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Evaluating the Success of Wetland Mitigation in West Michigan: A Macroinvertibrate-Based Approach

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EVALUATING THE SUCCESS OF WETLAND MITIGATION IN WEST MICHIGAN: A MACROINVERTEBRATE-BASED APPROACH

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ABSTRACT

EVALUATING THE SUCCESS OF WETLAND MITIGATION IN WEST MICHIGAN: A MACROINVERTEBRATE-BASED APPROACH

By James Neal

Michigan's wetlands, and their invaluable natural services, were declining at an alarming rate; therefore, the process of wetland mitigation was introduced in 1979 to offset this loss. From 2003-2006, a series of mitigated wetlands were installed to compensate for the wetlands removed during the construction of the M-6 highway south of Grand Rapids, MI. The objectives of my research were to determine whether these man-made wetlands function similarly to natural wetlands in terms of biological integrity, further develop methods for a macroinvertebrate-based index of biological integrity for inland wetlands, and provide pragmatic suggestions for wetland mitigation practices. Macroinvertebrate, plant, and water samples from mitigated and natural wetlands were collected. Macroinvertebrate samples were applied to appropriate bioassessment indices developed by Uzarski et al. (2009) while plant samples were applied to analyses developed by the Michigan Department of Natural Resources (Herman et al. 2001) to indicate biological integrity. Floristic quality analyses yielded varying results, yet the

mitigated and reference wetlands were statistically similar in terms of invasive plant coverage, floristic quality index, and species richness. The macroinvertebrate index of biological integrity scores for the reference wetlands indicated significantly healthier ecosystems than their mitigated counterparts. The macroinvertebrate and plant bioassessments yielded different results, which is concerning since most agencies use floristic quality assessments as the primary means to evaluate wetlands. This divergence can be attributed to dissimilarities in ecosystem characteristics between mitigated and natural wetlands, some of which are successional processes and heterotrophic or autotrophic dominance. Suggestions for wetland mitigation practices include: wetland location chosen near large pre-existing wetlands to facilitate connectivity and interaction of more species, inoculation of less motile macroinvertebrate species for isolated wetlands to help expedite colonization, and prolonged maintenance of established wetlands to limit invasive species.

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INTRODUCTION

Wetlands are extremely valuable ecosystems which provide numerous natural services to humans and the environment. Some of the services that wetlands provide to humans include sediment, nutrient and toxic organic material removal, wastewater treatment, peak flow reduction, recreational hunting and fishing, erosion control, aesthetics, and climate regulation (Johnson et al. 2002; Vymazal 2011). Water quality improvement is one of the most economically important attributes of wetlands since it encompasses such important processes as denitrification and wastewater treatment. In terms of ecological benefits, wetland ecosystems are a major source of primary productivity and offer provision of wildlife habitat which helps maintain biodiversity (Ghermandi et al. 2010). Palustrine, or isolated inland wetlands, provide crucial feeding, nesting, and breeding grounds for migratory birds. In Michigan alone, there are at least 41 state-listed, threatened, and endangered species of animals dependent upon wetlands during some point in their life cycle; it is also estimated that 49% of Michigan's rare plant species only thrive in wetlands (Kost et al. 2007). The annual per acre value for west Michigan's wetlands is estimated to be between $1,391 and $4,203, not including water regulation services (Sterrett-Isely et al. 2007). A meta-analysis by Costanza et al. (1997) found that the natural services wetlands provide are estimated to be trillions of dollars per year globally; thus, wetlands are extremely valuable for both humans and the environment.

Wetlands have historically been one of the habitats affected most by anthropogenic activities (EPA 2002). Prior to settlement, wetlands occupied ~11.2 million acres in Michigan (Dahl 1990). Currently only ~6 million acres remain, a reduction in Michigan's wetland acreage of ~40%. Most of the losses sustained are due to drainage, logging, and conversion to agricultural land (Comer 1996); however, the largest current threat to wetlands is due to landscape alterations. Additionally, the ecological integrity of many of the remaining wetlands has been degraded (EPA 2001). Tiling, drainage ditches, and agricultural conversion can harmfully modify the hydrology of nearby wetlands by lowering the water table. Other factors such as construction of highways, dikes, dams, and railroads also contribute to altered flood duration and water depth which shifts plant and animal communities in wetlands (Burgin 2010). Inland, palustrine wetlands are especially susceptible to changes in hydrology since they are not directly connected to larger, more stable sources of water (Cowardin et al. 1979; Uzarski, unpublished; Zweig and Kitchens 2009). Many of the wetlands that retain their original hydrology are still affected by changes in water quality from industrial, agricultural, and urban runoff, as well as airborne pollutants, storm water and wastewater influx. The addition of impervious surfaces such as parking lots, roads, and sidewalks increases the liklihood of those pollutants to runoff into wetlands. This runoff can cause nutrient loading and possibly eutrophication, which is an extremely detrimental process and can lead to a state of hypoxia.

A less direct anthropogenic effect which plagues wetlands is the introduction of invasive species such as purple loosestrife (*Lythrum salicaria*) and narrow-leaf cattail (*Typha angustifolia*), which out-compete native species and diminish the biological integrity of the ecosystem. Wetlands are particularly susceptible to invasive species since they act as landscape sinks and have large canopy gaps. This threat can impact the wetlands' economic values through losses in recreational value, water filtration capabilities and modifications of natural food webs (Zedler and Kercher 2010). Furthermore, changes in water quality and hydrology can make it easier for invasive species, such as the aggressive reed canary grass (*Phalaris arundinacea*), to invade and overrun native wetland species (Kercher and Zedler 2004).

To alleviate the degradation and loss of wetlands, the process of wetland mitigation was initiated. Mitigation involves the construction of new, man-made wetlands to offset the loss of natural wetlands. The practice was introduced to the U.S. as part of the Clean Water Act in 1972, and established the "no net loss of wetlands" policy, since wetlands are required to be mitigated for an equal or greater area than the original wetland (Burgin 2010; Ghermandi et al. 2010). In accordance, Michigan legislature passed the Geomare-Anderson Wetlands Protection Act in 1979, which was revised in 1994 and is now Part 303, Wetlands Protection, of the Natural Resources and Environmental Protection Act, Public Act 451 (DEQ 1994). The Michigan Department of Environmental Quality is the regulatory agency that is responsible for the interpretation and administration of Part 303.

The mitigation process typically involves four main steps: determining mitigation goals and objectives, a grading plan, planting plan, and monitoring plan (Wyant 2011). The mitigation goals are simply the designated objective/s for that specific wetland such as wetland functions that include water regulation services, wildlife habitat, etc. The grading plan consists of classifying the site in terms of existing hydrology, biota, soil type, and surround landscape. Identifying the appropriate seed mixture for the location and creating a suitable planting arrangement are the two main factors considered in the planting plan. The monitoring plan evaluates whether the wetland is functioning correctly after construction. Often, this requires periodic restoration efforts to monitor native plant species and limit invasive plants through treatment and removal (Batzias et al. 2007). To ensure mitigation goals have been met, the Michigan Department of Environmental Quality has to “sign-off” on mitigation projects. This typically occurs ~5 years after construction of the wetland, at which monitoring ceases (Campbell et al. 2002).

Since wetland mitigation was implemented, ecologists have worked to evaluate the success of these constructed wetlands. Wetland mitigation success is measured by functionality, which includes sediment, nutrient and toxic organic material removal, provision of wildlife habitat, wastewater treatment, primary production, and peak flow reduction (Johnson et al. 2002; Vymazal 2011). A wetland’s health should be viewed as how well it provides these services (Uzarksi et al. 2009). To measure the ecological functions of wetlands, floristic quality assessments have been one of the traditional methods agencies use since they are relatively cost effective, can be completed quickly, and do not require seasoned experts to perform. These assessments may employ the use of diversity indices, floristic quality indices, conservatism values, percent coverage of natives and invasive species, and rank abundance and species area curves to evaluate the floristic condition and composition. Water quality testing is another approach which is often utilized to determine the state of the ecosystem. Water quality analyses can often reveal abiotic wetland characteristics that may otherwise go overlooked, such as increased chloride levels from highway runoff, low dissolved oxygen levels, or increased nitrate levels due to agricultural runoff. The downfalls of water quality tests include their unpredictable nature and cost associated with lab analysis (Kashian and Burton 2000). Water tests are not the best indicators of ecological integrity since they're an abiotic measurement and don't directly represent the biota of a wetland.

Since plant communities in mitigated wetlands are controlled through the aforementioned planting scheme and monitoring efforts, they can be a misleading measure of the wetland's true ecological condition (Marchetti et al. 2011). In comparison, macroinvertebrates may be better biological indicators because they show a large spectrum of tolerance to impairments, react predictably to human influences, are a crucial part of the food web, and their limited dispersal restricts them to water for most of their life (Merritt et al. 2008). They are more sensitive indicators of a wetland's condition than plant communities due to their motility; shifts in macroinvertebrate communities often occur very quickly in response to disturbance. They can also be easily placed into ecological guilds such as functional feeding groups (FFGs), which can be formed into ratios to function as surrogates for ecosystem characteristics such as substrate stability (Cummins 1974). Aquatic insects are not only vital for evaluating lotic systems but are also important to improve the biodiversity and functionality of lentic systems (EPA 2002). Macroinvertebrates play pivotal roles in nutrient cycling and food web support in wetlands (Balcombe 2005).

Indices of biological integrity (IBIs) are a wide-spread and well known ecosystem evaluation method which uses plants and animals to indicate the ecosystem's health or condition. The concept of IBIs is based on the use of multiple biotic metrics which are thought to delineate healthy from degraded systems. Biological data is applied to this template to produce a score for each metric. The scores are then summed to produce an overall numeric value for the ecosystem; high values indicate a healthy system while lower values indicate a degraded system (Uzarski et al. 2009). IBIs were first developed using fish community properties as proxies of lotic system health (Karr 1981), and have since grown to include the use of birds, reptiles, and macroinvertebrates to indicate the health of lakes, streams, and wetlands. Macroinvertebrate indices of biological integrity (IBIs) have been successfully used in evaluating lotic systems for decades (Kerans and Karr 1994); however, their use as biological indicators in lentic systems is relatively unproven (Burton et al. 1999). Many states are in the process of developing standardized macroinvertebrate-based IBIs for wetlands. States such as Minnesota, Wisconsin, and Montana are at the forefront of this evaluation method and have fully developed macroinvertebrate IBIs for wetlands (Genet and Bourdaghs 2006). Over the past decade, Michigan has been in the process of developing and improving accurate macroinvertebrate-based IBIs for coastal wetlands (Burton et al. 1999; Kashian & Burton 2000; Bhagat et al. 2007), and has more recently began creating similar IBIs for inland wetlands (Uzarski et al. 2009).

