



Genetic Structure and Morphometric Variation among Fragmented Michigan Wild Rice Populations

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Abstract

Wild rice (*Zizania spp.*) has ecological and cultural importance in the Great Lakes region, but has been declining due to habitat loss and fragmentation. We investigated the potential impact of bed area and isolation by distance on genetic structure, reproductive effort, and morphometrics in populations of two wild rice species (*Z. palustris* and *Z. aquatica*) in Michigan. Leaves were analyzed with Amplified Fragment Length Polymorphisms (AFLPs) and stem height, leaf length, panicle length, and number of male and female flowers were recorded. Despite finding multiple genetic clusters in each species, we found no geographic pattern to the clusters or any isolation by distance. Correlations revealed no associations between bed area and AFLP band diversity, nor bed area and morphometric traits. The number of female flowers was positively correlated with AFLP band diversity, demonstrating a possible impact of genetic diversity on reproductive effort. The results of this study suggest that habitat loss is not yet affecting the genetic diversity of wild rice in Michigan, possibly because of long distance dispersal vectors facilitating gene flow. Nevertheless, the ongoing decline of populations shows their need for protection and restoration, such as through more seeding efforts.

Keywords Wild rice · Genetic structure · Great Lakes region · Plant morphometry · Habitat loss

Introduction

Habitat loss and fragmentation are known to have adverse effects on ecosystems, including a loss of species at both local and global scales (Fahrig 1997; Brook et al. 2008). One driver of such declines is decreased genetic diversity. Small population size and isolation resulting from human induced habitat changes can hinder dispersal between populations (Frankham 1995; Trakhtenbrot et al. 2005), which can increase inbreeding and decrease genetic diversity (Lande 1988; DiBattista 2008). Reduced genetic diversity is problematic for populations as it can lead to the expression of deleterious genetic

traits (Couvet 2002), reduced reproductive output (Frankham 2005), reduced survival and health, and an inability to adapt to environmental changes (Lande 1988; Bijlsma and Loeschcke 2012). As a result, populations are more prone to further decline and extirpation. These cascading effects are collectively termed “the extinction vortex” (Soulé and Mills 1998).

While all organisms are vulnerable to reduced genetic diversity via habitat loss and fragmentation, plants are particularly susceptible. They rely on extrinsic factors such as animals, wind, and water to transport pollen and seeds (Cain et al. 2000; Nathan 2008). The effectiveness of wind and water as dispersal vectors often decreases with distance from a source population (Vittoz and Engler 2007; Nathan 2008). Habitat fragmentation further decreases the ability of pollen or seeds to be transferred between populations (Rathcke and Jules 1993; Aguilar 2006), thus hindering gene flow and decreasing genetic diversity as a result. Reductions in genetic diversity can have negative effects on vegetative traits and reproduction. Williams (2001) observed decreased shoot growth and reproductive effort in populations of eelgrass with low genetic diversity. Moreover, de Vere et al. (2009) found that thistles (*Cirsium dissectum*) from

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genetically depauperate populations produced seedlings with significantly lower survival rates.

One plant whose populations may be experiencing the extinction vortex is wild rice (*Zizania* spp.). Wild rice is a wetland grass, with three annual species (*Z. aquatica*, *Z. palustris*, and *Z. texana*) occurring in North America and one perennial species (*Z. latifolia*) occurring in Asia (Oelke 1993; Guo et al. 2007). It is important to wetland ecosystems as it provides habitat for wetland fauna (Moulton 1979; Haramis and Kearns 2007) and enriches lake sediments with nutrients (Engel and Nichols 1994). Native Americans consider wild rice sacred. They harvest it in annual ceremonies and it plays an important role in their economies (Vennum 1988; Kjerland 2015).

Z. aquatica and *Z. palustris* are native to Great Lakes wetlands, but populations have been declining, particularly in Michigan (Michigan Natural Features Inventory 2009; Pillsbury and McGuire 2009), due to habitat loss and fragmentation (Engel and Nichols 1994), high water levels (Vennum 1988; Drewes and Silbernagel 2012), competition (Clay and Oelke 1987; Engel and Nichols 1994), herbivory (Moulton 1979; Haramis and Kearns 2007; Johnson and Havranek 2010), and agriculture (Engel and Nichols 1994; Madsen et al. 2008; Kjerland 2015). Habitat loss and fragmentation are the most concerning threats, as wild rice habitat has been reduced by over 30% in the past 100 years (Vennum 1988; Drewes and Silbernagel 2012). It has been associated with genetic diversity loss in wild rice populations in previous studies. Lu et al. (2005) found that small populations of *Z. palustris* in Wisconsin had lower isozyme diversity (protein diversity, an analogue for genetic diversity) and fewer seed panicles per plant than large populations. In a study on the Asian species, *Z. latifolia*, Chen et al. (2012) observed high heterozygosity and minimal genetic distance between populations using microsatellites despite habitat loss and fragmentation. Lastly, Xu et al. (2015) compared the genetic structure of *Zizania* populations in North America to those in East Asia using chloroplast DNA (cpDNA) and microsatellites and found much greater genetic differentiation among the North American populations; however, no populations in Michigan were assessed. Similar population genetics studies on Michigan wild rice are needed. Michigan is central to the Great Lakes region and links east coast and Midwest populations, yet the Lower Peninsula may also be isolated on three sides by the Great Lakes. Wild rice populations in Michigan are fragmented, and one of the species, *Z. aquatica*, is state threatened.

We examined the genetic structure, reproductive effort, and morphometric traits of wild rice in Michigan and potential impacts of bed area and isolation by distance. We also investigated the potential impacts of genetic diversity on reproductive effort and morphometry. *Z. aquatica* and *Z. palustris* genetic structure was investigated using Amplified Fragment

Length Polymorphisms (AFLPs). AFLPs involve the selective amplification of DNA fragments cut by restriction enzymes. This genetic analysis produces large quantities of polymorphisms and a high degree of resolution for quantifying genetic variation in a population (Vos et al. 1995; Meudt and Clarke 2007; Mutegei et al. 2016). No previous study has used these markers for analysis of wild rice populations. Altogether, the aim of this study is to investigate implications of habitat loss and fragmentation on Michigan wild rice and to understand how populations might be prone to further decline. The results will specifically help Native American tribes and state agencies make informed management decisions for the preservation of wild rice in Michigan. The overall approach of this study, however, may be more broadly applied to monitoring and management strategies of many native wetland plant species.

