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Project Abstract

PROJECT TITLE: Understanding Threats, Genetic Diversity, and Conservation Options for Wild Rice
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Overall Project Outcome and Results: Wild rice (Zizania palustris L.) was studied using DNA-based single sequence repeats and the tools of bioinformatics to determine the genetic diversity of wild rice among 70 populations across the state of Minnesota. This study had two objectives: 1) to document genetic diversity of wild rice populations; and 2) assess the usefulness of genetic information for the conservation of this important wild species in Minnesota. Results showed that genetic diversity of the populations in Minnesota is relatively high with a range of 0.37 to 0.73 in heterozygosity and a mean of 0.54. Hetereozygosity can range between 0.0 to 1.0 indicating that genetic diversity among wild rice populations is reasonably high. This also means that many populations are quite unique from a genetic standpoint. Two genetic phylograms are presented. These are figures that illustrate the genetic relationships among the populations using two different genetic models. Examples are given to illustrate how genetics may be used when restoring or rebuilding populations of wild rice.

Project Results Use and Dissemination: This project will be disseminated via a website report and via seminars and presentations both nationally and regionally. The data will be useful to resource managers across the state who are managing populations of wild rice. The genetics of wild rice in Minnesota has not been explored in detail, thus resource managers will now have another tool to use when making decisions about restoration of wild rice populations. The results will be published in a nationally recognized peer reviewed journal.

Final Report

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Introduction

Wild rice (*Zizania palustris* L.) is a culturally important and valuable aquatic plant that is native to Minnesota. It is recognized that wild rice is being threatened by changes in hydrology of streams, lakes and rivers, changes in seasonal housing along lakeshores, and competition from both native and exotic aquatic species. The most important threat is a loss of genetic diversity that is the direct result of the previously mentioned threats. As habitat declines, competition increases from exotic species forcing decreases in the size of native populations and their genetic diversity. Thus, natural wild rice populations decline and even disappear.

This results reported here were based on DNA-based simple sequence repeat (SSR) marker data (also called microsatellite markers). Using the powerful tools of bioinformatics, the marker data were analyzed to study the genetic diversity of wild rice within Minnesota by calculating the genetic distances and fixation indices among the sampled wild rice populations. Genetic distance is a measure of the genetic divergence between two populations and indicates whether populations are genetically different or alike. The fixation index is a measure of the amount of inbreeding that has taken place among members of a population and indicates how related any member of the population is to another member. When population fixation indices between two populations are compared, their historical relatedness can be inferred.

SSR markers are widely used in plant genetic studies and constitute an important genomic resource in the botanical sciences. The markers provide a valuable tool for genetic fingerprinting, linkage map development, quantitative trait locus (QTL) mapping, marker-assisted selection, parentage analysis, genetic diversity studies, gene flow studies, and evolutionary studies (Cavagnaro et al., 2010; Zhu et al., 2011). SSRs are also useful for

determining the population structure within and among natural populations and/or for identifying potential progenitors of a particular species.

Prior to this report, very little has been known about the genetic diversity of natural wild rice populations. Lu *et al*. (2005) used isozyme analysis of 17 populations of wild rice across northern Wisconsin, and showed that wild rice genetic diversity was moderate, compared to similar outcrossing grass species. Larger populations of wild rice in larger lakes expressed higher levels of genetic variability and smaller inbreeding coefficients than did smaller or more isolated populations; the study also noted that gene flow was limited between drainages. One important conclusion of Lu *et al*. (2005) was that small populations with high genetic diversity might demand special efforts in identification and conservation.

The techniques for SSR analysis are now well known and accepted for the study of genetic diversity in plants (Zalapa et al., 2012). Understanding genetic diversity is central to the conservation of plant genetic resources for future use and for future protection of plant species. This study had two principle objectives: 1) to document genetic diversity of wild rice populations within the state of Minnesota; and 2) to assess the usefulness of the genetic information for the conservation of wild rice in Minnesota.

Methods and Materials

Plant collections and DNA extraction. During August and September from 2006 to 2012, wild rice leaf tissue was collected from 70 locations on public waters in the state of Minnesota (Table 1). Sampled populations were primarily large, harvestable, continuous beds of wild rice in both lakes and rivers. At each population site, leaf tissue collections were made by kayak or canoe. Fifty different individuals were sampled from each population on parallel or long linear transects at approximately 10-meter intervals between sampled individuals. A GPS location was taken and recorded at the approximate center of each transect. Table 1 lists the exact location of each of the sites where wild rice populations were sampled. Figure 1 illustrates the locations of the sample sites across the state by county.

Six centimeter pieces of healthy, emergent leaf tissue were placed into sealable plastic bags. The leaf tissue samples were stored on ice and returned within 24 hours to the laboratory where they were frozen at -80° C until DNA extraction. Genomic DNA was extracted using a modified CTAB extraction protocol (Ausubel et al., 2002) or the BioSprint 96 chemistry and automated DNA extraction machine (Qiagen, Inc.).

SSR marker data analysis. Fifteen SSR loci developed by our labs (Zp5, Zp6, Zp7, Zp8, Zp9, Zp11, Zp12, Zp13, Zp15, Zp19, Zp23, Zp25, Zp28, Zp34, Zpr1) were amplified from purified genomic DNA templates of 47 individuals from each sampled population using standard polymerase chain reaction (PCR) conditions. DNA from a positive control wild rice individual was loaded in the 48th sample space for each population. Forward primers were 5' end-labeled with fluorophores (6-FAM, VIC, NED, or PET). The resulting PCR products were analyzed by capillary electrophoresis using the 3730 Genetic Analyzer (ABI, Inc.). Electropherogram peaks generated by the genetic analyzer were sized using the GeneMapper software package (ABI, Inc). Fragment sizes for each SSR locus were tabulated using Microsoft Excel. Population genetic analyses and construction of phylogenetic trees was carried out using the PowerMarker (Liu and Muse 2005) and MEGA 4.1 (Tamura *et al.* 2007)

Results and Discussion

Measuring wild rice genetic diversity and genetic distance. Genetic diversity is a measure of the allelic differences at genetic loci between and among populations. The genetic loci (or locations in the genome) analyzed for this report were SSRs. It is important to note that SSRs are neutral genetic markers, meaning they are not genes. Wild rice is a diploid species, meaning each genetic locus (*i.e.,* SSR) has two copies; one is from the paternal parent and the other is from the maternal parent. Therefore, it is expected that two genetic alleles will occur at each SSR marker locus for each individual. If the paternally inherited SSR marker allele is the same as

the maternally inherited allele, the individual sampled is homozygous at that SSR marker locus. If the two alleles are different, the individual is heterozygous at that SSR marker locus.

The frequencies at which different alleles occur at a given SSR marker locus are calculated among all sampled populations using a statistical model. The calculation results are estimates of the genetic distance (*i.e.,* change in genetic similarity over time) between populations. The following model (Nei, 1983; Takezaki and Nei, 1996) was used to calculate genetic distances among sampled populations for this report.

Figure 1. Genetic distance model (Nei 1983)

$$D_A = 1 - \Sigma_l \Sigma_u \sqrt{X_u Y_u} / L$$

Where: X is the first population, Y is the second population, L = total number of loci, $_u$ = the $_u^{th}$ allele, l = the l^{th} locus.

The genetic distance measurements show a relatively high amount of genetic variation (*i.e.,* diversity) among the 70 wild rice populations sampled. An average of 14 alleles was observed per locus and the average observed population heterozygosity was 0.54. These results indicate that there is significant genetic diversity among individuals within the majority of the sampled populations. Table 2 shows the summary statistics for the data gathered from this study.

The unrooted neighbor joining phylogram resulting from the allele frequency data shows that the 70 sampled wild rice populations can be divided into four major clades (Figure 2). Further, a total of 10 sub-clades further divide the set of populations based on the similarity of allele frequencies across the set of SSR loci assayed.

The Nei83 genetic distance model assumes that changes in population-level allele frequencies are due to mutation and/or drift (Nei, 1983). The model is linear over time and therefore may be used as a representation of the divergence time for populations which were once a single, panmictic population. Both mutation and genetic drift are random processes, therefore genetic distance is not necessarily a measure of relatedness. Genetic distance values vary between 0 and 1, where a distance of 0 means two populations have exactly the same allele frequencies across all loci; a distance of 1 indicates the two populations are "fixed" for different alleles at all loci. The genetic similarity coefficient among sampled wild rice populations using the Nei83 model varied from 0.22 to 0.83 indicating a wide amount of genetic variation at the population level.