These IBIs take time to develop because there are many types of wetlands (riverine, lacustrine, palustrine, marsh, swamp, etc.) that each support dissimilar assemblages of macroinvertebrates, are prone to different forms and degrees of impairment, and usually require an IBI developed specifically for that wetland type in that specific ecoregion. Once the metrics for an IBI are tested and the IBI is developed, it generally undergoes many modifications before being accepted as a proven bioassessment method (Karr 2006). Another problem that arises during development is that ecologists have varying definitions and categories of wetlands, which makes collaboration difficult. Wetlands also have highly variable plant communities which can have an effect on macroinvertebrate communities (Zimmer et al. 2000; Balcombe et al. 2005). However, with enough trials and fine-tuning these IBIs can prove to be great indicators of a system's biological and ecological condition.

Although much research has been conducted on wetland functionality and evaluation, relatively few studies have focused on the comparison of mitigated and natural wetlands. A study by Marchetti et al. (2010) compared the macroinvertebrate communities in restored and natural inland wetlands. Their data suggested characteristically different communities among and between these wetland sites, which was indicative of a haphazard or nonpredicatable community assembly process through time. However, their data was quantitative and thus ignored the full potential of macroinvertebrates as bioindicators; ecological importance of specific taxa such as FFGs, tolerance values, and consideration of indicator species can reveal trends in community structure, ecological quality, and ecosystem functionality that may otherwise be ignored using standard quantitative approaches (Kashian and Burton 2000).

Studies by Campbell et al. (2002) and Brooks et al. (2005) used a similar suite of metrics to evaluate mitigated and natural wetlands and found that created wetlands had a greater proportion of dominant plants that were invasive, and natural wetland had greater species richness. Brooks et al. (2005) concluded that wetland mitigation techniques result in a more homogeneous, degraded set of wetlands, while Campbell et al. (2002) found that created wetlands were more similar to degraded natural wetlands which were structurally and functionally similar to moderate to severely degraded natural wetlands. A meta-analysis by Ghermandi et al. (2010) compared the values of created and natural wetlands and concluded that created wetlands don't provide the same level of values as natural wetlands. However, human-made wetlands delivered high values associated with flood control, storm buffering, and water quality improvement. Marchetti et al. (2010) and Brooks et al. (2005) acknowledged that there has been a general deficiency of research in this area, as well as a lack of professional consensus in wetland evaluation methods, thus justifying more research on this topic.

This study investigated the biological integrity of mitigated wetlands as compared to natural, reference wetlands in western Michigan. Five sites, which were mitigated for the construction of highway M-6, were compared to three natural wetlands with similar features. Macroinvertebrate measurements were applied to a rapid bioassessment method established by Uzarski et al. (2009), specifically designed for inland depressional wetlands in Michigan. This is a relatively new method that requires more studies to evaluate its effectiveness as a tool for natural resource managers to use to evaluate wetland quality. The objectives of this study were to 1) determine if wetlands mitigated for the construction of a highway function similarly to natural wetlands by using macroinvertebrate assemblages, water quality, and floristic analyses as indicators of biological integrity, 2) further develop methods to assess the efficacy of using a macroinvertebrate IBI for inland, depressional marshes and 3) provide pragmatic suggestions concerning wetland mitigation practices.

METHODS

Study Sites

The criteria used to choose three natural and five mitigated wetlands were based on the U.S. Fish and Wildlife Service wetland classification system (Cowardin et al. 1979). This classification system delineates five wetland systems: marine, estuarine, riverine, lacustrine, and palustrine. All sites were classified as inland, palustrine marshes because they were dominated by non-woody, persistent emergent plants and were > 1 mile from a Great Lake (Uzarski et al. 2009). The three reference wetlands (R1-R3) were chosen as examples of natural wetlands present prior to development of the M-6 highway (Table 2). Five mitigated sites (M1-M5) were created by the Michigan Department of Transportation to offset the wetlands destroyed or altered during the construction of Highway M-6 in 2004, as required per part 303 of the Natural Resources and Environmental Protection Act (Figure 1). The mitigation sites varied in age from six to twelve years since construction and varied in size ~5 to ~20 ha. Two of the mitigated wetlands were within ~30 and ~15 m from the highway (M3 and M5, respectively), while the other three mitigated wetlands shared a border with agricultural land. Two of the natural remnant wetlands were located in Bysterveld Park, Allegan County. The R1 wetland dominated by reed canarygrass (*Phalaris arundinacea*) and was ~30 m from a major road. The R2 wetland was within ~100 m of agricultural lands to the east and west. The last wetland, R3, was the most unaffected by anthropogenic activities since it was >

400 m from the nearest road and agriculture. This large wetland was located in Hofma Park, Ottawa County, and was surrounded by upland forest ~60 m to the east and west.

Macroinvertebrate Sampling and Processing

Macroinvertebrate sampling took place May 22nd through June 12th, 2012. Three replicates were collected from each major flooded plant zones present: emergent, submergent, and floating leaved (Uzarski et al. 2009). Each plant zone was not present at every site. Samples were collected throughout the entire water column with standard 0.5 mm D-frame dip nets, for 40 person-minutes of effort per replicate. Replicates within each plant zone were then pooled. Specimens were preserved in 80% ETOH in the field and sieved through a 212 µm screen in the lab. Specimens were then sub-sampled using a fixed-count method of 100 specimens per plant zone. Sub-sample and total sample (all specimens collected from a site) macroinvertebrate data were applied to the IBI designed by Uzarski et al. (2009) with the intent of assessing the efficacy of the sub-sampling method (Table 1).

All specimens were identified to the taxonomic level appropriate for the IBI, which was Family for most taxa (Uzarski et al. 2009). Specimens were placed into FFGs and given tolerance values based on bioassessment protocols by Barbour et al. (1999). Twelve metrics were calculated and applied to the IBI (Table 1) developed for inland, depressional marshes (Uzarski et al. 2009). The IBI metrics were proposed metrics based on results of similar studies examining coastal wetlands (Burton et al. 1999), which were then tested on inland marshes. The 12 IBI metrics were summed and applied to a scoring system to indicate the quality of the marsh. Plant zone specific IBI scores were calculated for each site to explore the possibility of certain plant zones being better indicators of ecological integrity. IBI scores in mitigated and reference wetlands were tested using a nonparametric Mann-Whitney Rank Sum Test. Similarly, the sub-sample and total specimen IBI scores were tested to determine the efficacy of the sub-sampling method. Inverse Simpson's index was calculated as a supplementary indicator of biological integrity (Magurran 1988). This index was chosen because it's widely applied to biological data sets and is relatively easier to interpret than other indices (Hill 1973). Using the inverse Simpson's diversity index, each wetland was given a value between zero and one; higher values indicated more diverse communities and lower values indicated less diverse communities. Diversity data were checked for equal variance and normality then t-tests were used to check for possible differences between mitigated and reference wetlands.

Functional feeding group ratios were calculated and used as surrogates of ecosystem characteristics (Cummins 1974). The ratio of predators / all other FFGs indicated whether the systems had a proper predator to prey balance. Scrapers + collector-filterers / shredders + collector-gatherers ratio was used as a proxy of habitat stability, which indicates exposed large woody debris, cobbles, boulders, and bedrock. The ratio of scrapers / all collectors + shredders was applied as an indicator of photosynthesis / respiration ratio, which was then used to estimate if the wetland was primarily autotrophic or heterotrophic. These FFG ratios were originally developed for river systems but have since proved useful in wetlands as well (Kashian & Burton 2000; Rader et al. 2001).

Bray-Curtis similarity scores were calculated for each wetland assemblage and were used with non-metric multidimensional scaling (NMDS) to assess wetland community variation, using R statistics software (version 2.14.1) (Bray and Curtis 1957). NMDS uses number of taxa and abundance of each taxon to examine assemblage similarity in two-dimensional space; the proximity of the points, which represent wetland sites, in the plot indicates the similarity of their communities (Marchetti et al. 2011). Two NMDS plots were used in this study: the first involved only communities in mitigated and reference wetlands, while the second investigated the similarity of communities found in different plant zones within each wetland. A post-hoc analysis of similarities (ANOSIM) test was performed to investigate possible differences between macroinvertebrate communities in mitigated and natural wetlands, as well as a similarity of percentages (SIMPER) test to point out the relative contribution of each taxon to the similarities among sites (Clarke 1993). Taxa occurring at less than 1% of all samples were not included in the NMDS and subsequent analyses due to the statistical effects of rarity on these procedures.

Floristic Quality Assessment

Floristic quality assessments of emergent vegetation at all sites were conducted in August 2012, at a time when most of these plants were flowering which facilitates identification. A fixed transect method was used with five sampling points. Three sampling quadrats (1x1 meter) were determined from each of the five sampling points at a randomly selected distance (1-10 meters) and compass direction (0-360^{o}), resulting in a standardized 15 quadrats per wetland. One transect was used if it could encompass all the present plant zones, if not, two transects were used per wetland in order to better represent the plant community. Plant zones included wet meadow, emergent, floating, and floating leaved zones. Given the variation in wetland sizes, distance between sampling points was established relative to the size of the total wetland acreage; larger wetlands had larger transects and subsequent increment sampling points (15-20 m) than smaller wetlands (10-15 m). Plants within each quadrat were identified to species and percent coverage estimated. Submergent plants were not identified in this study.

Floristic quality analyses were conducted based on standard practices used to assess wetlands by the Michigan Department of Environmental Quality. These methods were published by the Michigan Department of Natural Resources (Herman et al. 2001) and include native species and total species richness, invasive coverage, floristic quality index (FQI), and mean coefficients of conservatism. Mean coefficients of conservatism and FQI are closely related bioassessment methods that use plant community characteristics to determine habitat quality (Swink and Wilhelm 1994). Coefficients of conservatism are values (0-10) based on the probability that a plant is present in a relatively unaltered habitat, similar to pre-settlement conditions. Lower coefficient of conservatism values indicate plants that can be found in most wetlands while higher values are assigned to plants that are restricted to higher quality wetlands. FQI was calculated by multiplying the mean coefficient of conservatism by the square root of the total number of species (Taft et al. 1997; Herman et al. 2001). Additionally, Simpson's diversity index was calculated for each wetland. All floristic measurements were tested for equal variance and normality and t-tests were used to explore differences between mitigated and reference wetlands. If normality or equal variance were not met, a Mann-Whitney Rank Sum test was used.