Methods

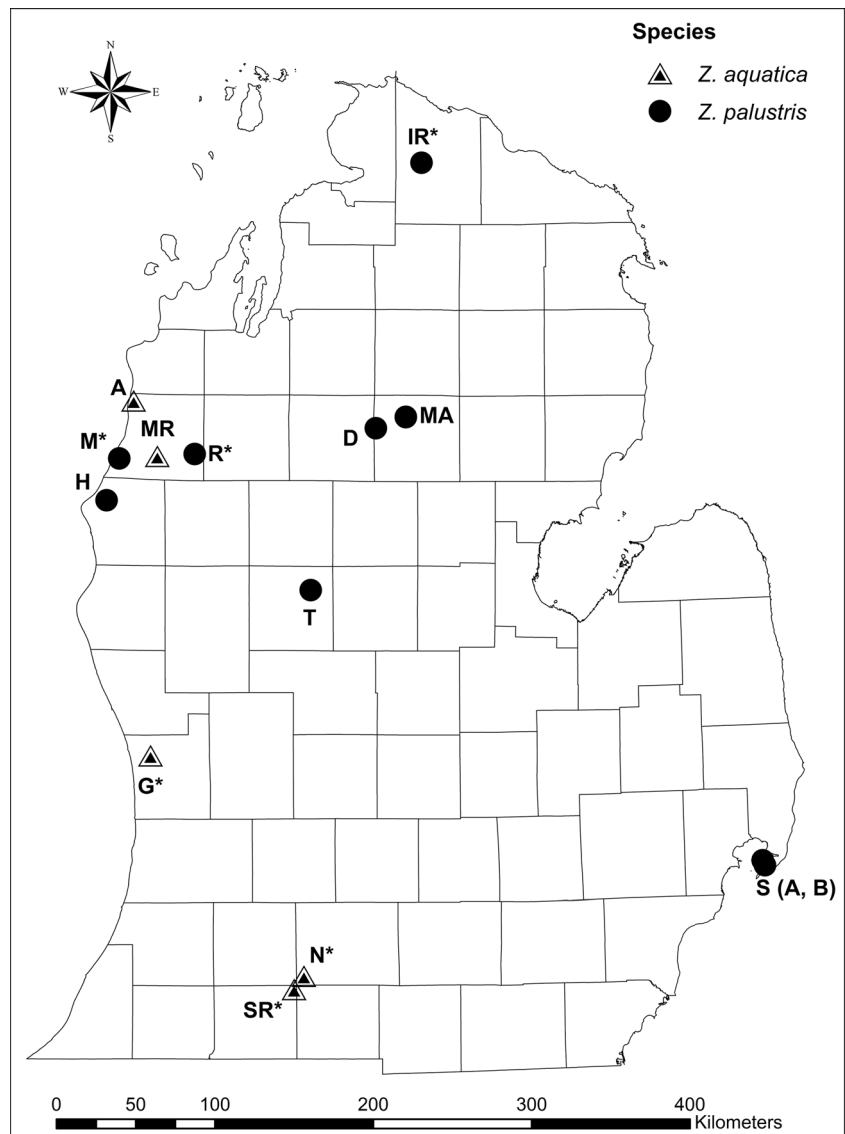
Study Species

Zizania palustris and *Z. aquatica* are annual plants that thrive in wetland environments, particularly lakesides and riverbanks (Oelke 1993; Drewes and Silbernagel 2012). Individuals are monoecious and have both male and female reproductive structures. On each plant, female flowers will open prior to male flowers, thus minimizing self-fertilization (Oelke 1993; Lu et al. 2005). Pollen is typically wind dispersed, and seeds can be dispersed via water or animals (Lu et al. 2005; Michigan Natural Features Inventory 2009). Species can be distinguished by their flowers and stem height. *Z. palustris* has purple flowers and short stems (<2 m tall), while *Z. aquatica* has yellow-green flowers and is taller (2–3 m). *Z. palustris* is most commonly found in shallow lakes, while *Z. aquatica* is often associated with riverine habitat (Terrell et al. 1997; Michigan Natural Features Inventory 2009).

Study Sites

We selected 14 sites to sample, which included most of the large wild rice populations in the Lower Peninsula of Michigan (Fig. 1). These sites represent most wild rice populations in the Lower Peninsula of Michigan. Nine of these sites contained *Z. palustris*: Indian River (Cheboygan County), Redbridge (Manistee County), Manistee Lake (Manistee County), Hamlin Lake (Mason County), Marl Lake (Roscommon County), Deadstream Flooding (Roscommon County), Tubbs Lake (Mecosta County), and two sites in the St. Clair River delta (St. Clair County). The remaining five sites contained *Z. aquatica*: Arcadia Marsh (Manistee County), Manistee River (Manistee County), Grand River

Fig. 1 Map of wild rice sites sampled for genetics and morphometrics in Michigan August–September 2013. IR = Indian River, A = Arcadia Marsh, M = Manistee Lake, MR = Manistee River, R = Redbridge, H = Hamlin Lake, MA = Marl Lake, D = Deadstream Flooding, T = Tubbs Lake, G = Grand River, S = Lake Saint Clair (2 sites), N = Nottawa Creek, SR = Short's Road. Asterisks (*) indicate known seeded sites



(Ottawa County), Nottawa Creek (Calhoun County), and Short's Road (St. Joseph County). Only one species is present at each site, thus minimizing concerns of sampling hybrids.

Sampling Methods

Sampling occurred from August 2013 through September 2013 when plants were producing seed. We sampled twenty wild rice plants from each site, with the exceptions of Hamlin Lake and Lake Saint Clair. Because of its large size, we divided the rice bed at Hamlin Lake into three sub-sites: north, middle, and south. We sampled ten plants at each sub-site (30 total plants). St. Clair contained two distinct patches of wild rice separated by 2.5 km, so we sampled 20 plants from each. Plants were randomly selected throughout the entire area of the bed, with a minimum separation of 5 m between samples. We measured stem height (from sediment to first male

flower branch) and the length of the topmost leaf. Then we removed the panicle and stored it for later flower count. At some *Z. aquatica* sites, where seeds had not yet fallen, we took photographs of the panicle to prevent flower loss in this threatened species. Because of the difficulty in getting an accurate seed count, we used flowers as a proxy for reproductive effort. One leaf was removed from each plant for genetic analysis. We kept samples on ice until they could be brought back to the laboratory, where we stored the panicles at 4 °C and the leaves at –80 °C.