Another measure of genetic distance between populations is Wright's Fixation Index (also called coancestry coefficient or F_{ST}). It is the most widely used model for describing genetic difference between populations. The F_{ST} model shown below and the resulting phylogram (Figure 3) compares the observed heterozygosity of each population to the expected total heterozygosity across all populations to determine the amount of genetic divergence. The longer a population has been in isolation, the more inbred a population becomes resulting in a net loss of heterozygosity (or a net gain in homozygosity). Fixation indices, unlike genetic distance models like the Nei83 model discussed above, do not compare allele frequencies among all sampled populations, but rather is based on pairwise comparison of between all pairs of sampled populations. Further, as F_{ST} values are a measure of population relatedness since alleles that are in common between two populations are assumed to be identical by descent from a common ancestor.

Figure 2. Wright's F_{ST} (Wright 1969)

$$F_{ST} = \frac{\sigma^2_S}{\sigma^2_T} = \frac{\sigma^2_S}{\bar{p}(1-\bar{p})}$$

Where: σ^2_s = variance of allele frequency in subpopulations, σ^2_T = variance of allele frequency in the total population, p = average allele frequency in the total population.

In practice, the the F_{ST} model of Wright is not able to be precisely measured and several other improved models have been developed to estimate pairwise fixation indices. For this project, the model developed by Weir and Cockerham (1984) was used.

Generally, F_{ST} values of 0.0 – 0.05 indicate very little genetic difference between populations. Values between 0.05 and 0.25 indicate moderate genetic difference and values greater than 0.25 indicate large genetic variation. Too many missing marker data result in negative coefficients, which make it impossible to generate a complete phylogram. Due to some missing data, the Zp5 and Zp12 marker data were omitted when calculating the reported F_{ST} coefficients. Fifteen populations (CRL, DRL, HAL, JOP, LIR, PNL, SHL, TNWR-DL, TNWR-TL, VER, MOR, ITL, MRL, URL, SKM) were also omitted from the F_{ST} calculations and resulting phylogram due to some missing data. Figure 3 shows the unrooted F_{ST} phylogram for the sampled wild rice populations. F_{ST} measurements divide the populations into six clades.

Conservation/restoration of wild rice and population genetic diversity. Wild rice (*Zizania palustris* L.) is a native aquatic annual plant species found in the wetlands of many counties in Minnesota, although most populations occur in the north central portions of the state. The plant is an annual diploid and is principally a wind pollinated, out-crossing species.

The exact nature of wild rice species across North America is not yet fully understood, nor is the amount of genetic similarity among wild rice species. For example, some authors identify wild rice in the Midwest as either *Zizania palustris* or *Z. aquatica* based on their relatively easily identifiable morphological characteristics. However, others still use the older classification system of Dore (1969) who recognized varieties of wild rice including *Z. palustris* var. *palustris*, and var. *interior*; and *Z. aquat*ic var. *aquatica*, var. *brevis*, and var. *subbrevis*. Experimental hybridizations demonstrated that crosses between *Z. palustris* and the other varieties are all fertile and crosses between *Z. palustris* and *Z. aquatica* produced some fertile hybrids at a low frequency (Duvall and Biesboer, 1988).

Differences in morphological characteristics and the observation that interbreeding occurs suggests that these varietal types might occur in Minnesota (personal observations by the investigators). They may be only distinguishable by genetic analysis because growing conditions influence the morphology of this very plastic species. For example, both genetics and ecology influence the biomass of seeds per square meter of wild rice populations. In the case of seed biomass, types of water bodies or other factors such as sediment composition appear to account for 71.3% of the variance. Genetic diversity possibility accounts for the rest (Eule-Nashoba et al., 2010). For the purposes of this study, collected plants are identified as *Z. palustris* and varietal names are not used. Morphological types that could be clearly identified as *Z. aquatica* were not included in this study.

Wild rice has been and is still considered a traditional source of food for regional Native Americans (Johnson, 1969; Kahler 2010) and has become a semi-domesticated crop in recent times (Hayes et al., 1989). As previously mentioned, habitat loss, disruptions of hydrological regimes, competition from invasive plants, etc., have caused natural populations of this species to disappear from the state of Minnesota (see *e.g.* Meeker, 1993; Kern and Kahler, 2014). The importance of wild rice to Native Americans and its ecological role in wetland ecosystems as a food source for many wildlife species make the conservation of extant natural populations and preservation of their genetic variability a serious concern (Waller et al., 2000).

How can we take conservation/restoration practices into account when discussing genetic variation? A population might be considered for conservational attention if it has high genetic diversity in comparison to other populations. It is locally adapted, survives well, and grows well in its habitat. Thus, it can serve as a reservoir for future restoration efforts simply because of its genetic diversity.

For restorations, it has been pointed out that two distinct issues must be considered (Clewell, 2000). First, genetic accuracy might be the point of a restoration but if an original population is completely lost, then perfect accuracy is not attainable. Thus, some value judgments must be made as to what might be a close genetic match to the lost

population based on available research. Secondly, it might be the aim of a restoration to produce a functional population, *i.e.,* one that survives and reproduces well in the restored area. A range of genotypes might be tried to rebuild the population. It should be pointed out, that *Zizania palustris* is a very common species in Minnesota. The species has many weedy characteristics including an ability to rapidly colonize suitable habitats, it is a prolific seed producer, it produces seeds even under difficult growing conditions, its seeds can persist from year to year, and it exhibits rapid growth. It can be found in roadside ditches, smaller streams, and around isolated spring fed ponds. Probably for many restorations in Minnesota, with its large populations of wild rice, it should be relatively easy to find genetically similar populations to replace those that are declining.

Some very good and basic guiding principles for the restoration of natural populations can be found in Falk et al., 2001. These are enumerated below and closely paraphrased from the original publication.

1) Wild rice is a species that from this study has a reasonable amount of genetic diversity. Individual populations of *Zizania palustris* may vary in their dispersal rates and distances from other populations to which a specific population can interbreed. These differences in genetic diversity may correlate with life-histories of specific populations. We note that the species colonized the State after the last glaciation that ended ca. 10,000 to 13,000 years before the present, ample time for this species to change genetically.

2) Value judgments must be made to determine if a restoration will be historically accurate or more broadly, only a functional one. Natural populations will experience genetic changes over time and space. However, as Falk et al. (2001) note, even where the emphasis is on a functional restoration, historic accuracy should be considered to anchor restoration attempts within the natural range of variability. Historical accuracy is also complicated by that fact that people have introduced wild rice to many lakes and rivers over time. It has been done locally many times by sportsmen introducing wild rice into ponds for waterfowl hunting, by local people who just desire to have wild rice in their lakes and ponds, and certainly by native people over much longer periods of time. Records of those introductions can rarely be verified. Additionally, waterfowl feeding in wild rice beds move seeds from one water body to many others.

3) Restorationists often use geographic distance from seed sources for establishing new populations (*i.e.*, it seems to make sense to find a population in the same watershed not far from the restoration site). But it can be a crude substitute for patterns of gene flow among populations and may not reflect the genetics of the population being restored. If populations are strongly selected to local habitats, then habitat similarity may outweigh distance as a selection criterion. However, we often do not know how well a population is adapted to its habitat.

4) Large genetically diverse populations are generally preferable to small isolated populations as a source of seeds for restorations, even when those small populations are geographically closer to the restoration site. It is probably preferable to combine seed sources from several suitable sites to capture a wider array of genetic diversity that can succeed in the new location.

5) On the other hand, small, localized populations can be "swamped" by the introduction of highly, genetically diverse seed sources. If an existing population is to be increased or augmented, the number of seeds from other locations should not be so large as to overwhelm the local genotype. In other words, some care must be taken in determining exactly which populations might be most suitable for introductions.

Before discussion of the findings of this report, we should point out that the most important consideration for conserving or restoring wild rice in Minnesota is the conservation of and/or the rebuilding of wild rice habit. If wild rice is declining in a watershed, the reasons for its decline must be discovered. Populations are susceptible to water pollution; fluctuations in water levels that are man-made or caused by beaver dams; competition from native weeds (such as *Nymphaea* spp. (water lilies), *Typha latifolia* L. (cattail) or *Alisma triviale* Pursh. (water plantain); large boat wakes along shorelines that tend to favor establishment of cattails, and physical removal of wild rice by land or resort owners can severely impact local populations where wild rice is not abundant. Populations may also be impacted occasionally by biological agents such as fungal diseases or insects *e.g*, *Apamea apamiformis* Guenee (the rice worm). Hydrologically, wild rice requires the presence of shallow relatively clear water from a depth of 0.5 feet to 3 feet. The best sediments are organic muck at least several inches deep but we have observed wild rice populations growing on sandy or even marly lake bottoms. Wild rice

seems to grow best in aquatic systems that have some flowage, *i.e.,* lakes with an inlet and outlet, or at the edge of rivers.