Water Quality Measurements

Water samples were collected on June 18th, 2012 and June 20th, 2013. Samples could not be collected for the R1 site due to dry weather conditions and extremely low water levels in June 2012. Collections were conducted via two methods: composite grab samples and sonde measurements. Composite grab samples were collected, filtered through 0.45 μm syringe filter, put on ice, and then transported to Grand Valley State University's Annis Water Resources Institute laboratory for analysis of soluble reactive phosphorus (SRP), sulfate, chloride, and nitrate concentrations. Due to financial constraints, composite grab samples were only collected once per year, near the middle of the wetland, at mid-depth. Additionally, at each site, three measurements were taken at mid-depth using a YSI multiprobe meter (probe model: 600QS; meter model: 650 MDS) to measure specific conductance, dissolved oxygen, and pH. Water quality in mitigated and reference sites were compared using standard t-tests if normality and equal variance met, if not, a nonparametric Mann-Whitney Rank Sum Test was used in both 2012 and 2013. To determine differences in results between years, a paired t-test was performed on each metric. If normality or equal variance were not met, a signed rank test was used instead.

RESULTS

Macroinvertebrate Assemblages

The most abundant taxa found in the reference wetlands were 56% *Isopoda*, 13% *Amphipoda*, and 9% *Gastropoda* (Figure 2). Comparably, the mitigated wetlands had fewer crustaceans and more aquatic insects. Despite *Gastropoda* being the most plentiful taxon within the mitigated sites, constituting 31% of the total catch, the next three most abundant taxa were 16% *Hemiptera*, 16% *Coleoptera*, and 13% *Odonata*. There were 56 *Trichoptera* found in the reference wetlands while none were found in the mitigated wetlands. Trichopteran taxa were collected in all three of the reference wetlands and encompassed the *Limniphilidae*, *Phryganeidae*, and *Molannidae* families. Isopods comprised over half of the specimens collected in the reference wetlands, however, they comprised < 1% of the specimens in the mitigated wetlands.

The sub-sampled macroinvertebrate IBI scores were significantly greater for the natural wetlands than the mitigated wetlands ($P < 0.001$; Table 3). Likewise, the IBI scores for all specimens were significantly greater for the natural wetlands than the mitigated wetlands ($P < 0.001$; Figure. 3). The IBI scores from the sub-sampling method were not significantly different from the IBI scores for all specimens. The results of the 1st NMDS (stress = 0.06) and ANOSIM test showed that the macroinvertebrate communities in the mitigated sites were significantly different from the natural sites ($R = 0.87$; $P = 0.021$; Figure. 4). The SIMPER test revealed the taxa that influenced this result the most were *Isopoda* and *Amphipoda*, which accounted for over 60% of the cumulative contribution. The results of the 2nd NMDS (stress = 0.17) and ANOSIM test indicated that the macroinvertebrate communities found in three plant zones were not statistically different from each other (R = -0.14; P = 0.873; Figure. 5). The Inverse Simpson's diversity indices for the mitigated wetlands were statistically greater than the natural wetlands (Table 3).

The functional feeding group (FFG) ratios, which served as proxies of ecosystem characteristics, revealed that 4/5 of the mitigated wetlands had stable substrate; however, none of the reference wetlands shared this substrate stability (Table 4). All three of the natural, reference wetlands had a healthy predator / prey balance while the mitigated wetlands had an over-abundance of predatory taxa such as *Pleidae*, *Coenagrionidae*, and *Hydrophilidae*. Three of five of the mitigated wetlands were extremely autotrophic, while all of the reference wetlands were considered extremely heterotrophic.

Floristic Quality Assessment

The non-native narrow-leaf cattail (*Typha angustifolia*) was the most dominant species in the mitigated wetlands, comprising an average coverage of approximately 30%. The native, yet highly invasive reed canary grass (*Phalaris arundinacea*) was the most dominant plant in the reference wetlands with an average coverage of approximately 53%. The mitigated wetlands had a more diverse floral composition, although not statistically significant. However, the R3 reference wetland harbored northern reedgrass (*Calamagrostis lacustris*), which is considered a threatened species in Michigan, in 20% of the sampled quadrats (Herman et al. 2001).

None of the floristic quality assessment metrics comparing mitigated and reference wetlands were significantly different ($P > 0.05$; Table 5). The mean invasive plant coverage for the mitigated wetlands was greater than the natural wetlands, but these differences were also not statistically significant. Measurements of the floristic metrics were more variable for the reference wetlands than for the mitigated wetlands.

Water Quality

Water quality data was highly variable between sites and thus lacked obvious trends. In 2012, dissolved oxygen was low in the R2 site (Table 6).. Chloride levels were notably higher in the M3 and M5 mitigated wetlands. Four of five of the mitigated wetlands had elevated levels of nutrients (nitrate, SRP, sulfate), while the R2 reference wetland had the highest sulfate level.

In 2013, the pH in the M3 site was alarmingly alkaline at 10.3 (Table 7). Chloride levels were elevated in M2 and R1 sites. Nutrient measurements were high in the M1, M3, and M5 mitigation site. Nitrate levels in the R2 wetland were a full order of magnitude greater than all the other wetlands.

The t-tests and Mann-Whitney Rank Sum tests applied to both the 2012 and 2013 data to compare mitigated and reference wetland water quality resulted in no significant differences ($P > 0.05$) for each of the water quality metrics. The paired t-tests and Signed Rank tests used to compare water quality metrics between years also produced no significant differences.

DISCUSSION

The biological integrity of the mitigated and reference wetlands is substantially different. The macroinvertebrate IBI indicates that the reference wetlands are high to excellent quality while the mitigated wetlands are poor to average quality (Figure 3). The first NMDS illustrates that the macroinvertebrate assemblages in the natural wetlands are statistically different from the mitigated wetlands (Figure 3), while the supplemental FFG ratios also show some distinct trends. The IBI combines many bioassessment indices to estimate the overall biological integrity of each site, while the NMDS and ANOSIM test provide a more statistical approach to comparing community composition between sites. The use of both the macroinvertebrate IBI and the NMDS/ANOSIM allows for a more thorough and accurate bioassessment approach. Combined, these results indicate that the macroinvertebrate communities in the mitigated and natural wetlands were statistically different in terms of biological integrity and community composition. These results coincide with similar studies examining mitigated and natural emergent wetlands (Campbell et al. 2002; Brooks et al. 2005; Ghermandi et al. 2010).

Macroinvertebrates have proven themselves as effective measures of bioassessment in lotic systems (Cummins 1974; Plafkin et al. 1989; Kerans and Karr 1994) and my results strongly suggest that they can be equally important tools for evaluating lentic systems (Burton et al. 1999; Kashian and Burton 2000). The macroinvertebrate FFG ratios, IBI, and 1st NMDS indicate a distinct separation of biological integrity between mitigated and reference wetlands, which was not detected using standard water quality and floristic analyses. Macroinvertebrates are mid-level consumers so they are affected by both top-down and bottom-up trophic interactions, which makes them great indicators of current trophic conditions (Merritt et al. 2008). Presence or absence of sensitive taxa, such as most *Trichoptera*, can be an indication of physical and/or chemical impairments. There were 56 trichopterans collected in the natural wetlands while none were found in the mitigated wetlands; over 90% of those trichopterans belonged to the family *Limniphilidae*, which is an intolerant, sensitive taxon (Merritt et al. 2008). Macroinvertebrates' usefulness as a bioassessment tool extends to include estimates of autotrophic / heterotrophic dominance, substrate stability, and photosynthetic food availability via FFGs and FFG surrogate ratios. Using these ratios I identified an overabundance of predatory taxa in the mitigated wetlands (Table 4), a trophic imbalance also observed by Brown et al. (1997) in a study examining newly flooded restored wetlands.

The Simpson's diversity index reveals that the macroinvertebrate communities in the mitigated wetlands were more diverse than the natural wetland communities (Table 3). Many ecologists have traditionally considered species diversity as an effective indicator of a system's health and biological stability (Magurran 1988); however, the results of the macroinvertebrate IBI indicated that the mitigated wetlands were of poor to average quality in terms of biological integrity. This contradiction could stem from the fundamental flaws associated with the use of diversity indices. Diversity indices utilize two unrelated aspects: taxa composition and abundance of each taxon. Composition in a given area is dependent upon factors such as historical climatic shifts, seasonal variations, dispersal events, and proximity to other bodies of water (Hubbell 2001; Voshell 2002). Abundance of each taxon is dependent upon factors such as predation, competition, life history traits, and food availability. The combination of these two aspects into an index, such as Simpson's diversity, results in a single value which can be difficult to interpret and less ecologically meaningful (Barrantes and Sandoval 2009). For example, the mitigated wetlands had macroinvertebrate communities which were more diverse; however, they had a fewer specimens which were deemed intolerant (metric 2, Table 1). These intolerant, sensitive species are better ecological indicators because they can only persist in relatively pristine habitats (Barbour et al. 1999). When diversity indices are applied to a set of data, the taxa are considered numeric and thus nothing can be inferred of their ecological role and importance (Barrantes and Sandoval 2009). Alternatively, multimetric indices are specifically designed to assimilate information from the ecosystem, community, population, and individual specimen level into an easy to interpret estimation of an ecosystem's condition (Barbour et al. 1999).

The rapid bioassessment IBI used in this study utilizes a variety of practical metrics such as tolerance values, specific taxa richness, ecological roles (FFGs), and presence of indicator species to assess the biological integrity of inland marshes (Table 1). I recommend this IBI as a rapid bioassessment tool for inland wetlands with the exception of metric 1, "% of total catch that were intolerant" and metric 2, "% of total catch that were tolerant" (Table 1). These two metrics tested poorly in this study. It seems counter-intuitive that a higher percentage of tolerant taxa would be deserving of a higher IBI score, since most tolerant taxa can thrive in degraded systems. Macroinvertebrate tolerance values have potential to be helpful indicators of a system's condition with proper application (Barbour et al. 1999); thus, I recommend that the values for each disturbance level be revised to better reflect disturbance upon further testing.