We mapped the rice bed using the program TerraSync on a Trimble Juno SC GPS unit (Trimble Navigation Limited, Sunnyvale, CA) to calculate bed area for use in later analyses. In most cases, we created a polygon feature by traveling along the perimeter of the rice bed. In some instances, however, parts of the rice bed perimeter were impossible to access and we plotted point features at accessible corners of the rice bed.

We exported GPS polygon and point features collected at each bed to ArcMap v.10.1 (ESRI, Redlands, CA) for analysis. We determined complete perimeters for those beds with incomplete polygons by overlaying field data on to the ESRI World Imagery layer in ArcGIS (LANDSAT satellite imagery provided by NASA and USGS, accessed in 2014) at 3 m resolutions. Once all boundaries had been completed, we recorded the bed area from the file's attribute table.

In the laboratory, we measured panicle length between the node of the lowest male flower branch and the pedicel of the topmost female flower. We estimated the number of male and female flowers by counting pedicels because many flowers had fallen before sampling. Male pedicels are much smaller than female pedicels, so we distinguished the sexes with ease.

Genetic Analysis

The wild rice leaves were ground in a lysis buffer using a Qiagen TissueRuptor (Qiagen Inc., Valencia, California, USA) and extracted DNA using a NucleoSpin Plant II DNA extraction kit (Macherey-Nagel Inc., Bethlehem, Pennsylvania, USA). We assessed DNA concentration and quality using a NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific, Waltham, Massachusetts). In cases of poor DNA quality, we performed a gel extraction using a NucleoSpin Gel and PCR Clean-up kit (Macherey-Nagel Inc., Bethlehem, Pennsylvania). Amplified Fragment Length Polymorphisms (AFLPs) were used to assess genetic structure of Michigan wild rice sites. We digested extracted DNA with *EcoRI* and *MseI* restriction enzymes, followed by a pre-selective PCR amplification and a selective PCR amplification. For detailed AFLP methods, see Online Resource 1.

We analyzed the AFLP fragments using an ABI310 Genetic Analyzer (Thermo Fisher Scientific, Waltham, Massachusetts) and GeneMapper (Thermo Fisher Scientific, Waltham, Massachusetts). Peaks were produced for every fragment of a unique length detected. Since signal sensitivity varied among plates, we scored peaks differentially. We scored peaks on plates with relatively low signal sensitivity if they were greater than 100 fluorescence units, while we scored peaks on plates with relatively high signal sensitivity if they were greater than 300 fluorescence units. This normalization produced data where the average number of fragments per individual was consistent for all plates. Additionally, we reran 5 randomly selected individuals from each population to determine the error rate in band detection. The resulting dataset was a presence/absence matrix of individuals and their fragments.

Population Genetic Structure

We used the program Structure (Pritchard et al. 2000) to assess the number of genetic clusters for both species (Poelchau and

Hamrick 2012; Gao et al. 2016; Zaya et al. 2017). The settings we used for Structure were no prior location information, an admixture model with correlated allele frequencies, and 100,000 burn-in iterations followed by 100,000 collection iterations. We tested a range of 2–9 genetic clusters (K). After five replicates were completed, we used the program Structure Harvester to determine K and likelihood (ΔK ; Evanno et al. 2005). We also looked for convergence in the MCMC runs. We performed a Principal Coordinate Analysis (PCoA; Nguyen et al. 2013; Zaya et al. 2017) in R (R Foundation for Statistical Computing, Vienna, Austria) using Jaccard distances of the AFLP band data, which allowed us to compare the results of Structure to the results of a model not based on population genetics. Using the program GenAEx 6.5 (Peakall and Smouse 2006), we performed an analysis of molecular variance (AMOVA) to determine the variance among and within sites. We also used GenAEx to calculate Nei's genetic distance, which we compared to geographic distance using a Mantel Test (Árnason et al. 2014; Tang et al. 2016; Zaya et al. 2017).

Morphometric Variation and Correlation Analyses

We evaluated differences in mean morphometric traits between wild rice species using a t-test or Mann-Whitney U Test (if data were not normal) in Minitab v.16 (Minitab Inc., State College, PA). We performed Pearson correlations to look for relationships between bed area, AFLP band diversity, and morphometric traits. For these correlations, we averaged morphometric characteristics at each site to investigate population level effects. Since there were few *Z. aquatica* sites, we only performed correlations using *Z. palustris* data. Bed area did not have a normal distribution and had to be log transformed. Because number of male and female flowers are not continuous variables, Spearman rank correlations were used to test these variables for associations. In order to reduce the risk of Type I errors, we applied Bonferroni corrections to t-test and correlation analyses.

Results

Genetic Structure

We were able to amplify and score 195 AFLP bands for both species. The mean percentage of polymorphic loci within sites was $52.54 \pm 3.28\%$ for *Z. palustris* and $43.90 \pm 5.89\%$ for *Z. aquatica*. Our quality control measures for band identity across the 70 individuals was 97%. Structure indicated that there were most likely 3 genetic clusters of *Z. palustris* from the 9 sites across the lower peninsula of Michigan ($\Delta K = 141.52$; Fig. 2a). Alpha values converged to a range of approximately 0.1, which is well within the range of alpha

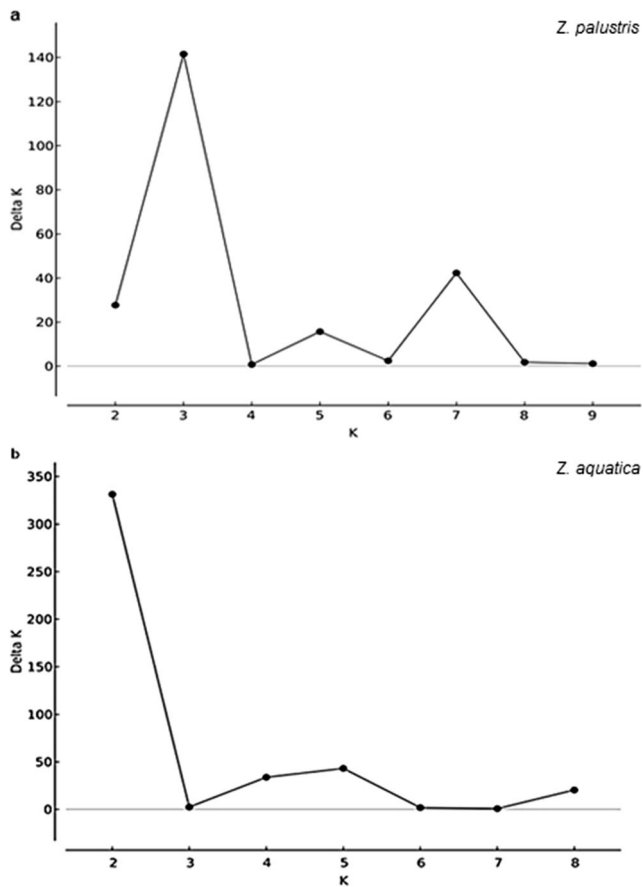


Fig. 2 Delta K plots for *Z. palustris* (a) and *Z. aquatica* (b) sites sampled in Michigan in 2013. They show possible number of genetic clusters (K) and their likelihood (Delta K) and were generated using the program Structure Harvester