With the 5 guiding principles noted above in mind, we now turn to the lakes and rivers sampled in this study (Table 1) and the unrooted phylogram using Nei83 genetic distance values for those same wild rice populations (Figure 3). Figure 3 is a phylogram where branch length indicates genetic distance between populations as shown by the scale at the lower edge of the figure. The lake and rivers collected in this study are indicated on the phylogram by a short abbreviation corresponding to the populations listed in Table 1. As mentioned, genetic distances vary between 0 and 1, where a distance of 0 means two populations have exactly the same allele frequencies across all loci, they are not genetically diverse; a distance of 1 indicates the two populations are "fixed" for different alleles at all loci and would be genetically diverse.

With the 5 guiding principles noted above in mind, we now turn to the lakes and rivers sampled in this study (Table 1) and the unrooted phylograms using Nei83 genetic distance and F_{ST} values for those same wild rice populations (Figures 3 and 4). Figure 3 is a phylogram where branch length indicates genetic distance between populations as shown by the scale at the lower edge of the figure. The lake and rivers collected in this study are indicated on the phylogram by a short abbreviation corresponding to the populations listed in Table 1.

Groups of species that are most genetically similar to each other form clusters or clades that branch from the main root of the phylogram. For example, LRL, LOL, LAL, BRL and BSB form a clade on the upper side of the phylogram. On the right side, TNWR-TL, TNWR-RL, RLNWR-W TNWR-DL and RLNWR- R form another distinct clade. At the lower side of the phylogram, it can be noted that some larger distinct clades exist such as FOL, LAO, HTW, RIL ORK, TAL, ANL, and PIL.

By inspection, several observations can be made from the phylogram. Three populations that are highly genetically diverse are Crooked Lake in Pine County (CRL), Lake Plantagenet (PTL), and Upper Rice Lake (URL) in Clearwater County. They exhibit the most genetic diversity of the populations of wild rice in this study.

Several other populations also have high diversity but not as significant as the previous three. These include an entire group at the lower end of the phylogram and include Marsh Lake (MSL) in Cook County, Little Indian Sioux River (LIR) in St. Louis County, Grass Lake (GSL) in Cass County, Decker Lake (DEL2) in Itasca County, Mud Lake (MUL) in Morrison County and Pine Lake (PNL) in Clearwater County.

Another, perhaps third tier of genetic diversity is the clade at the upper left of the phylogram. These include St. Louis River, North Bay (BSB) in St. Louis County, Big Rice Lake (BRL) in St. Louis County, Laura Lake (LAL) in Cass County, Lows Lake (LOL) in Crow Wing County, and the Pike River (LRL) in St. Louis County.

One surprising clade uncovered in this study is the clade that includes all of the National Wild Life Refuges sampled in this study. They included Rice Lake NWR – River (RLNWR – R) in Aitkin County, Rice Lake NWR – West (RLNWR – W) in Aitkin County, Tamarac NWR – Rice Lake (TNWR- RL) in Becker County, Tamarac NWR – Tamarac Lake (TNWR – TL) in Becker County. All populations grouped together indicating a very similar and relatively low genetic diversity between these refuges.

The Nei83 tree can be useful, from a genetic standpoint, to restore populations or create new ones in a restored lake/wetland system. As a general observation, when rebuilding a population it would be wise to look for nearest neighbors in a clade that are genetically similar. An example might be the restoration of YAL (Yaeger Lake in Wadena County). Yeager Lake is a relatively isolated forest lake. Nearest neighbors would be SHL (Shell Lake in neighboring Becker County) or UNL (an unnamed lake in Stearns County). Populations suitable for a genetic restoration certainly may not be nearest neighbors in a watershed. Over-riding decisions, when genetics is being considered in a restoration may be hydrology of the system, whether or not the population should be harvestable or not, lake bed type, managing waterfowl populations, etc.

In contrast to using Nei83 genetic distances to make wild rice restoration or population introduction decisions, it may be more appropriate, in some cases, to use F_{ST} data. As stated earlier, fixation indices estimate the population

structure (or relatedness) between populations. Therefore, if two populations cluster very closely on the F_{ST} tree, they are likely related by a more recent ancestral population than are two populations that cluster more distantly. An example of two closely related populations, based on the F_{ST} tree in Figure 4, are GOL (Grove Lake) and TAL (Tamarac Lake). Those two populations are in separate clades on the Nei83 tree in Figure 3. This is an excellent illustration of the difference between genetic distance and genetic difference, or population structure. Two populations may have very similar allele frequencies due to genetic drift leading to an unrooted phylogram that places them far apart (or genetically distant) from one another, while having allele frequency variances within and between members of the two populations that leads to an unrooted phylogram that places them very close together (*i.e.*, genetically similar).

Another potential application of this genetic technology is in identifying wild rice populations that are most divergent (i.e., "unique") from other populations in the state, or even in a given genetic clade. The identification of these particular populations should allow resource managers to identify populations that deserve the highest priority for protection, given their genetic characteristics. Frequently, these "genetic outlier" populations are considered to have special status as a unique natural resource. From a natural history perspective, the use of Fst values is probably the most relevant measure of identifying these unique populations. While it must be emphasized that our analyses indicate relatively large degrees of divergence across most populations (in other words, each wild rice population is "unique", from a genetic perspective), our analyses indicate certain specific populations are more genetically distant than most populations from an ancestry perspective. Examples in Figure 4 include the Brule River and Marsh Lake populations in Cook County, the Pike River in St. Louis County, and the Otter Tail River in Otter Tail County. Interestingly, these "most-divergent" populations are all river sites. Due to missing data points, our team will perform additional data analyses on other river sites in the study to determine if this trend continues.

If one were interested in restoring a population based on its ancestral history, it may be more appropriate to base wild rice seed source decisions on the F_{ST} data. However, if the goal of the restoration effort were to augment an existing population or create a new population, it may be more appropriate to base seed source decisions using genetic distance data.

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Table 1. Lakes and rivers sampled for this research. Table 1 includes population name, ID code, collected counties and GPS coordinates for each collected population.

Population Name	ID Code	County	GPS Coordinates
Rice Lake	ARL	Crow Wing	46.596317-94.269867
4th Crow Wing Lake	CW4	Hubbard	46.880133 -94.8571
Ann Lake	ANL	Kanabec	45.922133 -93.404417
Bass Lake	BAL	Itasca	47.289967-93.63235
Big Rice Lake	BRL	St. Louis	47.692100 -92.470110
Breda Lake	BEL	St. Louis	47.3367 -91.870933
Brule River	BRU	Cook	47.90729 -90.25804
Cramer	KRL	Lake	47.52242 -91.09924
Crooked Lake	CRL	Pine	46.12298 -92.55029
Decker Lake	DEL2	Itasca	47.63584 -94.40399
Deer Lake	DRL	Itasca	47.82083 -93.39562
Dora Lake	DOL	Itasca	47.73821 -94.04760
Flowage Lake	FOL	Aitkin	46.68911 -93.33364
Garden Lake	GAL	Crow Wing	46.51217 -94.20393
Goose Lake	GSL	Cass	47.21655 -93.970733
Grass Lake	GRL	Otter Tail	46.4011-95.523167
Grove Lake	GOL	Pope	45.6038 -95.1822
Hart Lake (Necktie River)	HAL	Hubbard	47.29298 -94.74791
Hesitation WMA	HTW	Crow Wing	46.339117 -93.897017
Itasca	ITL	Clearwater	47.22720 -95.19674
Josephine Pool (Sherburne NWR)	JOP	Sherburne	45.45957 -93.68416
Kettle	KEL	Carlton	46.63439 -92.78819
Lake Onemia	LAO	Mille Lacs	46.081583-93.676683
Laura	LAL	Cass	46.98230 -94.01608
Little Birch	LBL2	Cass	47.03739 -93.86696
Little Indian Sioux River	LIR	St. Louis	48.133083 -92.209
Little Puposky	LIP	Beltrami	47.7099 -94.93577
Lows Lake	LOL	Crow Wing	46.743760 -93.844520
Mallard Lake	MAL	Mille Lacs	46.40408 -93.72700
Mark Lake	MRL	Cook	47.78658 -90.60119
Marsh Lake	MSL	Cook	47.83522 -90.81302
Miss. River-Green's Pt.	MRG	Crow Wing	46.43868 -94.12140
Moose Horn River	MHR	Carlton	46.43855 -92.78425