The sub-sampling methods used in this study yielded IBI scores which are statistically similar to the IBI scores of the complete samples, which verifies the efficacy of this sub-sampling method, or a similar method with a fixed count minimum of 100 specimens per plant zone. If only one plant zone is present, a fixed count method of 200 specimens is suggested to achieve an accurate representation of the macroinvertebrate assemblage (King and Richardson 2002). Previous studies have stressed the importance of evaluating wetland macroinvertebrate communities in a stratified manner based on plant zones such as emergent, submergent, and open water areas (Balcombe et al. 2005; Streever et al. 1995). These studies, which evaluated macroinvertebrate abundance and diversity, also indicated that pooling of plant zone specific data could mask potential differences between created and natural wetlands that lie solely within these plant zones. To test that approach, the methods employed in this study used both plant zone specific data and pooled data, so a comparison could be made. Based on the locations of the points in the second NMDS plot (Figure 4), it can be concluded that the macroinvertebrate communities in this study are not heavily influenced by plant zone;

instead, they are more influenced by site. Additionally, the plant zone specific IBI results were not significantly different from the pooled plant zone IBI results (Table 3). Taken together, these results indicate that plant zone specific data may not be as important as originally thought. Furthermore, plant zones within wetlands can often be difficult to delineate because of the high amount of overlap between zones (Zimmer et al. 2000; Balcombe et al. 2005), which can reduce the confidence of plant zone specific data. Many taxa thrive in these different microhabitats and for that reason it is recommended that all plant zones be incorporated, but pooled, while sampling macroinvertebrates in wetlands. This approach should contribute to the speed of this rapid bioassessment method without hindering its efficacy.

A plausible explanation for the differences between macroinvertebrate communities in mitigated and reference wetlands could stem from ecosystem characteristics which can often go undetected using standard approaches. The macroinvertebrate FFG ratios employed in this study act as proxies of such ecosystem attributes and revealed some substantial trends between mitigated and reference wetlands. For example, the reference wetlands are all extremely heterotrophic while 3/5 of the mitigated wetlands are extremely autotrophic (Table 4). This can be explained by the influence that succession has on substrate and algae. Four out of five of the mitigated wetlands had stable substrate while all of the reference wetlands had unstable substrate (Table 4). Stable substrate encompasses the large woody debris, cobbles, boulders, and bedrock that most algae require as a natural growth medium. Algae are a major contributor of autotrophy in aquatic systems (Minshall 1978). Anderson et al. (2005) found that as created wetlands age, there is a general shift in organic matter from algae to macrophytes. Notably, there were more algae visually observed in the mitigated wetlands than in the reference wetlands. As successional processes progress, algae may lose its light availability due to an increase in emergent plants, as well as be buried from cumulative sedimentation; a process which reduces the area of standing water in wetlands (Uzarski, unpublished; Zweig and Kitchens 2009). This scenario would provide for a more heterotrophic system. Using the same FFG ratio method while testing potential IBI metrics on reference and degraded wetlands, Kashian and Burton (2000) found that an impacted wetland was primarily autotrophic while their reference site was heterotrophic. Their rationale for this was an increase in nutrient levels in the impacted wetlands, a trend that was also found in some of the mitigated sites in this study. Regardless of whether the mitigated wetlands were primarily autotrophic due to natural successional processes or increased nutrient levels, my findings reveal that they do not possess some of the ecosystem characteristics of natural wetlands.

There are some notable limitations of this study. Although rapid bioassessments typically involves just one sampling period, the lack of a temporal series in this study means there is no account for seasonal or annual variation. The level of identification of macroinvertebrates (mostly family level) resulted in underestimated values for the diversity measurements. This is a standard, pragmatic IBI approach for rapid bioassessment and since level of identification was constant across all sites, it should not have affected comparison of diversity between the mitigated and natural wetlands in this study. The absence of soil sampling in this study may have limited my ability to discern the mechanisms driving the discrepancies between floristic and macroinvertebrate results. Many studies report that soil composition can heavily influence vegetation (Kentula et al. 1992; Stauffer and Brooks 1997) as well as the macroinvertebrate communities in a wetland (Merritt et al. 2008; Voshell 2002). It's worth noting that this study is limited to ecological and biological integrity and thus ignores other important wetland functions such as flood control and filtration processes. The minimal number of reference sites is reason for some concern in this study since it could influence results comparing mitigated and natural wetlands. Financial and time constraints, as well as lack of proximal natural wetlands were factors that limited the number of reference sites used in this study. For similar studies, I recommend a greater sample size.

The floristic quality assessments in this study were chosen based on methods which agencies actively use to evaluate wetland plant communities. The results revealed little in the way of trends between mitigated and natural wetlands. The mean floristic quality index (FQI) and mean coefficient of conservatism values were slightly greater for the mitigated sites (although not significant); yet, the R3 site had the best floristic quality scores overall. The assessment of more natural sites may have revealed trends similar to Kellog and Bridgham (2002) and Balcombe et al. (2005), in which the natural wetlands had a more diverse vegetative structure than the mitigated wetlands. Interestingly, the mitigated sites had a higher percentage of invasive plant coverage despite the vegetative monitoring and invasive plant treatments which are required as per the mitigation performance standards; a trend that is also found in similar studies (Brooks et al. 2005; Campbell et al. 2002). These results could be contributed to the invasion strategies of exotic plants. Many invasive plants out-compete native plants during early succession of the community (Bryant et al. 1992; Herben et al. 2004). The mitigated sites in this study are still undergoing early succession and therefore may still have greater dominance by invasive plants. One possible solution to this problem is to require vegetative monitoring for a longer period of time than is currently required and, if necessary, more invasive species control (Campbell et al. 2002).

Despite the FQA not revealing trends between the two types of wetlands, these analyses are important for estimating floristic community structure, invasive species estimates, and identifying rare or endangered species (Herman et al. 2001). The mean coefficient of conservatism and FQI analyses offer a flexible bioassessment method based on species' indicator values, or "C" value (Swink and Wilhelm 1994). FQI can be used for comparison of sites of different sizes and complexity, while mean coefficient of conservatism can be employed to assess the quality of similar study sites (Taft et al. 1997). These analyses can easily be applied to the floristic monitoring data which is standard for mitigation practices. The only noticeable downfall of the floristic methods described by the Michigan Department of Natural Resources (Herman et al. 2001), which are commonly used by monitoring agencies, is the delineation of "native" or "adventive species". For example, reed canary grass (*Phalaris arundinacea*) can have devastating effects on wetland communities (Kercher and Zedler 2004); yet, this species is considered native in most of North America, including Michigan (Hansen et al. 1995; Herman et al. 2001). For the sake of invasive species monitoring and treatment, it may be more practical to classify species as “invasive” or “not-invasive” as pertaining to a certain habitat.

Water quality results appeared highly site specific and lacked overall trends between both wetland type and sampling years. There were instances of elevated SRP and nitrate levels in both mitigated and reference wetlands, which can be a precursor of detrimental eutrophication processes. Water quality can be an abiotic measure of biological integrity in wetlands since it greatly influences the conditions in which organisms live; however, the results are prone to inconsistencies due to natural atmospheric fluctuations, photosynthetic / cellular activity, water depth changes, and collection time (Uzarski et al. 2009). Extremely low water depth, experienced at some of my study sites, can result in a higher than average concentration of compounds such as nitrates, SRP, and phosphate. The short-term sampling approaches used in this study limit the interpretation of my results, which show no obvious trends between mitigated and reference wetlands. Long-term evaluation of water quality can account for episodic pollution and seasonal changes but is often not feasible due to financial and time constraints (Kashian and Burton 2000). For rapid bioassessment of wetland biological integrity, I recommend macroinvertebrate bioassessment in lieu of water quality analyses since they respond to both periodic and long-term abiotic changes (Karr 1993; Kashian and Burton 2000).

Of all factors considered while creating a wetland, location is of the utmost importance (Campbell et al. 2002). Location will dictate influential factors such as pre-existing hydrology, soil properties, and established flora and fauna. Additionally, wetland location relative to land usage such as urbanization, forests, and agriculture will affect wetland functionality. In accordance with the metapopulation / metacommunity theory (Wilson 1992), site location relative to other wetlands may be the most important factor influencing biological integrity. This is especially true with inland, palustrine marshes which, by definition, are more isolated than lacustrine and riverine wetlands. The metapopulation theory, as pertaining to wetlands, involves a set of wetland communities interacting together based on habitat size, species dispersal capabilities, wetland proximity and connectivity. I recommend that mitigated wetlands be located near large, pre-existing wetlands and preferably a high level of connectivity between these wetlands, properties which were not observed in the wetlands chosen for this study. Connectivity depends on the life history traits of the taxa being considered; for example, wetland connectivity for many amphibians would be high if isolated wetlands were connected via forests, since many amphibians require forested habitats for part of their life cycle (Batzer and Sharitz 2006). Many macroinvertebrate taxa live their entire life in water; for these macroinvertebrates, connectivity would be influenced by the presence of permanent and seasonal bodies of water such as flood plains and vernal pools (Voshell 2002). A study investigating the biological integrity of a series of wetlands that represent a gradient of proximity and connectivity to other wetlands may help reveal the extent of the influence of these factors.

In this study the macroinvertebrate communities found in the mitigated wetlands were significantly different than those found in the natural wetlands. The question is whether these results are truly due to differences in biological integrity. An argument could be made that the presence or absence of specific taxa could be due to the limited dispersal of some invertebrates. Macroinvertebrates, although widespread, have a varied range of motility; thus, if a taxon hasn't been exposed to a newly created wetland, either due to limited dispersal ability or wetland isolation, then it shouldn't be present there. Results of a similar study examining macroinvertebrates in restored and natural wetlands indicated a haphazard or nonpredicatable community assembly process of macroinvertebrates in wetlands (Marchetti et al. 2010). Community assembly may be an unpredictable process; however, installing man-made wetlands near other large wetlands with high levels of connectivity will help facilitate the colonization of all viable macroinvertebrates, including those with low dispersal capabilities. To help jumpstart this community assembly process, inoculation of less motile and nonaerial taxa can be implemented during the mitigation process (Brown et al. 1997). This will nullify any differences between mitigated and natural wetland which may exist due to macroinvertebrate dispersal and community assembly processes and should work to strengthen the long-term biological integrity of these wetlands.

The reference sites chosen in this study were not selected based on observable quality and/or biological diversity, rather as a representation of what natural wetlands may have looked like prior to the development of highway M6. Brooks et al. (2005) found that sets of natural wetlands exhibit greater heterogeneity as compared to created wetlands and I believe the reference sites chosen for this study accurately reflect this pattern. The wetland mitigation sites used in this study were of notably higher quality than many observed mitigated wetlands that were not chosen as study sites. The use of supplemental snags, multiple tiers, water level control, and diverse seeding helps facilitate microhabitat and species diversity. Yet, based on my findings, these mitigated wetlands clearly are not functioning similarly to natural wetlands in terms of biological integrity. Despite the enormous significance of wetlands and all the considerations that go into mitigation planning, Burgin (2009) describes mitigation outcomes as modestly successful at best, based on a suite of metrics which ecologists use to evaluate mitigation success. Likewise, the EPA (2001) stated that many created wetlands fail to replace the diverse plant and animal communities that are removed during the destruction of the original wetland. The suggestions provided in this study should facilitate more successful mitigation projects if implemented into standard mitigation practices. I also recommend new approaches to wetland evaluation techniques; current techniques are not measuring the mitigation goals, rather specific aspects of the wetland's condition. The mitigation goals, which are wetland functions, are measured by the services it provides such as wastewater treatment and provision of wildlife habitat, while most mitigated wetland are mainly measured by percent coverage of invasive plant species. The macroinvertebrate methods employed in this study further measure trophic structure and can be used as surrogates of wetland functionality.