values that suggest convergence in the MCMC models of alpha ranging within 0.2 (Pritchard et al. 2000; Gilbert et al. 2012). Further inspection of the Q-plot, however, suggested that this structuring is weak (Fig. 3a). All sites except Hamlin Lake and Marl Lake contained a mixture of the genetic clusters. Between 25 and 60% of individuals in these sites could not be assigned to a single genetic cluster with assignment probability of $\geq 80\%$ (Sacks et al. 2004; Menchari et al. 2007; Baumgartner et al. 2010). The Principal Coordinate analysis (PCoA) supports these results (Fig. 4a). AFLP band composition is similar among 6 sites. Hamlin Lake and Marl Lake have AFLP band combinations distinct from those 6 sites; however, Marl Lake has a band combination similar to that of Indian River. Weak genetic structure in *Z. palustris* was further supported by AMOVA results. Though significant (d.f. = 8, $\Phi_{PT} = 0.152$, $p = 0.01$), variation among sites only explained 15% of total genetic variation. Nei's genetic distance (0.005–0.021) indicated little genetic differentiation between sites (Table 1a), and was not related to geographic distance (Mantel test, $p = 0.230$, $R^2 = 0.0393$; Fig. 5a).

All AFLP bands observed at *Z. palustris* sites occurred with a frequency $\geq 5\%$, except for Hamlin Lake, where only

half of the bands occurred at a frequency of 5% or more (Fig. 6a). Hamlin Lake and Marl Lake had the lowest band diversity, while Deadstream, Manistee Lake, and Saint Clair B had the highest band diversity. The proportion of private bands was low (0–2.5%) at all sites (Fig. 6).

Structure revealed two genetic clusters of *Z. aquatica* from the 5 sites across the lower peninsula of Michigan ($\Delta K = 331.20$; Fig. 2b) and alpha values converged to a range of approximately 0.1. However, structuring was also weak. Based on the Q-plot, geographic populations did not include single genetic clusters, with the possible exception of Grand River and Short's Road (Fig. 3b). Between 10 and 30% of individuals could not be assigned to a genetic cluster with confidence based on an assignment probability of $\geq 80\%$. The Principal Coordinate analysis (PCoA) supports these results (Fig. 4b). AFLP band composition is similar among all 6 sites, although Grand River and Short's Road appear somewhat distinct. Weak genetic structure in *Z. aquatica* was further supported by AMOVA results. Though significant (d.f. = 4, $\Phi_{PT} = 0.134$, $p = 0.01$), variation among sites only explained 13% of total genetic variation. Nei's genetic distances (0.014–0.035) indicated little genetic differentiation between sites (Table 1b), and similarly to *Z. palustris*, genetic distance was not related to geographic distance (Mantel test, $p = 0.08$, $R^2 = 0.0995$; Fig. 5b).

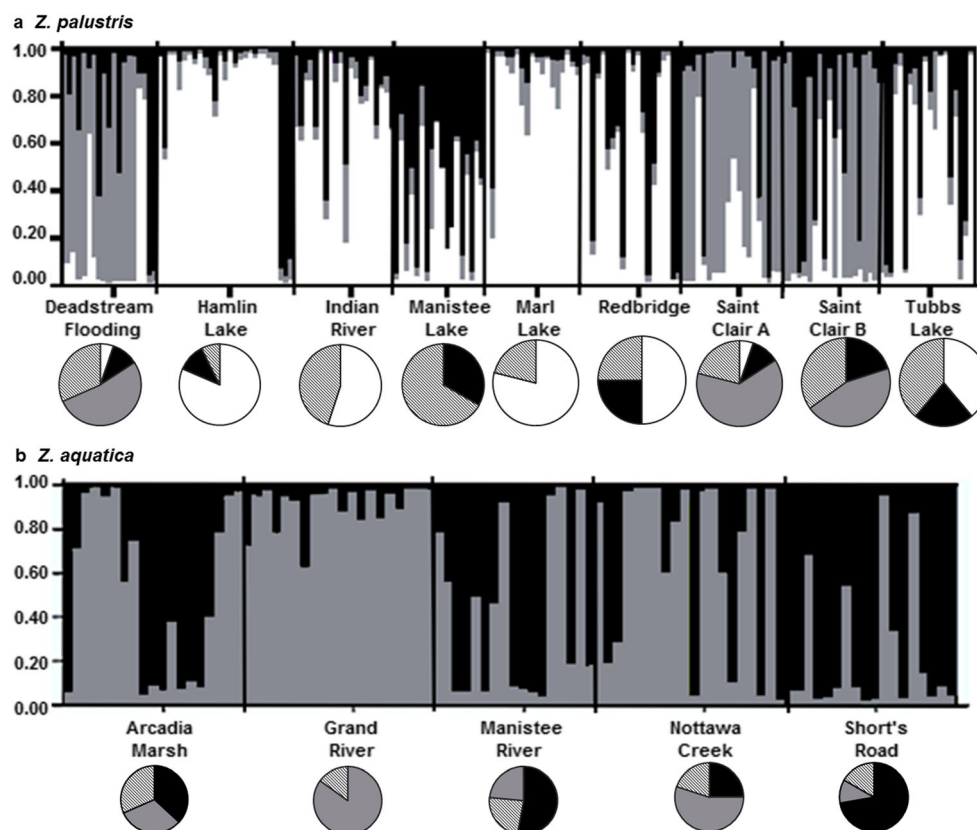
At *Z. aquatica* sites, all AFLP bands occurred at a frequency $\geq 5\%$ or more (Fig. 6b). Average band diversity was relatively consistent across sites, except for Grand River which had half the average band diversity of the other sites. The proportion of private bands was variable (0–19%) among sites, with Arcadia Marsh having the greatest number and Grand River the fewest (Fig. 6).