Moose Lake	MOL1	Aitkin	46.86818 -93.63522
Moose River	MOR2	Aitkin	46.88630 -93.60325
Mud Lake	MUL	Morrison	45.916183-94.225583
Mud Lake	MUT	Todd	46.118783-94.77345
Nature's Lake	NAL	Itasca	47.68406 -94.10658
Orrock Lake (Sherburne NWR)	ORK	Sherburne	45.45044 -93.74360
Otter Tail River	OTR	Otter Tail	46.46629 -95.58395
Pickerel Lake	PIL	Anoka	45.334017 -93.448267
Pike River	LRL	St. Louis	47.567700 -92.373830
Pine Lake	PNL	Clearwater	47 41.141 -95 31.367
Pine River	PIR	Cass	46.752033-94.407167
Plantagenet	PTG	Hubbard	47.36751 -94.91633
Platte River	PLR	Morrison	45.944383-94.249083
Prairie River	PRR	Itasca	47.25789 -93.48588
Rat House Lake	RHL	Aitkin	46.86701 -93.22751
Red Sand Lake	RSL	Crow Wing	46.376383-94.30175
Rice	RLI	Itasca	47.67572 -94.05171
Rice Lake	RIC	Crow Wing	46.463167-93.927817
Rice Lake	RCH	Hubbard	47.03084 -95.03268
Rice Lake NWR-River	RLNWR-R	Aitkin	46.562340 -93.355060
Rice Lake NWR-west	RLNWR-W	Aitkin	46.508880 -93.406570
Round Island Lake	RIL	Lake	47.61389 -91.29342
Shell	SHL	Becker	46.94757 -95.48517
St. Louis River, North Bay	BSB	St. Louis	46.65160 -92.23740
St. Louis River, Skibo Mill	SKM	St. Louis	47.47355 -91.91627
Star Lake	STL	Otter Tail	46.521967 -95.848067
Stone Lake	SOL	St. Louis	47.49940 -91.88809
Tamarac Lake	TAL	Stearns	45.460933-95.086017
Tamarac NWR-Rice Lake	TNWR-RL	Becker	47.49940 -91.88809
Tamarac NWR-Tamarac Lake	TNWR-TL	Becker	46.922020 -95.682720
unnamed	UNL	Stearns	45.7117-94.905367
unnamed	UNC	Cass	47.208567 -93.773333
unnamed	UNN	Norman	47.2302 -96.08896
Upper Rice Lake	URL	Clearwater	47.39101 -95.28571
Vermilion River	VER	St. Louis	48.240017 -92.59165
White Elk Lake	WEL	Aitkin	46.806317 -93.693217
Yaeger Lake	YAL	Wadena	46.70565 -94.969233

Figure 1. Locations of sampled populations of *Zizania palutris* L. across the State of Minnesota.

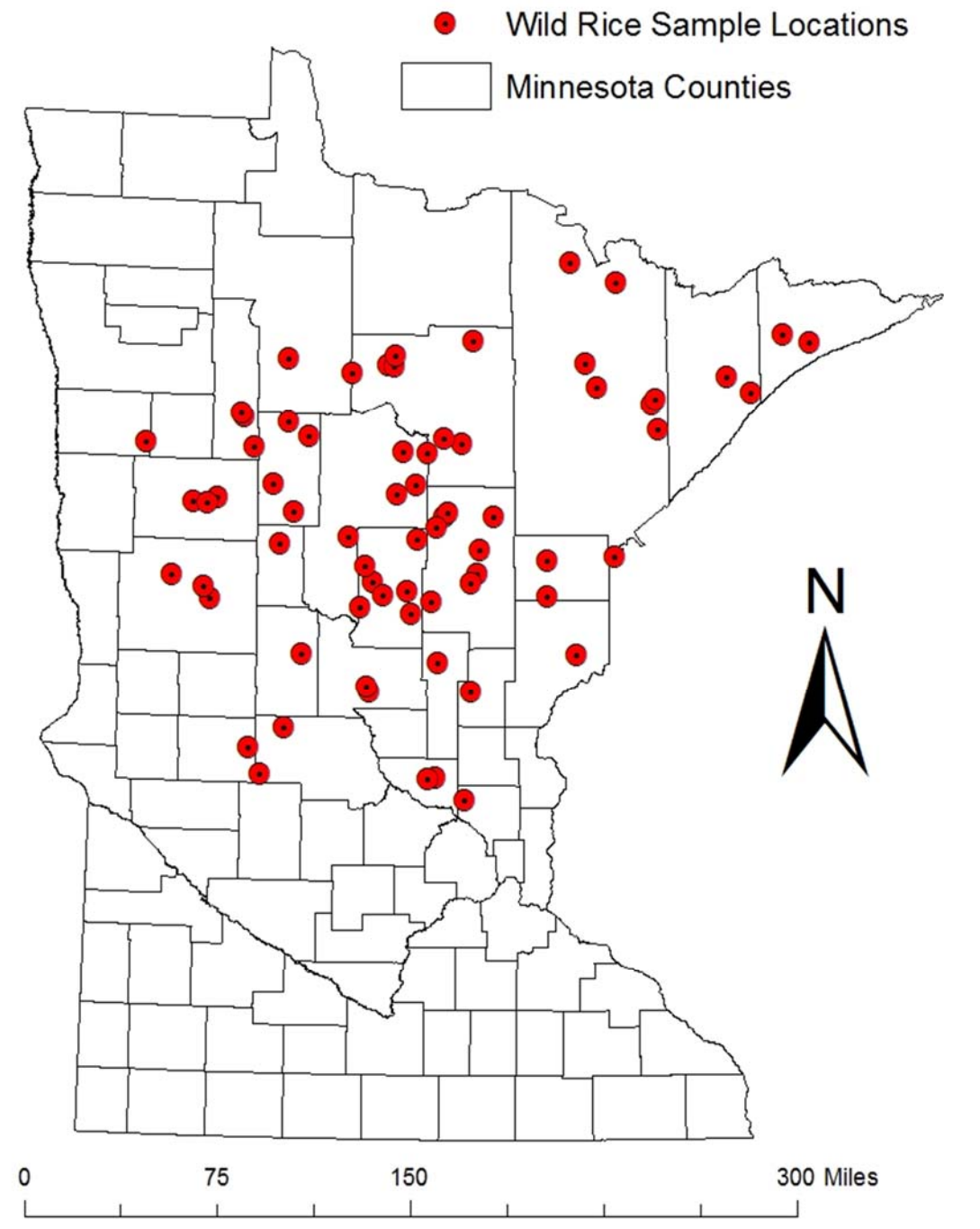


Table 2. Population summary statistics for this study. See Table 1 for population abbreviations.

