458	0.23	4.12	17.62	0.00	2.06	0
7459	0.00	0.00	6.00	0.62	1.86	2

IDCPCB	percent collector filterers	percent omni-vores	percent predators	percent scrapers	percent shred-ders	count parasitic taxa
7460	0.52	0.78	15.06	0.26	4.94	0
7461	0.00	2.13	10.64	28.72	1.70	1
7462	0.97	0.00	18.76	4.64	7.35	1
7463	2.27	0.62	44.74	2.06	2.68	2
7464	0.00	0.00	17.68	1.57	16.70	0
7468	0.43	0.87	19.05	1.08	4.98	1
7469	5.71	0.00	13.41	2.64	3.30	0
7470	0.88	0.00	11.23	1.32	2.42	0
7471	3.76	0.00	30.52	0.00	1.88	0
7472	0.00	0.00	28.83	5.26	8.92	0
7473	0.00	3.20	49.66	6.18	2.06	0
7474	0.00	0.00	36.58	4.44	3.17	1
7475	4.39	0.00	38.38	0.22	2.41	0
7476	0.78	0.00	20.59	1.18	1.37	0

APPENDIX E: METRICS (CONTINUED)

IDCPCB	count scraper taxa	percent bur-rowers	percent clingers	percent sprawlers	percent swimmers
7101	2	48.42	1.30	19.67	17.63
7102	3	61.85	6.77	12.31	10.46
7103	4	10.00	2.50	35.83	17.71
7104	1	52.69	0.36	37.28	1.97
7105	3	51.68	1.19	26.73	6.53
7106	2	49.33	7.24	31.62	2.48
7107	3	66.47	5.65	14.81	2.14
7108	1	77.03	1.45	9.95	7.41
7109	2	67.78	0.83	22.37	2.67
7110	3	23.65	2.70	30.41	29.22
7111	4	8.80	9.01	19.96	23.82
7112	2	40.95	1.26	28.14	19.10
7113	3	34.85	3.50	31.70	22.07
7114	4	40.82	2.04	8.78	35.31
7115	1	19.35	18.89	42.63	3.92
7116	1	26.52	6.30	26.74	8.70
7117	2	44.04	1.62	15.15	14.95
7434	3	45.87	1.10	17.06	22.75

IDCPCB	count scraper taxa	percent bur-rowers	percent clingers	percent sprawlers	percent swimmers
7435	2	30.55	0.55	10.18	54.00
7436	5	70.37	3.33	13.33	1.30
7437	1	74.06	1.33	15.52	7.76
7438	6	37.03	0.00	43.83	6.98
7439	4	47.90	0.35	36.19	5.42
7440	1	67.27	20.14	8.63	1.62
7442	2	81.84	0.00	10.57	2.44
7443	4	81.75	0.26	11.57	0.77
7444	0	78.73	0.61	10.84	9.61
7445	3	87.37	0.86	5.35	3.21
7446	2	64.50	0.72	7.21	9.19
7447	2	74.10	4.03	17.41	1.49
7448	5	43.13	3.77	38.23	9.60
7449	2	35.56	1.01	20.81	18.79
7451	5	46.59	6.09	31.90	5.56
7452	3	72.96	4.85	11.48	2.30
7453	2	66.30	6.56	2.91	8.38
7454	2	45.95	0.78	4.96	26.11
7456	6	61.28	0.60	23.95	0.60
7457	2	17.83	1.33	21.17	21.33
7458	0	30.21	2.06	54.23	10.76
7459	1	63.98	1.86	6.21	25.05
7460	1	72.92	10.94	11.72	2.08
7461	5	58.09	2.34	5.32	2.13
7462	5	59.19	12.38	8.70	1.35
7463	5	52.27	1.86	30.17	6.40
7464	3	64.83	8.45	11.98	10.22
7468	2	35.28	4.98	37.23	13.20
7469	1	52.53	1.32	33.19	1.10
7470	2	32.82	1.10	46.48	11.23
7471	0	81.46	1.88	4.23	6.57
7472	4	57.67	7.09	19.22	6.18
7473	4	17.39	1.83	57.21	6.86
7474	3	31.50	2.54	34.67	22.62
7475	1	59.65	0.88	25.66	5.70
7476	4	10.39	1.18	73.53	9.41