Morphometric Variation and Correlation Analyses

There were significant differences in all 5 morphometric traits between species and variation in traits within each species was apparent (Table 2). *Z. aquatica* was taller, had longer leaves, longer panicles, and produced more male and female flowers. These differences remained significant after Bonferroni corrections, although differences in panicle length became marginally significant. The coefficient of variation (CV) for each trait, except number of flowers, was similar between the two rice species. The CV for number of male and female flowers was higher for *Z. aquatica* than *Z. palustris*.

Correlations were performed to look for relationships between bed area, AFLP band diversity, and morphometrics in *Z. palustris*. Rice bed area ranged from 40 m² (Manistee Lake) to 2.7 million m² (Hamlin Lake), and bed area was more variable for *Z. palustris* than *Z. aquatica* (Fig. 7). No significant relationship was found between bed area and AFLP band diversity ($p = 0.060$). Bed area was positively related to stem height ($p = 0.047$), but this relationship was deemed

Fig. 3 Structure Q-plots for *Z. palustris* ($K=3$) (a) and *Z. aquatica* ($K=2$) (b) sites sampled in Michigan in 2013. The horizontal axis shows individuals and their site locations. The vertical axis shows the proportion of each individual's genetic make-up that belongs to each genetic cluster, represented by different colors. The corresponding pie charts show assignment of individuals to genetic clusters. An individual was assigned to a genetic cluster if 80% or more of its genetic make-up could be attributed to that cluster. Wedges with parallel lines indicate individuals who could not be assigned based on this benchmark



insignificant after a Bonferroni correction. No other plant traits were correlated with bed area. AFLP band diversity was not significantly associated with stem height, leaf length, panicle length, or number of male flowers. However, the correlation between AFLP band diversity and number of female flowers was marginally significant after a Bonferroni correction (corrected $\alpha = 0.005$, $p = 0.006$, $r = 0.828$; Fig. 8).

Discussion

The results of this study show that habitat size and geographic distance have little impact on the genetic structure, reproductive effort, and morphometrics of Michigan wild rice. Structure and Nei's genetic distance suggest that *Z. palustris* and *Z. aquatica* sites are connected through gene flow despite geographic distance and have maintained similar levels of genetic variation regardless of bed area. We did, however, observe lower genetic diversity compared to other studies evaluating grasses with AFLP markers (Ozbek et al. 2007; Wang et al. 2010; Todd et al. 2011). Thus, Michigan wild rice may not be at risk of extirpation by means of the extinction vortex, although some isolated populations may be vulnerable to genetic drift in the future. Our data show that reproductive effort (number of female flowers) decreases in beds with low AFLP band diversity. While evidence for gene flow is a promising indication that genetic diversity can be maintained in

populations, excessive amounts of gene flow may be deleterious because introduced genes can break apart coadapted gene complexes (Burton et al. 1999; Stelkens et al. 2015; Arnold 2016).

Weak genetic clustering in Structure and the genetic similarity of sites implies gene flow among *Z. palustris* sites. Two of the sites, Hamlin Lake and Marl Lake, consisted of a single dominant genetic cluster. These sites also had the lowest genetic diversity, which suggests that these sites may be receiving few alleles through gene flow and may be vulnerable to genetic drift (Slatkin 1987; Frankham 2005). Marl Lake, a small site, had a low number of AFLP bands and lacked both rare and private bands. When few bands dominate the gene pool of a small group of individuals, genetic drift may be occurring, as the strength of genetic drift is inversely related to effective population size. Hamlin Lake, a large site, presents a different situation as it has maintained private and rare bands despite low AFLP band diversity. This site is likely less affected by genetic drift. Although no significant correlation existed between AFLP band diversity and bed area, the massive bed area of Hamlin Lake might protect it from the negative effects of genetic drift and inbreeding.

Likewise, weak genetic clustering and genetic similarity also implies gene flow among *Z. aquatica* sites. The Short's Road and Nottawa Creek sites were dominated by different genetic clusters. However, these sites are on the same stream less than 8 km apart. So, it is unlikely that they represent

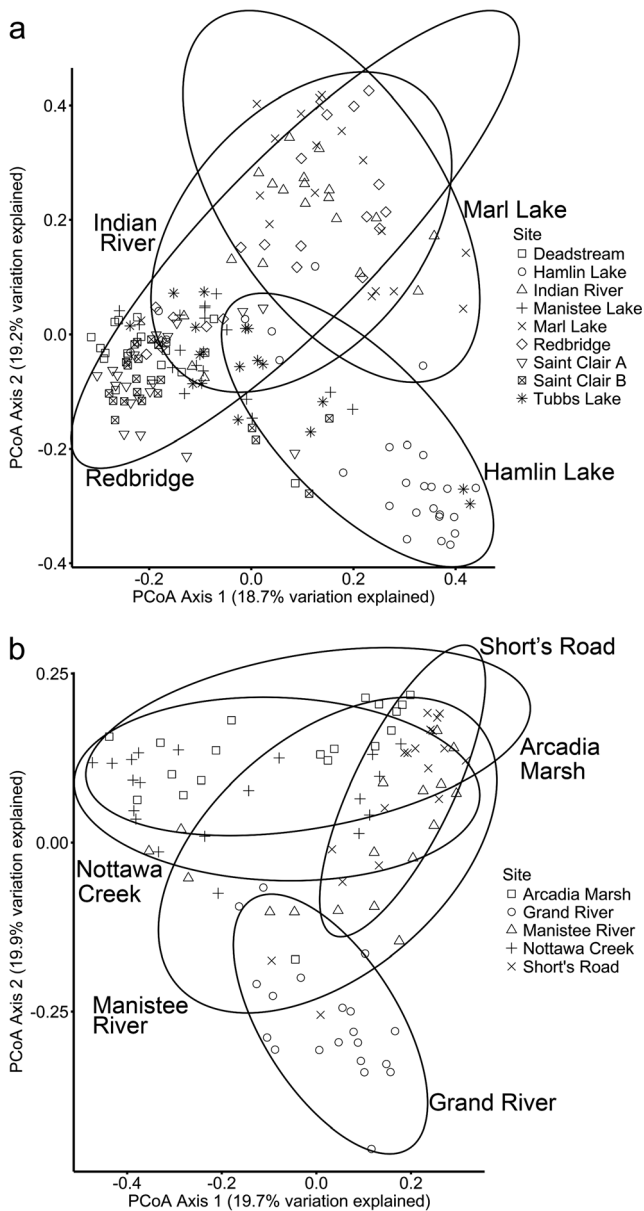


Fig. 4 Principal Coordinate Analysis (PCoA) of AFLP bands from *Z. palustris* (**a**) and *Z. aquatica* (**b**) individuals sampled in Michigan in 2013. AFLP band data were initially a presence/absence matrix, but were transformed into a Jaccard distance matrix prior to analysis. Symbols for individual points represent site locations. Ninety-five percent confidence ellipses are shown for select sites that appear distinct

naturally occurring distinct genetic strains. Dispersal should connect these sites, albeit likely in one direction only, and maintain genetic similarity. In addition, both sites were historically sown (Fig. 1), likely with seed from the same source. Indeed, Nei's genetic distance (0.035) indicated little genetic differentiation. So, we argue that only one genetic cluster truly exists for *Z. aquatica* in Michigan. Dominance by one distinct genetic cluster at the Grand River site could be explained by low genetic

diversity (AFLP bands) compared to other *Z. aquatica* sites. Among *Z. aquatica* sites, Grand River is most in danger of the extinction vortex. The high percentage of private AFLP bands at Arcadia Marsh (50%) suggests that this site may have recently developed from a seed bank with novel genetic diversity.