Population	MAF	# Genotypes	Sample Size	# Observations	# Alleles	Expected Heterozygosity	Observed Heterozygosity	PIC	f
ANL	0.27	23	47	37	15	0.84	0.59	0.83	0.31
ARL	0.40	17	47	31	12	0.72	0.50	0.70	0.33
BAL	0.35	22	47	39	15	0.76	0.49	0.74	0.36
BEL	0.34	22	47	37	15	0.79	0.55	0.77	0.32
BRL	0.41	17	47	40	11	0.72	0.56	0.69	0.24
BRU	0.55	13	47	38	9	0.58	0.41	0.55	0.31
BSB	0.33	19	47	34	13	0.79	0.50	0.77	0.38
CRL	0.39	17	46	34	14	0.76	0.49	0.74	0.37
CW4	0.35	21	47	36	15	0.79	0.49	0.77	0.39
DEL2	0.29	17	47	24	13	0.79	0.44	0.77	0.47
DOL	0.31	23	47	39	16	0.81	0.60	0.79	0.28
DRL	0.40	9	47	12	8	0.73	0.57	0.69	0.28
FOL	0.30	23	47	36	16	0.79	0.51	0.77	0.36
GAL	0.31	24	47	38	16	0.83	0.60	0.81	0.29
GOL	0.45	15	94	62	9	0.66	0.45	0.64	0.33
GRL	0.37	20	47	35	14	0.76	0.52	0.74	0.33
GSL	0.34	20	47	36	13	0.77	0.52	0.74	0.35
HAL	0.25	27	47	46	16	0.85	0.67	0.83	0.22
HTW	0.26	27	47	44	18	0.85	0.57	0.83	0.33
ITL	0.37	18	47	27	13	0.71	0.46	0.69	0.37
JOP	0.28	20	47	33	14	0.82	0.56	0.80	0.33
KRL	0.29	24	47	39	16	0.82	0.60	0.80	0.29
LAL	0.34	23	47	43	16	0.79	0.60	0.76	0.25
LAO	0.29	23	47	36	16	0.82	0.50	0.80	0.40
LBL2	0.32	22	47	35	14	0.78	0.55	0.75	0.30
LIP	0.38	20	47	36	14	0.75	0.47	0.73	0.38
LIR	0.35	17	47	25	14	0.72	0.44	0.69	0.42
LOL	0.33	21	47	46	14	0.78	0.73	0.75	0.07
LRL	0.37	19	47	45	13	0.75	0.70	0.71	0.08
MAL1	0.29	23	47	37	16	0.82	0.56	0.81	0.36
MHR	0.26	25	47	41	17	0.85	0.57	0.83	0.34
MOL1	0.32	17	47	25	13	0.78	0.61	0.75	0.31
MOR2	0.31	22	47	38	15	0.79	0.64	0.76	0.25
MRG	0.32	22	47	32	15	0.80	0.51	0.77	0.39
MRL	0.38	19	47	32	13	0.73	0.54	0.71	0.30
MSL	0.41	15	47	39	11	0.71	0.51	0.69	0.30
MUL	0.32	18	47	27	14	0.79	0.55	0.77	0.34
MUT	0.31	24	47	38	15	0.80	0.63	0.78	0.27
NAL	0.29	22	47	32	16	0.79	0.56	0.77	0.30
ORK	0.28	24	47	38	15	0.83	0.49	0.81	0.41
OTR	0.41	16	47	36	13	0.74	0.56	0.71	0.26
PIL	0.28	21	47	32	13	0.83	0.59	0.81	0.31

PIR	0.31	20	47	32	14	0.80	0.50	0.78	0.39
PLR	0.38	10	47	13	9	0.73	0.47	0.70	0.40
PNL	0.17	26	47	35	18	0.90	0.73	0.89	0.21
PRR	0.29	20	47	35	13	0.82	0.59	0.80	0.31
PTG	0.44	15	47	28	11	0.65	0.38	0.64	0.43
RCH	0.36	18	47	36	12	0.78	0.52	0.76	0.35
RCI	0.37	21	47	37	14	0.76	0.53	0.74	0.32
RHL	0.35	18	47	30	13	0.76	0.51	0.74	0.37
RIL	0.27	23	47	36	15	0.83	0.56	0.81	0.34
RLNWR-R	0.31	22	47	36	16	0.79	0.53	0.78	0.34
RLNWR-W	0.34	19	47	32	15	0.76	0.45	0.74	0.42
RSL	0.39	18	47	33	12	0.73	0.50	0.71	0.35
SHL	0.18	25	46	35	15	0.89	0.60	0.88	0.33
SKM	0.40	17	47	35	10	0.71	0.48	0.68	0.34
SOL	0.48	15	47	36	9	0.63	0.46	0.60	0.35
STL	0.36	19	47	34	13	0.77	0.46	0.75	0.41
TAL	0.37	23	47	41	16	0.78	0.49	0.76	0.38
TNWR-DL	0.28	16	47	26	13	0.83	0.37	0.81	0.56
TNWR-RL	0.26	23	47	39	16	0.85	0.49	0.84	0.43
TNWR-TL	0.27	20	47	35	15	0.82	0.48	0.81	0.44
UNC	0.28	26	47	39	22	0.85	0.58	0.83	0.33
UNL	0.32	19	47	35	11	0.79	0.52	0.76	0.35
URL	0.33	23	47	35	15	0.78	0.57	0.75	0.33
VER	0.23	24	47	33	17	0.87	0.72	0.86	0.19
WEL	0.34	23	47	41	14	0.79	0.54	0.77	0.33
YAL	0.31	24	47	40	15	0.81	0.54	0.79	0.34
RANGE					8 to 22	0.58 to 0.90	0.37 to 0.73	0.55 to 0.89	0.07 to 0.56
MEAN					14	0.78	0.54	0.76	0.33

Figure 3. Nei83 unrooted neighbor joining phylogram.

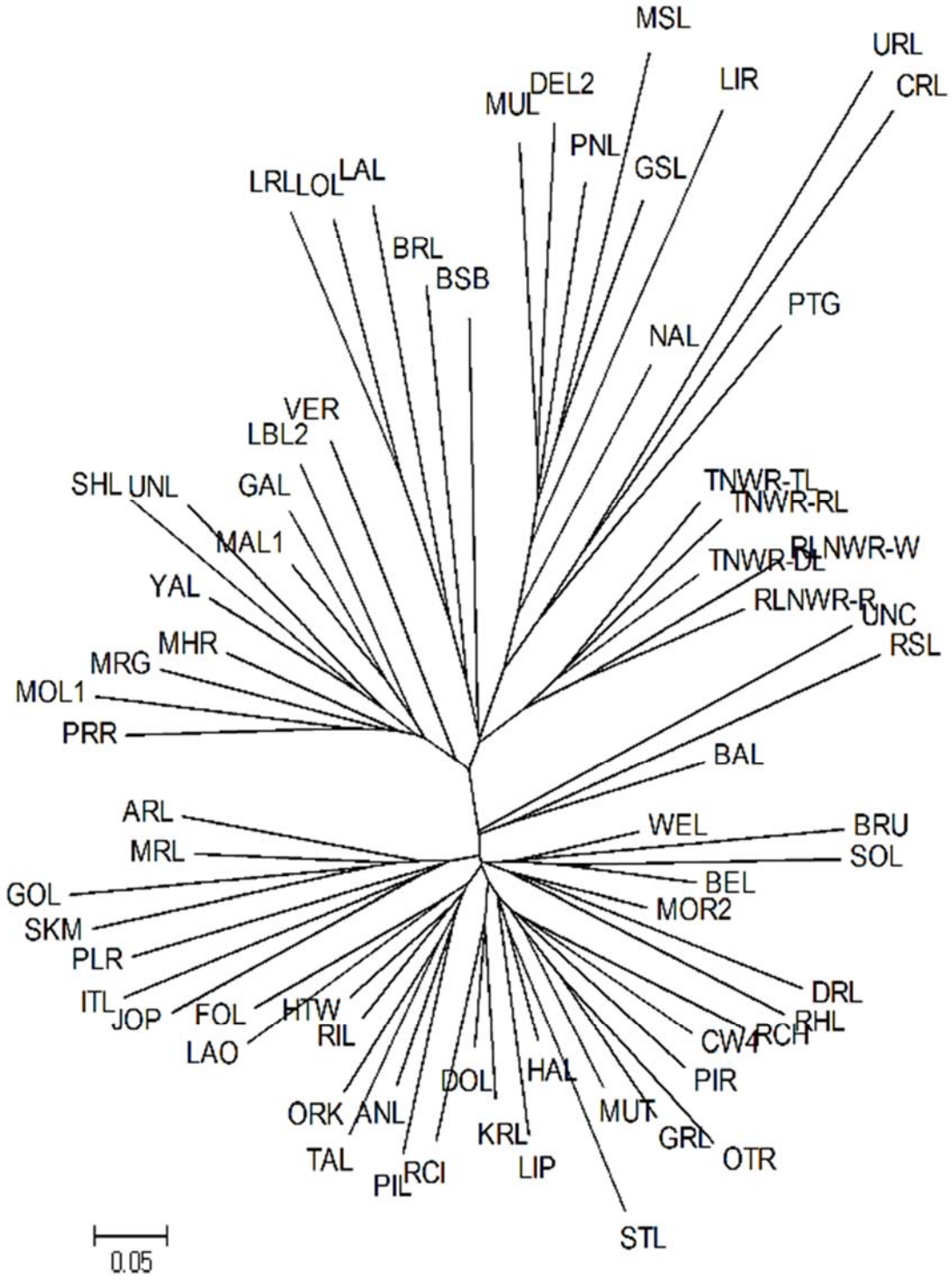
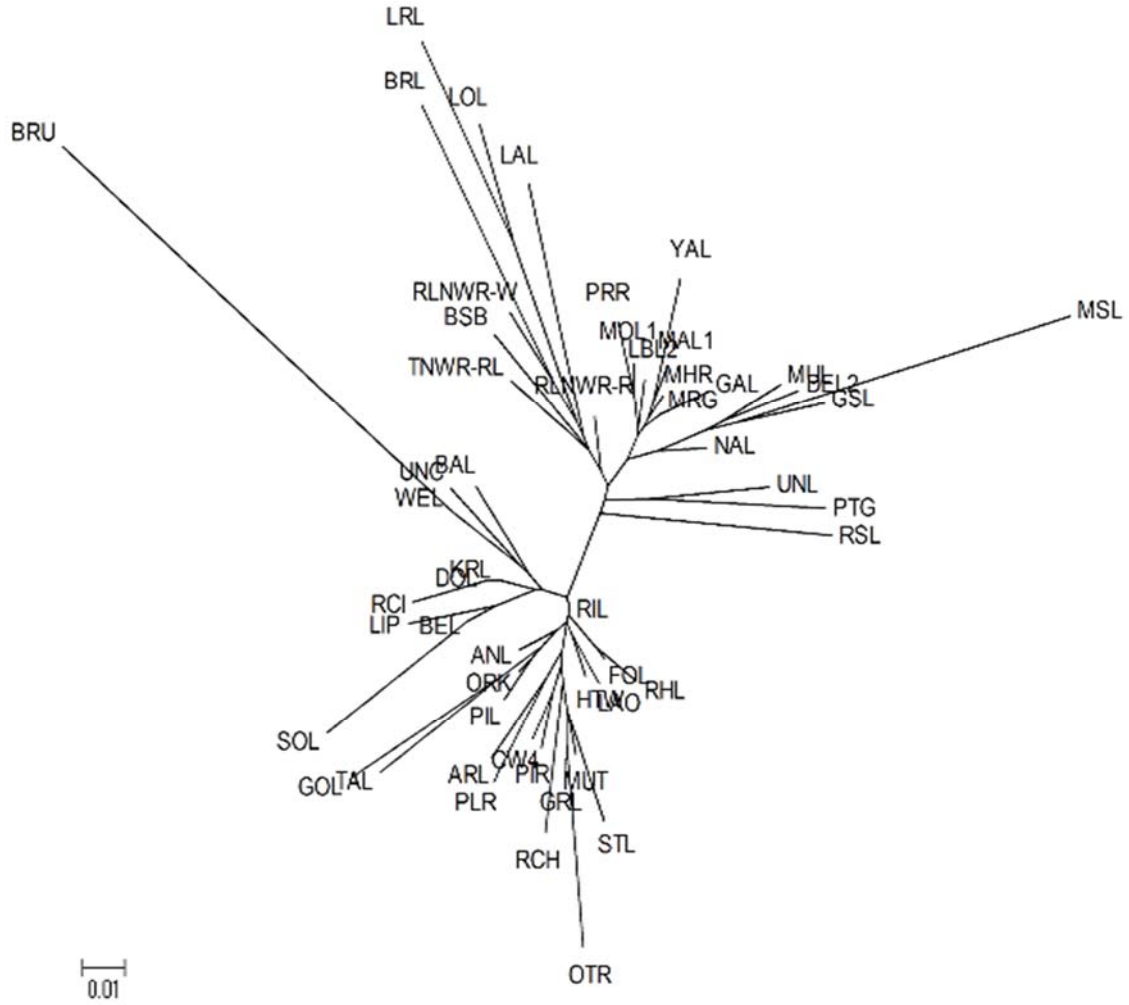