Table 1: An invertebrate index of biological integrity developed by Uzarski et al. (2009), which was designed specifically for inland, depressional marshes. Invertebrate scores were summed for 12 metrics and placed into categories: 48 - 60 = excellent quality marsh, 36 - 48 = average or higher quality marsh, 24 - 35 = below average quality marsh, degraded, 12 - 23 = Poor quality marsh, heavily degraded.

Metric	**Degraded**	**Moderately Impacted**	**Reference**
	Score = 1	**Score = 3**	**Score = 5**
1. % of total catch that were tolerant taxa	0 - 1 %	2 - 5 %	> 5 %
2. % of total catch that were intolerant taxa	0 - 0.10 %	0.11 - 0.75 %	> 0.75%
3. Number of "species" of beetles (*Coleoptera* taxa richness)	> 3	-----	0 - 3
4. % of total catch that were shredders	0 - 2.5 %	-----	> 2.5 %
5. % of total catch that were predators	> 25 %	12.1 - 25 %	0 - 12 %
6. % of total catch that were Crustacea + Mollusca	> 20 %	10.1 - 20%	0 - 10 %
7. Caddisflies (*Trichoptera*)	absent	-----	present
8. % of total catch that were water boatmen (*Corixidae*)	> 1 %	-----	0 - 1 %
9. % of total catch that were mosquito larvae (*Culicidae*)	> 1.5 %	-----	0 - 1.5 %
10. % of total catch were predaceous diving beetles (*Dytiscidae*)	> 2.5 %	-----	0 - 2.5 %
11. % of total catch that were crawling water beetles (*Haliplidae*)	> 3.0 %	1.1 - 3.0 %	0 - 1.0 %
12. % of total catch that were damselflies in the family *Lestidae*	> 5 %	3.1 - 5 %	0 - 3 %

Table 2: Characteristics of eight wetlands in western Michigan. “R” sites indicate natural, reference wetlands while “M” sites indicate mitigated wetlands. Average depth was calculated using averages of 2012 and 2013 sampling periods.

Site	Years Since Construction	Avgerage Depth (m)	Hydrology	GPS Coordinates
M1	6	0.21	Surface flow	42°47'42.89"N, 85°59'9.71"W
M2	9	0.74	Groundwater	42°48'8.52"N, 85°41'39.86"W
M3	8	0.63	Groundwater	42°51'18.52"N, 85°44'43.47"W
M4	9	0.72	Surface flow	42°49'20.88"N, 85°35'51.39"W
M5	11	0.43	Groundwater	42°51'9.67"N, 85°35'28.60"W
R1	-	0.17	Surface flow	42°43'24.47"N, 85°40'44.37"W
R2	-	0.06	Surface flow	42°43'11.48"N, 85°40'37.11"W
R3	-	0.45	Surface flow	43° 1'19.13"N, 86°11'18.22"W

Table 3: The index of biological integrity (IBI) scores for eight wetlands in western Michigan. “R” sites and values indicate natural reference wetlands while “M” sites and values indicate mitigated wetlands. Twelve IBI metrics were summed to comprise each IBI score. Sub sample and total IBI scores were included so a comparison could be made to test the efficacy of the fixed, 100-count per plant zone sub sampling method. IBI scores in the 3 mitigated and 5 reference wetlands were tested using a Mann-Whitney Rank Sum test. Both the sub-sampling and total sampling methods revealed that the reference wetland IBI scores were significantly greater than the mitigated wetland IBI scores. Floating and submergent plant zones were not statistically tested due to lack of replicates. A t-test indicated that the Inverse Simpson’s diversity indices for the mitigated wetlands were statistically greater than the natural wetlands.

Site	Sub sample IBI score	Total IBI score	Plant Zone Specific IBI Scores			Inverse Simpson's D
			Floating	Emergent	Submergent	
M1	36	30	36	28	-	0.78
M2	32	30	32	36	-	0.84
M3	34	34	38	38	-	0.73
M4	36	36	-	36	40	0.90
M5	32	32	-	36	36	0.80
R1	46	50	46	48	-	0.48
R2	50	48	-	48	-	0.54
R3	46	46	-	46	48	0.74
Mitigated Mean Std Deviation	34.0 ± 2.0	32.4 ± 2.6	35.3	34.8	38	0.81 ± 0.06
Reference Mean Std Deviation	47.3 ± 2.3	48.0 ± 2.0	46	47.3	48	0.59 ± 0.14

* denotes a significant value ($P < 0.05$)

Table 4: Functional feeding group ratios for eight wetlands located in western Michigan, which serve as proxies of ecosystem characteristics. "R" sites and values indicate natural, reference wetlands while "M" sites and values indicate mitigated wetlands. Shading is used to illustrate trends within data.

Site	Habitat Stability	Predator / Prey Balance	Photosynthesis / Respiration
M1	1.71	0.25	1.47
M2	0.62	0.76	0.56
M3	2.34	0.21	1.41
M4	0.14	0.79	0.14
M5	2.16	0.56	1.85
R1	0.14	0.13	0.12
R2	0.04	0.03	0.03
R3	0.20	0.11	0.16
Evaluation	Habitat Stability	Predator / Prey Balance	Autotrophic vs. Heterotrophic system
Criteria Levels	Stable Substrate Plentiful > 0.6	Normal balance <0.15	Autotrophic > 0.75

Table 5: The results of floristic quality assessments for eight wetlands in western Michigan. “R” sites and values indicate natural, reference wetlands while “M” sites and values indicate mitigated wetlands.

Site	Native species richness	Total species richness	Invasive plant cover (%)	Mean coefficient of conservatism	Floristic quality index
M1	11	15	29.0	4.27	14.17
M2	16	21	11.2	2.69	10.75
M3	30	33	22.4	3.33	18.26
M4	22	26	8.6	3.27	15.35
M5	11	14	31.9	3.36	11.16
R1	4	5	0.9	1.75	3.50
R2	9	10	4.1	2.89	8.67
R3	24	26	39.9	4.71	23.07
Mitigated Mean Std Deviation	18.0 ± 8.0	21.8 ± 7.9	20.6 ± 10.4	3.39 ± 0.57	13.93 ± 3.10
Reference Mean Std Deviation	12.3 ± 10.4	13.7 ± 10.9	14.9 ± 21.7	3.11 ± 1.49	11.74 ± 10.13

Table 6: Water quality data for seven wetlands in western Michigan which were sampled in May 2012. "R" sites and values indicate natural reference wetlands while "M" sites and values indicate mitigated wetlands. Using standard t tests and Mann-Whitney Rank Sum tests, no significant differences were detected between mitigated and reference wetland water quality ($P > 0.05$). Note: no water quality data could be collected from R1 due to low water levels.

Site	Specific Conductance (uS/cm)	Dissolved Oxygen (mg/L)	pH	Chloride (mg/L)	Sulfate (mg/L)	Nitrate (mg/L)	Soluble Reactive Phosphorous (µg/L)
M1	774	6.35	8.0	76	0.3	0.04	59.7
M2	430	9.94	7.9	5	13.0	4.44	4.6
M3	754	7.59	8.2	152	5.0	0.31	11.5
M4	212	10.97	7.8	3	0.2	0.15	6.4
M5	860	7.49	8.4	149	7.0	0.31	8.1
R2	635	3.87	8.0	37	32.0	0.51	5.5
R3	386	11.13	8.3	33	12.0	0.35	4.5
Mitigated Mean	543	8.71	8.0	59	5	1.24	20.6
Reference Mean	511	7.50	8.1	35	22	0.43	5.0

Table 7: Water quality data for eight wetlands in western Michigan which were sampled in May 2013. "R" sites and values indicate natural reference wetlands while "M" sites and values indicate mitigated wetlands. Using standard t tests and Mann-Whitney Rank Sum tests, no significant differences were detected between mitigated and reference wetland water quality ($P > 0.05$).

Site	Specific Conductance (µs/cm)	Dissolved Oxygen (mg/L)	pH	Chloride (mg/L)	Sulfate (mg/L)	Nitrate (mg/L)	Soluble Reactive Phosphorus (µg/L)
M1	658	18.84	9.6	44	12	0.19	116.9
M2	642	4.33	7.8	97	6	0.57	7.1
M3	767	13.13	10.3	29	33	0.99	7.5
M4	295	2.45	7.7	44	12	0.19	11.7
M5	530	3.98	7.6	67	3	0.07	4.7
R1	905	3.47	7.7	142	7	0.61	4.6
R2	378	8.60	8.1	10	16	12.34	7.7
R3	251	5.86	7.1	30	24	0.70	9.4
Mitigated mean	578	8.55	8.6	56	13	0.40	30
Reference mean	511	5.98	7.6	61	16	4.55	7.2