Lu et al. (2005) performed a similar study on Wisconsin *Z. palustris* populations using isozymes and found much different results. Low genetic diversity and high genetic distances between populations were revealed, and bed size and isolation appear to drive these trends. In contrast, *Z. latifolia* populations in China were found to have high genetic diversity and weak genetic structuring (Chen et al. 2012) with microsatellite markers. Xu et al. (2015) found significant genetic structure in *Zizania* populations across North America with two genetic clusters corresponding to *Z. aquatica* and *Z. palustris*. Each species also separated out into two clusters. Within the Great Lakes region, however, each species was considered a single genetic population. Interestingly, the findings of the latter studies are more similar to our results than the Wisconsin study. This suggests that population response to habitat fragmentation varies across localities and is not necessarily consistent across closely related species. Demographic and evolutionary factors, such as effective population size and time since divergence, may play a role (Richardson et al. 2016) and genetic markers experience differential sensitivities to these effects.

Based on the genetic similarity of Michigan wild rice sites, it is clear that these populations are well connected via dispersal, either natural or anthropogenic. Wild rice is a wind-pollinated plant, and while wind can occasionally carry wild rice pollen long distances, most dispersal occurs within a site or among nearby sites (Lu et al. 2005). An experiment by Oxley et al. (2008) found that *Zizania texana* pollen rarely traveled more than 0.75 m from the source plant. If pollen dispersal was a main mechanism for gene flow, our data would have shown stronger differentiation of wild rice sites and a significant relationship between geographic and genetic distance. Therefore, other long-distance dispersal mechanisms must exist.

Seeds are likely the primary dispersing structures for wild rice, and it has been suggested that possible vectors for such dispersal include water and animals (Lu et al. 2005; Chen et al. 2012). Water is an unlikely vector over long distances, because the wild rice seeds are heavy and do not float for long (Kjerland 2015). Animals, particularly migratory waterfowl, are effective at carrying seeds long distances because of their mobility and ability to traverse different types of terrain (Nathan 2008). In the field, we frequently observed mute swans and Canada geese foraging in wild rice beds. It is possible that the long awns of wild rice seeds get lodged in the

Table 1 Nei's genetic distance between *Z. palustris* (a) and *Z. aquatica* (b) sites in sampled in Michigan in 2013

a.									
	Deadstream Flooding	Hamlin Lake	Indian River	Manistee Lake	Marl Lake	Redbridge	Saint Clair A	Saint Clair B	Tubbs Lake
Hamlin Lake	0.018								
Indian River	0.021	0.010							
Manistee Lake	0.012	0.010	0.012						
Marl Lake	0.020	0.013	0.009	0.016					
Redbridge	0.018	0.010	0.005	0.009	0.010				
Saint Clair A	0.011	0.014	0.014	0.012	0.021	0.013			
Saint Clair B	0.010	0.014	0.016	0.010	0.021	0.014	0.005		
Tubbs Lake	0.013	0.005	0.008	0.006	0.013	0.007	0.010	0.010	
b.									
	Arcadia Marsh	Grand River	Manistee River	Nottawa Creek	Short's Road				
Grand River	0.025								
Manistee River	0.021	0.015							
Nottawa Creek	0.021	0.025	0.025						
Short's Road	0.030	0.029	0.014	0.035					

feathers of waterfowl and are dispersed when the birds move between foraging sites (Vivian-Smith and Stiles 1994).

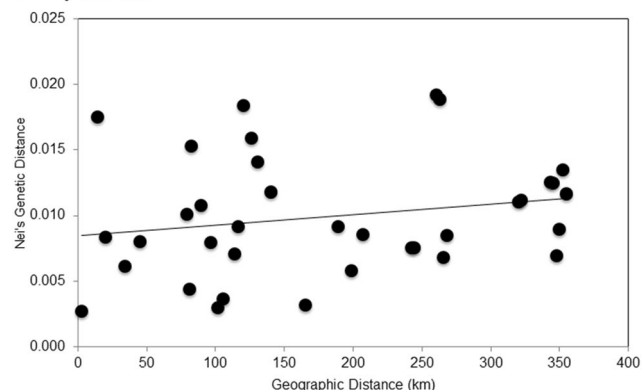
Humans may also be an important dispersal vector that functions independently of distance. As wild rice is an

important part of their culture, Native Americans in the Great Lakes region have planted rice seed to supplement populations and promote growth in new locations. Trade between tribes has also occurred (Vennum 1988; Drewes and Silbernagel 2005). In addition, managers and duck hunters are responsible for sowing wild rice seed, as this maintains stable wild rice populations and attracts waterfowl (Engel and Nichols 1994; Drewes and Silbernagel 2012). Sowed and traded seed from genetically distinct locations would facilitate gene flow, producing beds of mixed genetic origin.

Historical records document the planting of rice in Michigan (Fig. 1). The Indian River site (*Z. palustris*) was planted by the Michigan Department of Natural Resources in 1918, but the seed source is unknown. The Little River Band of Ottawa Indians planted Manistee Lake and Redbridge (*Z. palustris*) in 2003 with seed from Minnesota (personal communication, A. Smart, Little River Band of Ottawa Indians). The Grand River site (*Z. aquatica*) was sown by state funded environmental managers in the early 1900s, but their efforts were unsuccessful (personal communication, M. Manion, Ottawa County Parks and Recreation). The Nottawa Creek and Short's Road sites are believed to have been sown in the 1950s, but no information exists regarding seed source. These planting records support our hypothesis that humans are facilitating high rates of gene flow among wild rice sites. Human planting—with seeds from outside sources—and waterfowl mediated dispersal could explain why Michigan wild rice sites include a mixture of genetic clusters.