Figure 4. F_{ST} unrooted neighbor joining phylogram missing some data as indicated in the text.



Environment and Natural Resources Trust Fund (ENRTF)
M.L. 2011 Work Plan – Final Report

Date of Status Update: **6/30/2014**
Date of Next Status Update: Final Report
Date of Work Plan Approval: 6/23/2011
Project Completion Date: 6/30/2014 **Is this an amendment request? No**

Project Title: Understanding Threats, Genetic Diversity, and Conservation Options for Wild Rice

Project Manager: David Biesboer
Affiliation: U of MN
Address: 250 BioSci, 1445 Gortner Ave
City: St Paul **State:** MN **Zipcode:** 55345

Telephone Number: (612) 625-1799
Email Address: biesboer@umn.edu
Web Address: http://www.cbs.umn.edu/itasca

Location:

Counties Impacted: Statewide

Ecological Section Impacted: Lake Agassiz Aspen Parklands (223N), Minnesota and Northeast Iowa Morainal (222M), North Central Glaciated Plains (251B), Northern Minnesota and Ontario Peatlands (212M), Northern Minnesota Drift and lake Plains (212N), Northern Superior Uplands (212L), Paleozoic Plateau (222L), Red River Valley (251A), Southern Superior Uplands (212J), Western Superior Uplands (212K)

Total ENRTF Project Budget:		
	ENRTF Appropriation $:	195,000
	Amount Spent $:	195,000
	Balance $:	0

Legal Citation: M.L. 2011, First Special Session, Chp. 2, Art.3, Sec. 2, Subd. 04o

Appropriation Language:
$97,000 the first year and $98,000 the second year are from the trust fund to the Board of Regents of the University of Minnesota to research the genetic diversity of wild rice population throughout Minnesota for use in related conservation and restoration efforts. This appropriation is contingent upon demonstration of review and cooperation with the Native American tribal nations in Minnesota. Equipment purchased with this appropriation must be available for future publicly funded projects at no charge except for typical operating expenses. This appropriation is available until June 30, 2014, by which time the project must be completed and final products delivered.

I. PROJECT TITLE: Understanding Threats, Genetic Diversity, and Conservations Options for Wild Rice

II. PROJECT SUMMARY: Wild rice is a culturally important and valuable aquatic plant that is native to Minnesota. It is recognized that wild rice is being threatened by changes in hydrology of streams, lakes and rivers, changes in seasonal housing along lakeshores, and competition from both native and exotic aquatic species. The most important threat is a loss of genetic diversity, that is the direct result of the previously mentioned threats. As habitat declines, competition increases from exotic species, and genetic diversity decreases, natural wild rice populations have declined and even disappeared. This proposed research project seeks to utilize microsatellite DNA markers (also called simple sequence repeats, or SSRs) and the powerful tools of bioinformatics to study the genetic diversity of wild rice within Minnesota. The project methods will include collecting leaves from individual plants over two growing seasons. Population sites will include lakes where wild rice is rare or diminishing in addition to sites with healthy, robust populations. DNA will be isolated from the collected leaves and will be tested with SSR markers. The SSR marker data*, (i.e.,* information from wild rice DNA) will be used to calculate the genetic distances among the sampled wild rice populations. Genetic distance is a measure of the divergence of one population of wild rice from another and indicates whether populations are different or alike. The genetic information from this study may be used to directly assist natural resource managers in the conservation and restoration of wild rice. For example, as restoration of both currently and historically important wild rice populations is considered, the proper genetic type of wild rice can be re-introduced into a specific site. In addition, if unique genetic varieties of wild rice are discovered, appropriate conservation measures can be employed to ensure their protection.

Additionally, Drs. Biesboer, Kahler and Kern have been and continue to be actively involved in communicating with the Native American community about wild rice genetics research. Formal letters on letterhead from the University of Minnesota describing this project and accompanied by a proposal were sent to all tribal councils in Minnesota in November 2010. These stakeholders included: Bois Forte Tribal Government, Fond du Lac Reservation, The Minnesota Chippewa Tribe, Leech Lake Band of Ojibwe, Lower Sioux – Morton, Mille Lacs Band of Ojibwe, Prairie Island Indian Community, Shakopee Mdewakanton Sioux Community, Upper Sioux Community, and the White Earth Band of Ojibwe, A full copy of the proposal was sent to each of these native American tribes with an open invitation to participate in the project. It was noted at that time that we would not trespass on their reservation borders without their explicit approval. And, it was pointed out that all information about this project was publically available at the LCCMR website.