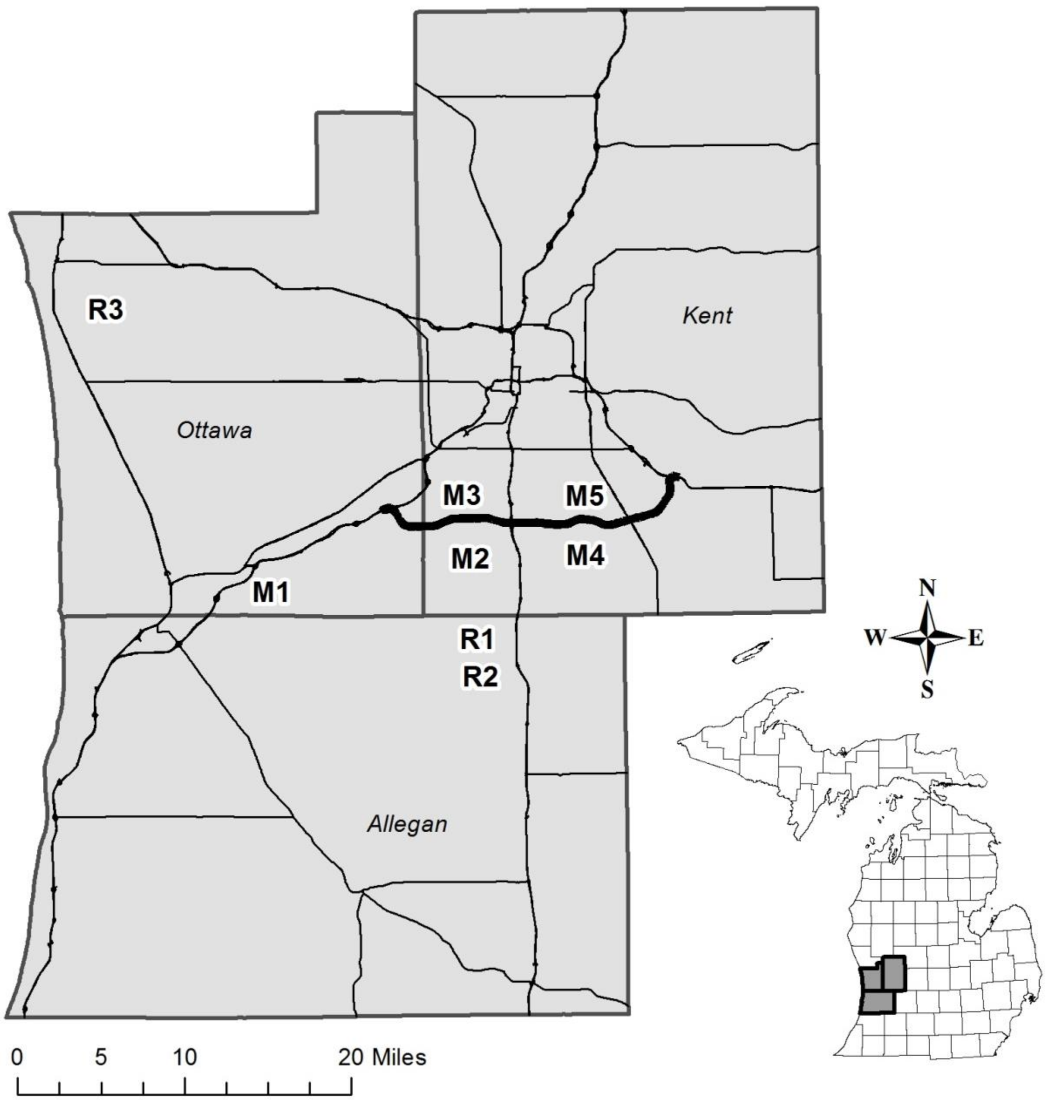

Figure. 1 Location of study sites in Allegan, Kent, and Ottawa counties, MI. M1-M5 sites were wetlands mitigated for the construction of highway M-6 (outlined here by a thick black line). R1-R3 sites served as natural, reference sites during this study.

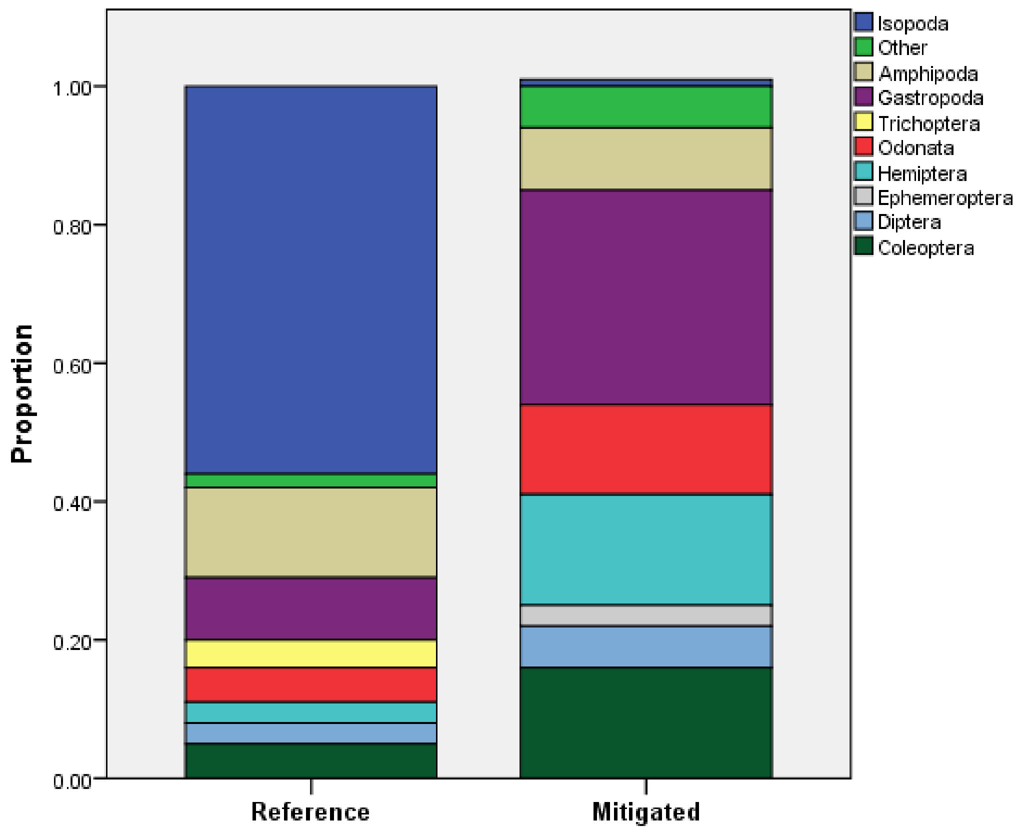

Figure. 2 Macroinvertebrate community composition in mitigated and natural, reference wetlands in west Michigan, expressed in a stacked bar graph. Data from three reference wetlands and five mitigated wetlands were pooled in each category. Proportion of total catch is on the Y axis and wetland type is on the X axis. N = 2859

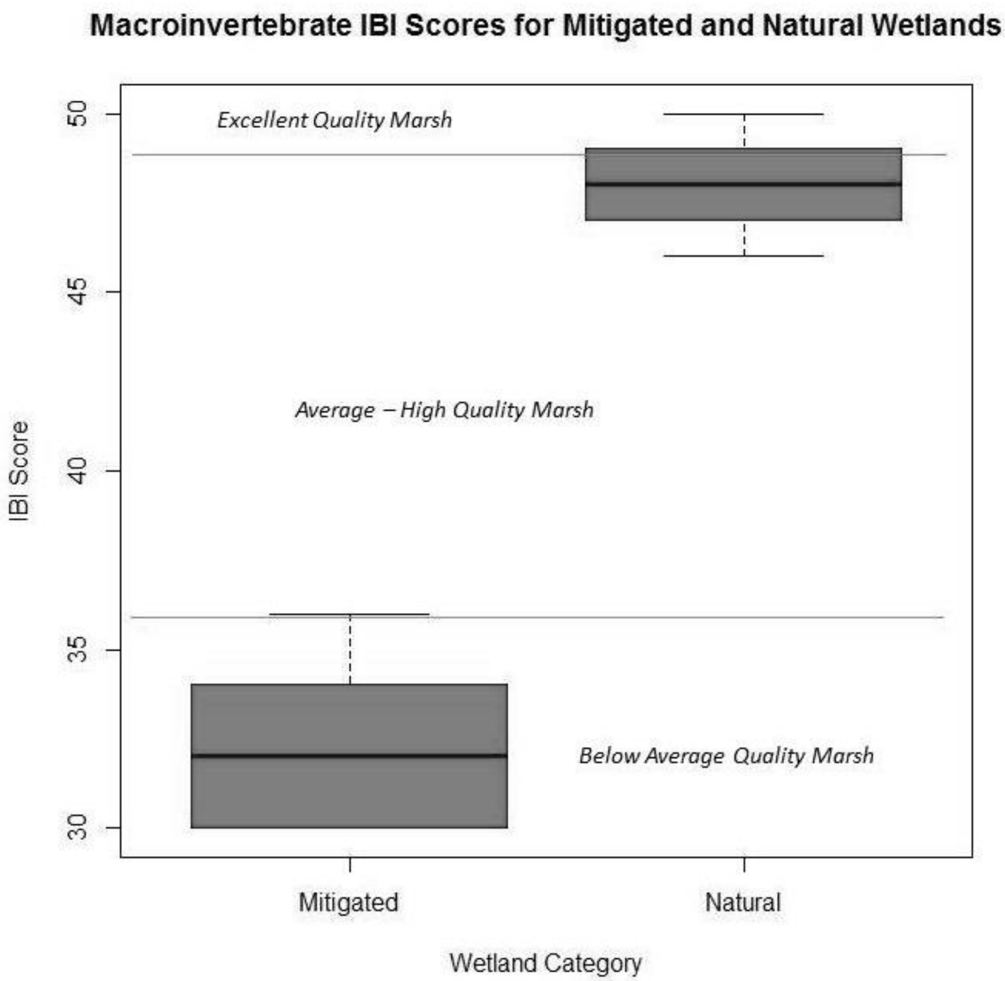

Figure. 3 The results of IBI scores for macroinvertebrate communities in five mitigated and three natural wetlands, expressed in a Box and Whisker plot. The IBI scores for the natural, reference wetlands were significantly greater than the IBI scores for the mitigated wetlands ($P < 0.001$). The thin black lines and italicized text indicate the wetland quality categories detailed in Uzarski et al. (2009). Invertebrate scores were summed for 12 metrics and placed into categories: 48 - 60 = excellent quality marsh, 36 - 48 = average or higher quality marsh, 24 - 35 = below average quality marsh, degraded, 12 - 23 = Poor quality marsh, heavily degraded.