Considering the history of seeding, we have developed new hypotheses to explain the current genetic structure of *Z. palustris* in Michigan. The Hamlin Lake and Marl Lake sites could represent an original Michigan *Z. palustris* genetic

a *Z. palustris*



b *Z. aquatica*

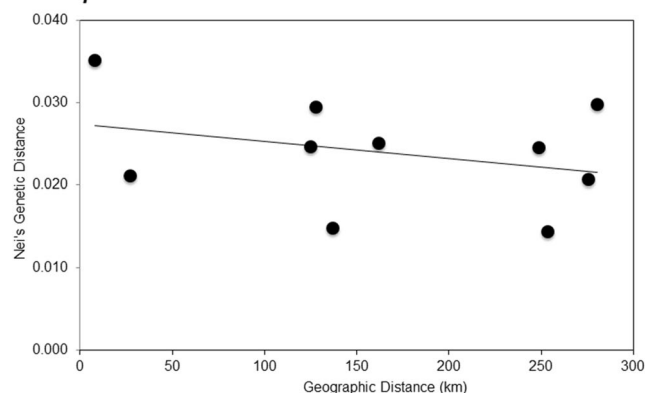
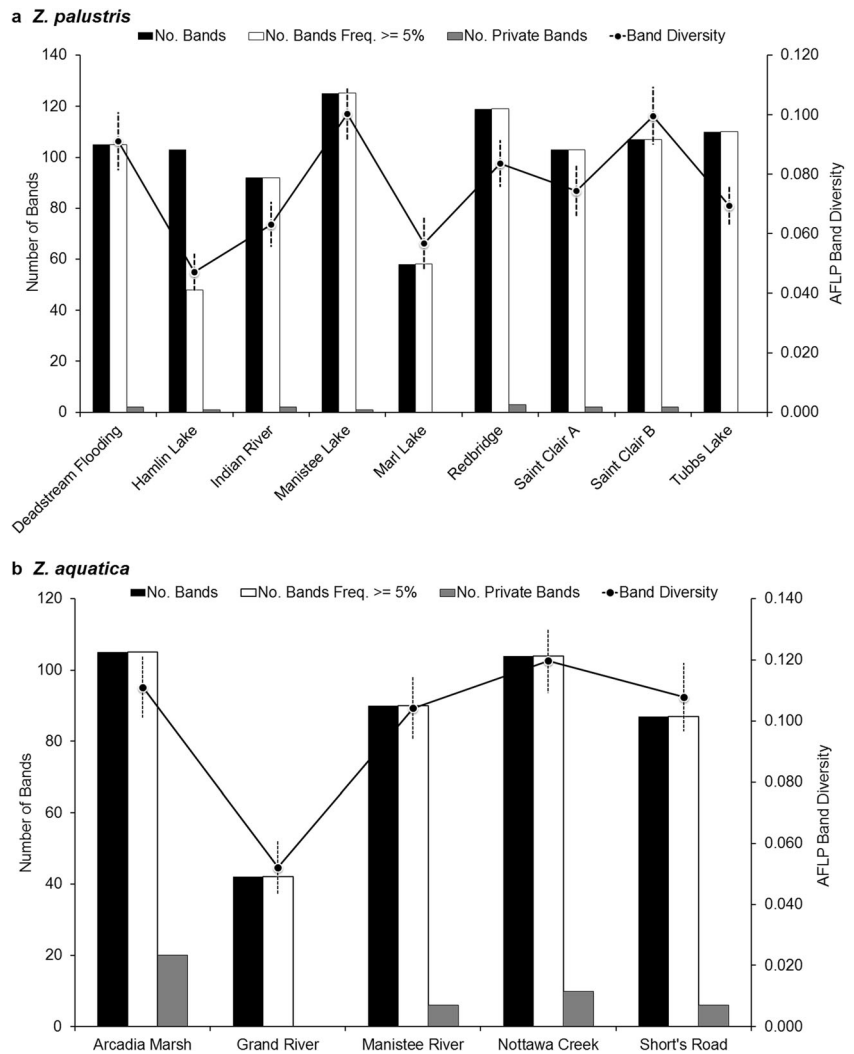


Fig. 5 Relationship between geographic distance and Nei's genetic distance for *Z. palustris* ($p = 0.230$, $R^2 = 0.0393$) (a) and *Z. aquatica* ($p = 0.08$, $R^2 = 0.0995$) (b) sites sampled in Michigan in 2013

Fig. 6 AFLP band frequencies and band diversity in *Z. palustris* (a) and *Z. aquatica* (b) sites sampled in Michigan in 2013



cluster because both contain the same genetic cluster of wild rice (white on the Structure Q-plot; Fig. 3a) and neither have any known seeding history. Since the Manistee Lake and Redbridges sites were sown with seed from Minnesota and have individuals that are likely from a second genetic cluster (black on the Structure Q-plot), we believe these sites could represent a Minnesota strain of wild rice. The Deadstream Flooding and Saint Clair sites contain individuals likely from a third genetic cluster. While we do not have information on other seed sources, we suggest that this could represent a Wisconsin or Canada source. Limited planting records and low genetic differentiation between sites provide little support for two genetic clusters of *Z. aquatica* in Michigan. At this time, it is most parsimonious to conclude that *Z. aquatica* represents a single genetic cluster. More information on planting attempts and seed sources of both *Z. aquatica* and *Z. palustris* sites, however, is necessary to support these conclusions. Comparing DNA from Minnesota, Wisconsin, and Canada wild rice to Michigan wild rice through next-generation DNA sequencing and SNP panels would provide

more concrete information about seed origin. The codominance of SNP markers would allow us to estimate observed heterozygosity and therefore inbreeding, which was a limitation of the current study.

Reproductive effort was the only plant trait that was correlated with genetic diversity. Wild rice populations with greater AFLP band diversity had increased female flower production. Lu et al. (2005) also observed increased reproductive output with greater genetic diversity in Wisconsin *Z. palustris* populations. Furthermore, they documented significant positive relationships between genetic diversity and vegetative traits, such as plant height and leaf length. Relationships between genetic diversity and physical traits have been observed in other wetland plants facing decline. Lienert et al. (2002) found a significant positive association between heterozygosity and several fitness traits in the endangered fen dwelling *Swertia perennis*. Likewise, Schmidt and Jensen (2000) observed significant positive correlations between genetic variation and seed capsules per plant, seeds per plant, and seedlings per plant in *Pedicularis palustris*, another rare fen species.