Dr. Biesboer personally disseminated the proposal for this project to members of the White Earth Band who attended an event at the Itasca Biological Station during the summer of 2010. He encouraged them to ask questions about and to provide feedback on the project. In August of 2009, Dr. Kern was invited to present the results of his wild rice research as part of the first annual Wild Rice Symposium held on the White Earth Reservation. Symposium participants included members of several Ojibwe bands in addition to those from White Earth and he has maintained a working relationship with several Ojibwe members since that time. Drs. Biesboer, Kahler and Kern are currently serving on the steering committee for the second annual Wild Rice Symposium being held on the White Earth Reservation in August of 2011. Dr. Kahler has been invited to present and discuss the results of the small, preliminary wild rice genetic diversity study that are the basis for this project. He has also agreed to participate in an informal discussion about wild rice genetics research with the symposium participants in a “talking circle” format. The finding from this project will be organized into a report that will be submitted to each of the Minnesota bands via their resource management directors. Further information about this project was presented to all participants including band representatives of an all day working group on the effects of sulfates on wild rice at the MPCA on 9 May 2011. Several representatives from various bands were present at this working group and no objections were raised concerning this project.

III. PROJECT STATUS UPDATES:

Project Status as of 21 December 2011: The progress of this proposed research is right on target. This past summer, leaves from 35 populations of wild rice were harvested from lakes in Minnesota, packaged and stored at -80 C for analysis during the second phase of Activity 1. Storage occurred in several places around the state. Most collections now have arrived on the St. Paul campus. Harvesting occurred between 1 August and September 15. The second activity will begin in January, 2012. The laboratory is currently being set up for DNA isolation and genotyping analysis. A list of these 35 lakes is appended (Attachment I) as the last page of this status update and has been reported to our website. No difficulties were encountered during the collections of wild rice in the state.

Project Status as of 29 June 2012: Project Status as of 29 June 2012: DNA has been extracted and quantified from the first thirty five populations of wild rice (see Attachment 1). Genotyping with the proposed set of fifteen SSR markers is currently under way. Nine populations have been genotyped with the complete set of fifteen SSR markers. The remaining sixteen populations are in various stages nearing completion with the full set of markers. All thirty-five populations will be completely genotyped by the middle of July, 2012. Normalization of the genotypic data has taken somewhat longer than anticipated. The project is on track to have the preliminary genetic distance analysis completed on the first thirty-five populations by the end of July 2012.

Summary Budget Information for Activity 1: ENRTF Budget: $97,000
Amount Spent: $97.000
Balance: $ 0

Project Status as of 30 December 2012: Our team has been successful, after a lot of travel, in collection of wild rice leaf tissue from across the state. At this point in time, we have met our goal of collecting 70+ lakes and rivers from most counties where wild rice is found in the state of Minnesota.

DNA has been extracted from the leaf samples from the 35 populations sampled during the summer of 2011 and we are extracting DNA from the 2012 collections. The genotyping of the first set of 35 populations is nearly complete with some samples needing to be re-run to clarify the results from some SSR markers. Genotyping of the population samples collected during the summer of 2012 will begin January 2, 2013. Analysis of genotypic data will become ongoing as more data are collected. The project is on track for all genotyping to be completed in time for an analysis of genetic diversity among all 70 wild rice populations by the end of June 2013.

Summary Budget Information for Activity 1: ENRTF Budget: $97,000
Amount Spent: $97,000
Balance: $ 0

Summary Budget Information for Activity 2: ENRTF Budget: $98,000
Amount Spent: $98.000
Balance: $ 0

Project Status as of 30 June 2013: Samples from all 70 lakes have been submitted for genotyping. Genotypic data for 45 lakes have been obtained and analysis is underway. The remaining genotypic data will continue to be obtained through July. This is a long process and provides a very large amount of data for analysis. Final genetic distance analysis will be completed by August 30, at which time a formal final report will be prepared. Several equipment failures significantly slowed the DNA extraction and genotyping of the last set of 35 lakes due to delays in equipment repair. To date, the quality of the data is excellent and no issues are expected in completing the analysis.

Project Status as of 18 March 2014: Sample analysis is complete. Genotyping for all 70 populations of *Z. palustris* has been entered into our data analysis program. The SSR marker data*, (i.e.,* microsatellite loci developed by our labs) is now being used to calculate the genetic distances among the sampled wild rice populations. Purified DNA from 50 individuals in each lake using standard polymerase chain reaction conditions with primer labeled with fluorophores on the 5' end of the forward primer. Resulting PCR products were analyzed and automated fragment analysis was conducted using standard procedures on the 3100 Genetic Analyzer at ABI, Inc. Electropherogram peaks generated by the 3100 Genetic Analyzer were compared to internal size standards and scored using the GeneMapper software packages at ABI, Inc. Resultant fluorescent base pair sizes associated with each peak were collected into a large Microsoft Excel spreadsheet. Now, population genetic analysis is being performed by the PowerMarker software package as originally proposed.

The data set is very large. Resulting genetic distance is a measure of the divergence of one population of wild rice from another and indicates whether populations are different or alike. This is a very sophisticated analysis and is time consuming and prone to data entering errors, however, those errors are now being corrected for a final analysis. Many iterations must be performed by the computer until the researchers can assess whether or not the data output is accurate for the final report.

Other tabular data will be included in the final report.

As an example of our progress, please refer to Attachment II near the end of this report. This is the principle form in which 100,00 data points will be expressed in the final report. This dendrogram is not a final representation of our data because it still has some slight errors in it. As noted, we are running other iterations, examining them carefully for errors, and will develop a very accurate interpretation of the dendrogram before the final report. Attachment III show a key that matches the genetic information presented in Attachment II. It is also being double checked for complete accuracy.

Attachment III is a map that shows our collections across the state by county. This map has yet to be keyed to our populations but that should be done in short order. This map was requested by the LCCMR to replace the Google Map that is also seen below.

The final report will be written within in a few weeks of this report. Certainly we hope to have it by the end of March or early April, 2014. We are being very careful in our analysis and what conclusions we might reach concerning this data because it may have far reaching implications for management of wild rice in the State of Minnesota.

IV. PROJECT ACTIVITIES AND OUTCOMES:

ACTIVITY 1: Initial collections of wild rice and initial SSR laboratory analyses

Description: Collection/Analysis 1. Activity 1 consists of two outcomes: a) collection of leaves from wild rice plants from 35 lake populations as the plants mature late in the summer and early fall; and b) DNA isolation and genetic analyses of collected samples. Thirty-five lakes will be identified for initial collections in Minnesota. Lakes will be collected from robust populations of wild rice, locations will be noted for each population, and leaves placed on ice for transport to the University of Minnesota for safe storage in -80 C freezer.

Daily rental of travel vehicles from University of Minnesota motor pool will occur as needed. Minor field equipment (*i.e.* GPS units to note locations, inexpensive two way radios for communication and on the water safety; miscellaneous disposable field supplies including ice, disposable styrofoam coolers, plastic bags) will be purchased to support the fieldwork. The website will be developed for data housing and interim progress reports.

Laboratory SSR analysis of first 35 wild rice populations will be performed in the Department of Agronomy and Plant Genetics.

Supplies including chemicals and reagents, and consumable plastics will be purchased to support the laboratory research. A PCR sealing machine for plates will be purchased to ensure quality of the isolated DNA (see justification below). The procedures will include automated DNA collection and PCR of DNA prior to mailing samples to Brookings, SD for genotyping services.

After return of the genotyping information, the data files will need to be converted and an initial analysis of allele frequency data will begin. Specific outcomes will include preliminary information on genetic distance analysis of the first collections and a summary to develop the 29 June 2012 status report.

Summary Budget Information for Activity 1:

ENRTF Budget: $97,000
Amount Spent: $97,000
Balance: $0

Activity Completion Date: 29 June 2012. Part 1 of Activity 1 is complete. Part 2 of Activity 1 is well under way. Due to time needed to normalize the genotypic data, analysis is about one month behind. The issue has been addressed and the project is advancing well and will be caught up by the end of July 2012. The genetic distance analysis is working well and relationships among populations are being observed. As soon as the preliminary analysis is completed on the first thirty-five populations in July, the data will be reported on the project web site.

Outcome	Completion Date	Budget
1. *Specific outcomes are: a) a formal report to the LCCMR of 35 collected lakes and their GPS locations; and b) reporting this information to the wild rice website.*	1 October 2011	$24,000
2. *Specific outcomes are: a) a formal report of the SSR analysis to the LCCMR of first 35 wild rice populations concerning the preliminary genetic distance analysis of those wild rice populations; and b) updates of these data will be reported to the interim data on website.*	29 June 2012	$73,000

ACTIVITY 2: **Collection/Analysis 2**. Activity 2 mirrors Activity 1 and consists of two outcomes: a) collection of leaves from wild rice plants from an additional 35 lake populations as the plants mature late in the summer and early fall; and b) DNA isolation and genetic analyses of collected samples.