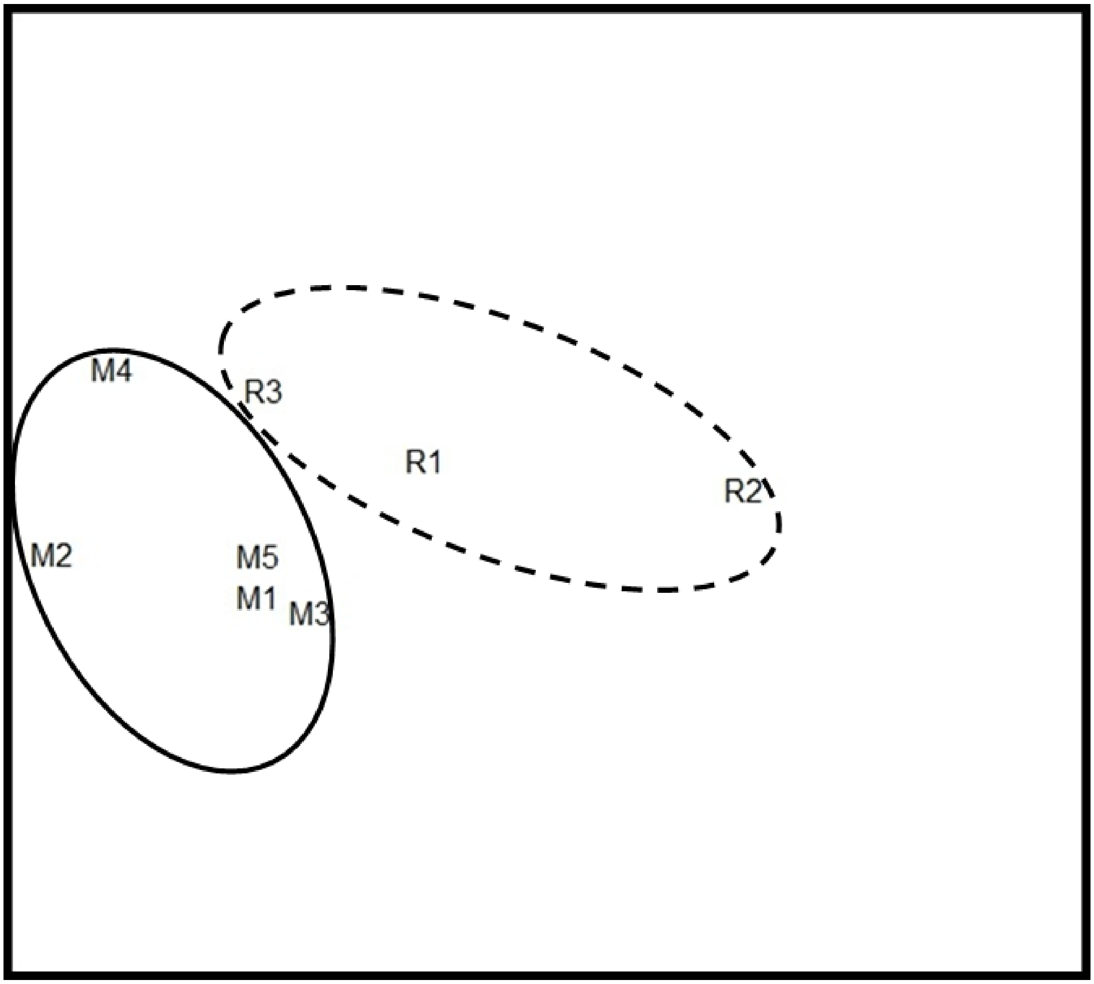

Figure. 4 NMDS ordination plot for eight wetland macroinvertebrate communities based on Bray-Curtis similarities (stress = 0.06). “R” sites indicate natural reference wetlands while “M” sites and values indicate mitigated wetlands. NMDS uses number of taxa and abundance of each taxon to examine assemblage similarity in multi-dimensional space; the proximity of the points, which represent wetland sites, indicates the similarity of their communities. Mitigated wetland communities were significantly different from the natural wetland communities according to the ANOSIM results ($R = 0.87$; $P = 0.021$). Both Bray-Curtis and ANOSIM tests were performed with 999 permutations.

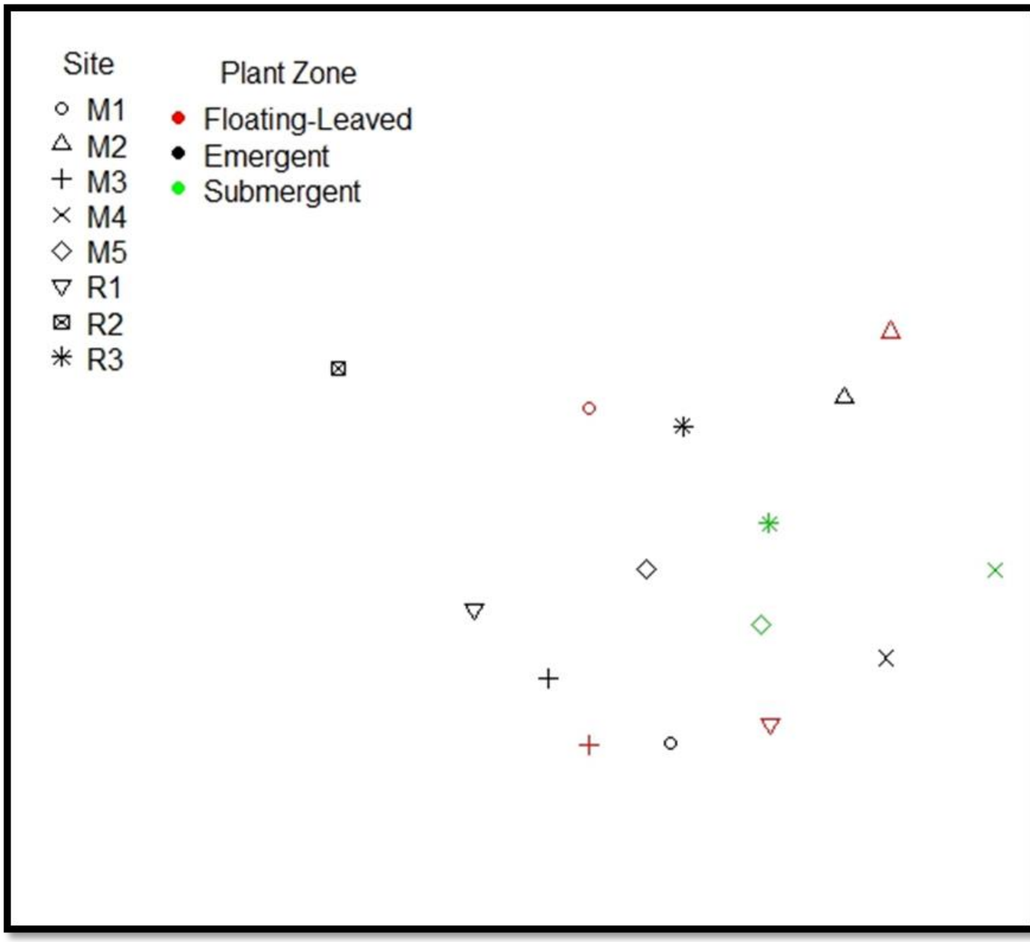

Figure. 5 NMDS ordination plot for macroinvertebrate communities collected in three different plant zones based on Bray-Curtis similarities (stress = 0.17). R1-R3 sites indicate natural, reference wetlands while M1-M5 sites indicate mitigated wetlands. Plant zones are differentiated by color, while wetland sites are represented by symbols. The ANOSIM test revealed that macroinvertebrate communities in the three plant zones were not significantly different (R = -0.14; P = 0.873). Both Bray-Curtis and ANOSIM tests were performed with 999 permutations.

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APPENDICICES

APPENDIX A

Macroinvertebrate Data

Taxon	Site							
	M1	M2	M3	M4	M5	R1	R2	R3
Aeshnidae	1	0	0	4	7	7	0	0
Amphipoda	0	80	0	28	0	0	0	186
Athericidae	0	3	0	0	0	0	0	0
Baetidae	0	0	0	12	0	0	0	0
Bivalvia	8	5	24	0	6	8	3	10
Caenidae	0	8	0	18	11	0	1	2
Calopterygidae	0	6	0	0	0	0	0	1
Ceratopogonidae	0	0	13	6	0	0	0	0
Chironomidae	16	4	7	25	6	13	11	8
Chrysomelidae	0	1	1	0	0	4	0	0
Coenagrionidae	1	89	0	8	9	14	0	3
Cordulegastridae	0	0	0	1	0	0	0	0
Corduliidae	0	0	0	0	0	0	0	1
Corixidae	36	28	3	17	11	0	0	12
Culicidae	1	0	1	2	1	0	0	0
Curculionidae	0	4	0	1	0	0	0	0
Decapoda	0	1	0	2	11	0	0	2
Dixidae	0	0	0	1	0	0	0	0
Dytiscidae	9	14	9	0	24	5	6	0
Elmidae	0	0	0	0	0	3	1	0

APPENDIX A

Macroinvertebrate Data (continued)

Taxon	Site							
	M1	M2	M3	M4	M5	R1	R2	R3
Gastropoda	0	77	121	18	113	63	6	59
Gerridae	0	0	0	1	1	0	0	0
Gomphidae	0	0	0	1	0	0	0	2
Haliplidae	12	4	9	16	19	20	2	9
Hirudinea	18	0	2	4	0	0	0	1
Hydrophilidae	11	1	10	17	10	12	1	4
Isopoda	0	0	0	8	0	500	164	133
Lampyridae	0	0	0	0	0	4	1	0
Lepidostomatidae	0	0	0	0	0	0	2	0
Lestidae	6	0	0	1	1	2	0	9
Libellulidae	5	3	5	8	28	16	0	10
Limnephilidae	0	0	0	0	0	6	45	0
Molannidae	0	0	0	0	0	0	3	0
Naucoridae	0	0	1	3	8	0	0	0
Phryganeidae	0	0	0	0	0	1	0	1
Pleidae	1	47	4	64	9	21	0	14
Psephenidae	12	0	0	0	0	0	0	0
Ptychopteridae	0	0	1	0	0	0	2	0
Sciomyzidae	2	0	0	0	0	0	0	0
Scirtidae	0	1	39	0	0	0	0	0
Stratiomyidae	0	1	1	2	0	0	3	0

APPENDIX B

Floristic Data

SCIENTIFIC NAME	ENGLISH NAME	C	NATIVE?	M1	M2	M3	M4	M5	R1	R2	R3
Lycopus americanus	American Water-horehound	2	Yes	0.0	0.4	0.6	0.0	0.0	0.0	0.0	0.0
Polygonum sagittatum	Arrow-leaf Tearthumb	5	Yes	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.3
Echinochloa crusgalli	Barnyard-grass	0	No	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Galium asprellum	Bedstraw	5	Yes	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.4
Brassica nigra	Black Mustard	0	No	0.0	0.4	0.0	0.0	0.0	0.0	0.0	0.0
Salix nigra	Black Willow	5	Yes	0.0	0.0	2.4	0.0	1.3	0.0	0.0	0.0
Verbena hastata	Blue Vervain	4	Yes	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0
Calamagrostis canadensis	Blue-joint	3	Yes	0.0	1.9	0.0	47.3	0.0	0.0	0.0	28.5
Salix pedicellaris	Bog Willow	8	Yes	0.0	0.0	0.0	0.3	0.0	0.0	0.0	0.0
Eupatorium perfoliatum	Boneset	4	Yes	0.0	0.0	2.6	0.0	0.0	0.0	0.4	0.0
Sagittaria latifolia	Broad-leaved Arrowhead	1	Yes	0.0	0.0	0.0	1.5	0.0	0.0	0.0	0.0
Typha latifolia	Broad-leaved Cattail	1	Yes	0.0	0.0	0.0	0.8	0.0	0.0	3.2	1.8
Andropogon virginicus	Broom Sedge	4	Yes	0.0	0.0	0.5	2.5	0.0	0.0	0.0	0.0
Cephalanthus occidentalis	Buttonbush	7	Yes	1.2	6.4	0.0	0.0	10.1	0.0	0.0	0.0
Cyperus esculentus	Chufa	1	Yes	0.0	3.6	0.9	0.0	0.0	0.0	0.0	0.0
Plantago major	Common Plantain	0	No	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.0
Ambrosia artemisiifolia	Common Ragweed	0	Yes	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0