Table 2 Summary of morphometric traits of *Zizania palustris* and *Zizania aquatica* and results of pairwise comparison (Mann-Whitney U-test or t-test) from plants sampled in Michigan in 2013. Measurements include mean ($\bar{x} \pm 1sd$), coefficient of variation (CV), and sample size (n)

	<i>Z. palustris</i>	<i>Z. aquatica</i>	<i>Z. palustris</i> vs. <i>Z. aquatica</i>
Stem Height (cm)	$\bar{x} = 140 \pm 38.4$ CV = 27.4% n = 189	$\bar{x} = 172 \pm 51.1$ CV = 29.7% n = 100	W = 17,604 $p < 0.0001$
Leaf Length (cm)	$\bar{x} = 26.5 \pm 14.0$ CV = 52.8% n = 184	$\bar{x} = 34.5 \pm 15.6$ CV = 45.2% n = 100	W = 17,479 $p < 0.0001$
Panicle Length (cm)	$\bar{x} = 37.3 \pm 10.8$ CV = 29.0% n = 189	$\bar{x} = 49.1 \pm 10.0$ CV = 20.4% n = 80	$t = 2.44$ $p = 0.016$
Number of Male Flowers	$\bar{x} = 80 \pm 47$ CV = 58.8% n = 117	$\bar{x} = 382 \pm 309$ CV = 80.9% n = 55	W = 7331.5 $p < 0.0001$
Number of Female Flowers	$\bar{x} = 36 \pm 21$ CV = 58.3% n = 188	$\bar{x} = 221 \pm 178$ CV = 80.5% n = 100	W = 19,053 $p < 0.0001$

Conclusions and Management Concerns

Although we expected to see evidence of genetic isolation by distance and genetic drift, our results indicate that most sample sites shared a similarly low amount of genetic variation. While bed area may have declined over time, inter-population dispersal may have buffered the negative effects of genetic drift. Nevertheless, efforts to preserve Michigan rice beds should still be taken because drift may eventually occur in small beds.

Fig. 7 Bed area of wild rice sites sampled in Michigan in 2013

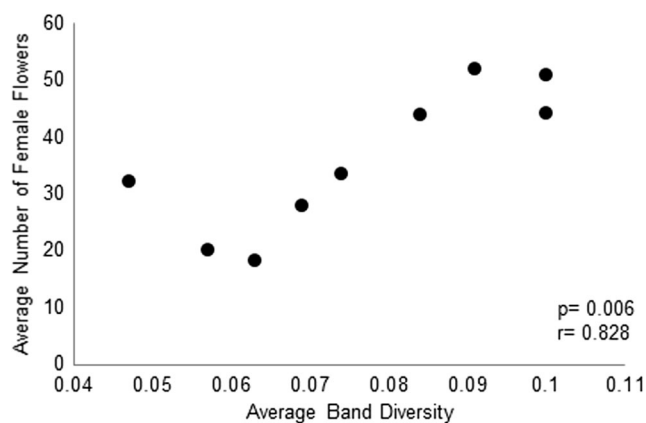
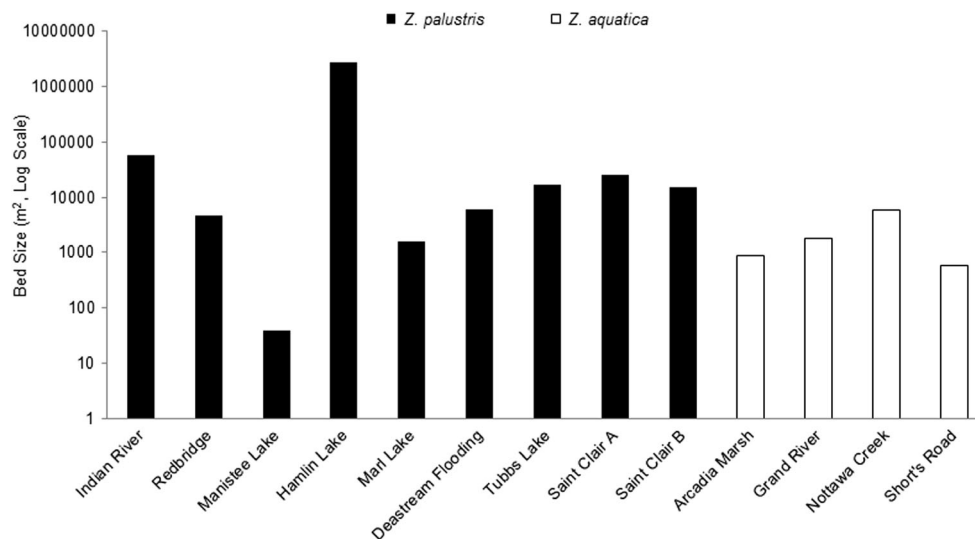


Fig. 8 Correlation between average AFLP band diversity and the average number of female flowers produced in *Z. palustris* sites sampled in Michigan in 2013. Statistical values were derived from a Pearson correlation

Furthermore, rice is culturally important to tribes and provides crucial habitat for waterfowl so small populations should be augmented.

Sowing efforts with seeds from genetically diverse beds could be a good management strategy to restore Michigan wild rice populations as well as other wetland plant populations facing decline. Monitoring growth and reassessing genetic diversity after restoration would help ensure that populations rebound. Several studies have explored the effectiveness of seeding in restoration efforts (Cobbaert et al. 2004; Nishihira et al. 2006; Ramp et al. 2006; Kettering and Galatowitsch 2011). However, caution must also be taken with such human mediated dispersal. While gene flow is often perceived as beneficial to populations, in some cases it can be detrimental by introducing maladaptive alleles. Consequently, adaptation to local conditions by means of natural selection becomes hindered (Hufford and Mazer 2003; Richardson et al. 2016). When attempting restoration of declining

populations of wetland plants, managers should use seed from local populations with similar environmental conditions to ensure success and the maintenance of adaptive genotypes (as described in McKay et al. 2005 and Vander Mijnsbrugge et al. 2010).

In addition to gaining a better understanding of seed selection for sowing efforts, future studies should investigate non-human dispersal vectors, such as waterfowl, to understand more natural gene flow mechanisms. Taking additional steps to study and preserve natural dispersal processes would help ensure that plant populations can thrive beyond initial restoration (Amezaga et al. 2002). Simultaneous examination of morphological traits and genetic structure within a fragmented population is an approach that is widely applicable for the conservation of wetland plant species across the globe. Following up with these suggested investigations could help create a more complete understanding of wetland plant population dynamics and evolutionary processes for the benefit of developing better restoration strategies.

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