APPENDIX E: FINAL METRIC AND MMI SCORES

IDCPCB	metric score count Hm intolerant taxa	metric score % burrowers	metric score intolerant SSS	metric score % hydrop-tilidae	MMI Score
7101	6.25	4.46	2.50	0.00	33.02
7102	5.83	2.92	5.00	0.00	34.39
7103	8.33	8.86	5.83	0.00	57.56
7104	7.08	3.97	3.33	0.00	35.96
7105	8.75	4.08	7.50	0.00	50.84
7106	5.42	4.35	0.83	6.66	43.15
7107	6.67	2.39	5.00	2.27	40.82
7108	3.75	1.18	0.83	0.00	14.41
7109	5.83	2.24	3.33	0.00	28.52
7110	7.50	7.29	5.00	1.97	54.4
7111	9.58	8.99	5.83	10.00	86.02
7112	8.75	5.31	4.17	0.00	45.57
7113	4.58	6.01	3.33	7.14	52.67
7114	7.50	5.33	4.17	0.00	42.49
7115	6.25	7.78	2.50	1.34	44.69
7116	9.17	6.96	5.83	2.53	61.24
7117	7.92	4.96	6.67	0.00	48.86
7434	5.00	4.75	1.67	0.00	28.54
7435	4.17	6.50	0.00	0.00	26.68
7436	4.17	1.95	2.50	0.00	21.53
7437	5.42	1.52	0.83	1.29	22.65
7438	7.08	5.76	2.50	1.04	40.97
7439	5.83	4.52	2.50	0.00	32.13
7440	6.25	2.30	3.33	0.00	29.71
7442	3.33	0.63	2.50	0.00	16.16
7443	4.17	0.64	1.67	0.00	16.19
7444	4.17	0.99	1.67	0.00	17.05
7445	3.33	0.00	0.83	0.00	10.42
7446	4.58	2.62	0.83	0.00	20.08
7447	5.00	1.52	3.33	1.24	27.72
7448	7.92	5.06	0.00	0.00	32.45
7449	5.83	5.93	5.83	0.00	43.99
7451	10.00	4.67	2.50	1.04	45.53
7452	5.42	1.65	0.83	0.00	19.75
7453	7.50	2.41	8.33	0.00	45.61
7454	7.08	4.74	10.00	0.00	54.56
7456	5.42	2.99	2.50	0.00	27.26
7457	7.08	7.96	1.67	0.00	41.77
7458	4.58	6.54	4.17	0.00	38.23
7459	2.50	2.68	0.00	0.00	12.94

IDCPCB	metric score count Hm intolerant taxa	metric score % burrowers	metric score intolerant SSS	metric score % hydrop-tilidae	MMI Score
7460	3.33	1.65	0.00	0.00	12.47
7461	3.33	3.35	0.83	0.00	18.8
7462	6.25	3.23	2.50	0.00	29.94
7463	7.50	4.02	5.00	0.00	41.29
7464	4.17	2.58	1.67	0.00	21.03
7468	5.42	5.96	0.83	1.26	33.68
7469	7.08	3.99	0.83	1.28	32.96
7470	6.67	6.24	0.00	2.57	38.69
7471	3.75	0.68	5.83	0.00	25.65
7472	5.42	3.40	4.17	0.00	32.46
7473	5.42	8.01	1.67	0.00	37.73
7474	5.42	6.39	1.67	0.00	33.69
7475	7.50	3.17	5.00	0.00	39.18
7476	8.33	8.81	1.67	1.14	49.88

APPENDIX F: LABORATORY MEASUREMENTS AND ANALYSES

Laboratory measurements and analyses

Parameters	Container	Instrument/Method	Method Citation	Detection Limit	Holding Time	Preservation
Total Phosphorus	1L Amber Glass	Persulfate digestion @ 250^{o}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^{o}C
Total Nitrogen	1L Amber Glass	Persulfate digestion @ 250^{o}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^{o}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^{o}C

Laboratory measurements and analyses						
Parameters	**Container**	**Instrument/Method**	**Method Citation**	**Detection Limit**	**Holding Time**	**Preservation**
Nitrate-N	1L Amber Glass	Automated cadmium reduction method using flow injection analyzer (Lachat QuikChem 8500)	20^{th} Ed. Standard Methods (4500-NO_3^- F)	0.01 mg/L	48 hours	4^oC
Nitrite-N	1L Amber Glass	Colorimetric method using automated flow injection analyzer (Lachat QuikChem 8500)	20^{th} Ed. Standard Methods (4500-NO_2^- B)	0.01 mg/L	48 hours	4^oC
Chlorophyll *a*	1L Amber Glass	Optical Tech. Devices, Ratio-2 System Filter Fluorometer	20^{th} Ed. Standard Methods (10200-H)	1.0 µg/L	30 days	4^oC
Atrazine	1L Amber Glass	Gas Chromatography/Mass Spectrometry	Thurman *et al*. 1990	0.05 µg/L	7 days	4^oC
Alachlor	1L Amber Glass	Gas Chromatography/Mass Spectrometry	Thurman *et al*. 1990	0.05 µg/L	7 days	4^oC
Metolachor	1L Amber Glass	Gas Chromatography/Mass Spectrometry	Thurman *et al*. 1990	0.05 µg/L	7 days	4^oC
Cyanazine	1L Amber Glass	Gas Chromatography/Mass Spectrometry	Thurman *et al*. 1990	0.1 µg/L	7 days	4^oC
TOC/DOC	1L Amber Glass	Shimadzu TOC Analyzer (TOC-5000A)	20^{th} Ed. Standard Methods (5310 B)	0.1 mg/L	7 days	4^oC, add H_3PO_4 pH < 2