APPENDIX B

Floristic Data (continued)

SCIENTIFIC NAME	ENGLISH NAME	C	NATIVE?	M1	M2	M3	M4	M5	R1	R2	R3
Populus deltoides	Cottonwood	1	Yes	0.0	0.7	0.1	0.0	1.3	0.0	0.0	0.0
Agrostis stolonifera	Creeping Bentgrass	0	No	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0
Sagittaria cuneata	Cuneate Arrowhead	6	Yes	0.0	0.0	0.0	0.0	0.0	0.0	0.0	5.2
Rumex crispus	Curly Dock	0	No	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Scirpus atrovirens	Dark-green Bulrush	3	Yes	0.4	1.9	9.1	0.8	0.0	0.0	0.0	0.0
Atropa belladonna	Deadly Nightshade	0	No	0.0	0.0	0.0	0.0	0.0	0.0	4.2	0.0
Juncus dudleyi	Dudley's Rush	1	Yes	0.0	0.0	0.2	0.1	0.0	0.0	0.0	0.0
Pilea pumila	Dwarf Clearweed	5	Yes	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0
Plantago lanceolata	English Plaintain	0	No	0.0	0.0	0.0	0.4	0.0	0.0	0.0	0.0
Boehmeria cylindrica	False Nettle	5	Yes	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.4
Mentha arvensis	Field Mint	3	Yes	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.1
Carex vulpinoidea	Fox Sedge	1	Yes	0.0	0.0	0.2	10.8	0.0	0.0	0.0	0.0
Triadenum fraseri	Fraser's St. John's-wort	6	Yes	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.7
Solidago gigantea	Giant Goldenrod	3	Yes	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0
Euthamia graminifolia (Solidago g.)	Grass-leaved Goldenrod	3	Yes	0.0	0.0	0.1	0.0	0.0	0.1	0.0	0.0
Aster pilosus	Hairy Aster	1	Yes	0.0	0.0	0.5	0.0	0.0	0.0	0.0	0.0
Aster pilosus var. pilosus	Hedge-nettle	5	Yes	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0
Typha glauca (hybrid)	Hybrid Cattail	0	No	0.0	0.0	0.0	0.0	0.0	1.1	0.0	0.0

APPENDIX B

Floristic Data (continued)

SCIENTIFIC NAME	ENGLISH NAME	C	NATIVE?	M1	M2	M3	M4	M5	R1	R2	R3
Eleocharis intermedia	Intermediate Spikerush	7	Yes	0.7	0.0	0.7	0.0	0.0	0.0	0.0	0.0
Polygonum persicaria	Lady's-Thumb	0	No	4.8	12.7	0.0	0.0	0.4	0.0	0.0	0.0
Thelypteris palustris (T. thelypteroides)	Marsh Fern	2	Yes	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.2
Asclepias incarnata	Marsh Milkweed	6	Yes	0.0	0.1	0.0	2.8	0.1	0.0	0.0	0.0
Ludwigia palustris	Marsh Seedbox	4	Yes	0.0	4.7	2.1	0.1	0.0	0.0	0.0	0.0
Caltha palustris	Marsh-marigold	6	Yes	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1
Polygonum hydropiperoides	Mild Water-pepper	5	Yes	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Typha angustifolia	Narrow-Leaf Cattail	0	No	47.5	0.1	30.3	10.1	63.2	0.0	0.0	0.0
Bidens cernuus	Nodding Beggar-ticks	3	Yes	0.5	3.1	0.0	0.0	0.0	0.0	0.0	0.0
Campanula aparinoides var. grandiflora	Northern Marsh Bellflower	7	Yes	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.8
Calamagrostis lacustris	Northern Reedgrass	10	Yes	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.8
Eleocharis obtusa	Obtuse Spikerush	3	Yes	0.0	1.6	0.0	0.0	0.0	0.0	0.0	0.0
Aster ontarionis	Ontario Aster	6	Yes	0.0	0.0	0.1	0.4	0.0	0.0	0.0	0.0
Impatiens capensis	Orange Touch-me-not	2	Yes	0.0	0.0	0.0	0.0	0.0	0.0	16.0	0.3
Aster lanceolatus (A. simplex)	Panicled Aster	2	Yes	0.0	0.1	1.0	0.1	0.0	0.0	0.1	0.0
Salix petiolaris	Petioled Willow	1	Yes	0.0	0.7	0.0	0.0	0.0	0.0	0.0	0.0

APPENDIX B

Floristic Data (continued)

SCIENTIFIC NAME	ENGLISH NAME	C	NATIVE?	M1	M2	M3	M4	M5	R1	R2	R3
Pontederia cordata	Pickerel-weed	8	Yes	0.0	0.0	0.0	0.0	0.0	0.0	0.0	4.1
Spartina pectinata	Prairie Cordgrass	5	Yes	0.0	0.0	7.1	0.0	7.0	0.0	0.0	0.0
Lythrum salicaria	Purple Loosestrife	0	No	0.0	2.9	1.4	0.0	0.8	0.0	0.0	17.8
Salix discolor	Pussy Willow	1	Yes	0.0	0.0	0.0	0.0	0.3	0.0	0.0	0.3
Fraxinus pennsylvanica	Red Ash	2	Yes	0.0	0.0	0.5	0.0	0.0	0.0	0.0	0.0
Acer rubrum	Red Maple	1	Yes	0.0	0.4	0.0	0.0	0.0	0.0	0.0	0.0
Eleocharis erythropoda	Red-foot Spikerush	4	Yes	0.0	0.0	0.0	0.0	4.8	0.0	0.0	0.0
Cornus stolonifera	Red-osier Dogwood	2	Yes	0.0	0.0	0.0	0.3	0.0	0.0	0.0	0.0
Phalaris arundinacea	Reed Canary Grass	0	Yes	23.0	0.0	13.0	0.0	1.5	98.1	61.5	0.0
Leersia oryzoides	Rice Cut-grass	3	Yes	6.7	0.4	19.6	7.3	4.4	0.0	3.4	0.0
Carex utriculata (C. rostrata)	Rostrate Sedge	5	Yes	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1
Salix exigua (S. interior)	Sandbar Willow	1	Yes	0.0	0.0	0.0	1.3	1.3	0.0	0.0	0.0
Onoclea sensibilis	Sensitive Fern	2	Yes	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2
Juncus effusus	Soft Rush	3	Yes	0.0	58.9	0.5	4.3	0.0	0.0	0.0	0.0
Scirpus validus (Schoenoplectus tabernaemontani)	Soft-stem Bulrush	4	Yes	11.9	0.0	0.0	0.1	3.4	0.0	0.0	0.0

APPENDIX B

Floristic Data (continued)

SCIENTIFIC NAME	ENGLISH NAME	C	NATIVE?	M1	M2	M3	M4	M5	R1	R2	R3
Eupatorium maculatum (Eupatoriadelphus m.)	Spotted Joe-pye-weed	4	Yes	0.0	0.0	0.0	0.0	0.0	0.0	7.1	0.0
Carex stipata	Stipitate Sedge	1	Yes	0.0	0.0	0.9	0.0	0.0	0.0	0.0	0.0
Carex stricta	Strict Sedge	4	Yes	0.0	0.0	0.0	0.0	0.0	0.0	0.0	11.5
Aster puniceus (incl. A. firmus)	Swamp Aster	5	Yes	0.0	0.0	0.0	0.0	0.0	0.0	3.9	0.0
Rosa palustris	Swamp Rose	5	Yes	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.9
Potentilla arguta	Tall Cinquefoil	8	Yes	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.7
Solidago altissima	Tall Goldenrod	1	Yes	0.0	0.0	0.0	4.2	0.0	0.7	0.0	0.0
Lysimachia terrestris	Terrestrial Loosestrife	6	Yes	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1
Bidens comosus (B. tripartitus)	Three-awned Beggar-ticks	5	Yes	0.0	0.0	0.0	0.4	0.0	0.0	0.0	0.0
Phleum pratense	Timothy	0	No	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.0
Juncus bufonius	Toad Rush	2	Yes	0.0	0.3	0.0	0.0	0.0	0.0	0.0	0.0
Scirpus torreyi (Schoenoplectus t.)	Torrey's Bulrush	10	Yes	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0
Juncus torreyi	Torrey's Rush	4	Yes	0.0	0.0	0.3	0.0	0.0	0.0	0.0	0.0
Carex tribuloides	Tribulus Sedge	3	Yes	0.0	0.0	0.5	0.0	0.0	0.0	0.0	0.0
Vaccinium myrtilloides	Velvetleaf Blueberry	4	Yes	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0

APPENDIX B

Floristic Data (continued)

SCIENTIFIC NAME	ENGLISH NAME	C	NATIVE?	M1	M2	M3	M4	M5	R1	R2	R3
Polygonum amphibium	Water Smartweed	6	Yes	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.5
Lycopus uniflorus	Water-horehound	2	Yes	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1
Polygonum hydropiper	Water-Pepper	0	No	0.0	0.0	0.0	0.0	0.0	0.0	0.0	11.0
Alisma plantago-aquatica (A. subcordatum)	Water-plaintain	1	Yes	1.2	0.0	0.7	0.0	0.0	0.0	0.0	0.0
Rumex verticillatus	Whorled Dock	7	Yes	0.0	0.0	0.2	3.6	0.0	0.0	0.0	0.0
Carex alata	Winged Sedge	10	Yes	0.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Scirpus cyperinus	Wool-grass	5	Yes	0.0	0.7	3.0	0.0	0.0	0.0	0.0	4.3
Salix eriocephala (S. rigida)	Woolly-headed Willow	2	Yes	0.0	0.0	0.9	0.0	0.0	0.0	0.0	0.0