Description:
Little description of Activity 2 is needed because it almost exactly mirrors Activity 1. Our focus will be on collecting an additional 35 lakes with special attention to lakes at the edge of the wild rice range in Minnesota. As noted above, we will follow the exact sequence of activities, i.e., collect, freeze samples, and perform SSR analysis of the last collections. Little equipment will be purchased for these later collections, and most of our focus will be on the purchase of laboratory supplies and performing the genetic analyses. A second round of salaries will be paid. Specific outcomes will include reporting final information on the genetic distances uncovered by SSR analysis for 70 lakes, final updating of the project website, and completion of the final LCCMR report. Following the completion of this project, the results will be written into publication format and will be submitted to a peer-reviewed scientific journal.

Summary Budget Information for Activity 2:

ENRTF Budget: $98,000
Amount Spent: $98,000
Balance: $ 0

Activity Completion Date: 30 June 2013

Outcome	Completion Date	Budget
1. *Specific outcomes are: a) a formal report to the LCCMR of the second set of 35 collected lakes and their GPS locations; and b) reporting this information to the wild rice website.*	1 October 2012	$22,500
2. *Specific outcomes are: a) a formal report of the SSR analysis to the LCCMR of the **second** 35 wild rice populations concerning the preliminary genetic distance analysis of those wild rice populations; b) a summarization and development of 29 June 2013 status report; and c) final LCCMR reports including genetic distances for total of 70 populations and d) updates of final data on website.*	30 June 2013	$75,500

Activity Status as of 1 October 2011: Activity 1, part 1, was completed as designed. Wild rice collections were begun on 1 August 2011 and completed on 15 September 2012. Thirty-five lakes were collected. The collection data is appended to this report.

Activity Status as of 1 March 2012: DNA has been extracted from the first 35 wild rice populations. Eleven of fifteen SSR markers have been run on 27 of the 35 populations. Genetic analysis has been completed on this preliminary data set in order to verify that the sampling and analysis processes are valid. The results indicate that the proposed methods are valid. More samples are being prepared for SSR analysis with most being completed by the end of April. Planning has begun for the next field season to accomplish our goal of 70 sampled lakes. A key to collection sites and a Google map that corresponds to those sites has been appended to the end of this report (Appendix II and Google map).

Activity Status as of 29 December 2012: Some samples from the first set of thirty five lakes collected during the summer of 2011 have been problematic to genotype. This is common when working with leaf samples of varying maturity and degree of health. These samples are being re-analyzed using additional DNA samples from the samples we have collected. The missing data have slightly hindered the completion of the genetic distance tree for the first set of populations as the models for calculating genetic distance do not account for missing data. The re-analyzed samples are expected to be completed by February 1, 2013. At that time, we will have a genetic distance tree for publication on our website. At the same time, genotyping of the population samples from Activity two will be well underway.

Activity Status as of 28 June 2013 Samples from all 70 lakes have been submitted for genotyping. Genotypic data for 45 lakes have been obtained and analysis is underway. The remaining genotypic data will continue to be obtained through July. This is a long process and provides a very large amount of data for analysis. Final genetic distance analysis will be completed by August 30, at which time a formal final report will be prepared. Several equipment failures significantly slowed the DNA extraction and genotyping of the last set of 35 lakes due to delays in equipment repair. To date, the quality of the data is excellent and no issues are expected in completing the analysis

Final Report Summary: The work plan for this project has been accomplished. As noted in this plan, the final scientific report to LCCMR will be done by August, 2013.

V. DISSEMINATION:

Description: All data and information gathered from this research will be reported to a section of the Itasca Biological Station and Laboratories website at: http://www.cbs.umn.edu/itasca/. A final written report will be submitted to the LCCMR. In addition, as time and opportunities arise, the project will be presented at seminars and professional meetings both state-wide or nationally. Finally, following full completion of the project and required reporting, the investigators will prepare the result in publication format and will submit the work to a peer-reviewed journal for publication.

Status as of 30 December 2011: All progress to date, which at this time only includes the list of collected lakes, has been reported to the web site listed above.

Status as of* 29 June 2012*: The list of thirty five wild rice populations included in Activity 1 and a map showing the locations in the state have been placed on the project web site (http://www.cbs.umn.edu/itasca). Immediately following the completion of preliminary genetic distance analysis of the first thirty-five populations, the genetic distance information will be placed on the web site. The project plan and status was presented by Dr. Alexander Kahler at the second annual wild rice symposium held at White Earth Reservation in August of 2011. Additionally, Dr. Kahler presented a poster summarizing the project at the Plant and Animal Genome Conference in January of 2012.

Status as of 30 December 2012: The list of seventy plus wild rice populations included in Activities 1 and 2 and a map showing the population locations in the state will be placed on the project web site in January, 2013 (http://www.cbs.umn.edu/itasca). At the end of this report, a list and map are included identifying the 72 populations that we have collected. Before the end of this project, a more sophisticated map using GIS information for each collection will be provided. Once missing data for the first thirty-five populations have been re-analyzed (see project status on page 3 above), the preliminary genetic distance tree will be added to the web site.

Status as of 28 June 2013: Dr. Kahler has continued to meet at regular intervals as part of a College of Food, Agriculture and Natural Resource Sciences committee that is focused in finding common ground between the University of Minnesota and the Ojibway concerning wild rice research. He has continued to openly discuss the goal of the research and the progress of the project. The final project report and published paper will be made openly available via the project web site.

Final Report Summary: This project is completed as designed. However, a full final report will be sent to LCCMR after a complete analysis of the data. Data of this type is quite extensive and requires many iterations of analysis via bioinformatics programs. We expect to have a significant scientific report and publication in August, 2012. It will be disseminated via website and public forums.

VI. PROJECT BUDGET SUMMARY:

A. ENRTF Budget:

Budget Category	$ Amount	Explanation
Personnel:	$40,666	Dr. Kahler (25% FTE – 2011/12 and 2012/13 Dr. Anthony Kern (2 months summer salary) - 2011/12 and 2012/13
Service Contracts	$113,400	Analysis of 70 lake populations with 15 SSR markers by Biogenetic Services, Inc.
Equipment/Tools/Supplies:	$20,670	Field supplies, DNA extraction and PCR
Capital Equipment over $3,500:	$9,000	PCR plate sealer, bioinformatics software

Printing and Postage:	$ 1,264	For publication or presentation purposes; sample shipments
Travel Expenses in MN:	$10,000	Personal vehicle @$0.51/mile or current U of M rate; daily vehicle rental as necessary; meals and hotels as needed
TOTAL ENRTF BUDGET:	**$195,000**	

Explanation of Use of Classified Staff: N/A

Explanation of Capital Expenditures Greater Than $3,500: The capital expenditures are to purchase a PCR plate-sealing machine and a proprietary software package that are crucial to the genetics research. The abbreviation refers to Polymerase Chain Reaction, a standard method in this type of research that is used to increase the amount of DNA in a sample for subsequent analysis of its sequences. The PCR plate-sealing machine will cost $4,000.00. The investigators do not currently have access to a PCR plate-sealing machine at the University of Minnesota. Largely due to liability issues, scientific equipment is not available for rent. The PCR plates must be sealed in order to ensure that the PCR reactions do not evaporate or contaminate each other during shipment to the testing laboratory, which would result in no data or false data respectively. The PCR plate-sealing machine will remain in the Department of Agronomy and Plant Genetics at the University of Minnesota following the completion of this project and will be made openly available for future research.

The GeneMapper software package is needed to convert the SSR genotyping information files. GeneMapper is a proprietary software package developed and sold by ABI, Inc. for use with the data files generated by their 3100 genetic analyzer, which is the system that is used for SSR genotyping. The cost for purchasing GeneMapper is $5,000.00. If the data files are converted by Biogenetic Services, Inc. instead of the investigators using GeneMapper, it would cost an additional $12,600.00.

Number of Full-time Equivalent (FTE) funded with this ENRTF appropriation: 1 person at 0.25% FTE per year for a total of **0.50%** over the two years of the proposal; 1 person at 0.08% FTE per year for a total of **0.16%** over the two years of the proposal. Total FTE for 2 years is: **1.32%** FTEs.

B. Other Funds:

Source of Funds	$ Amount Proposed	$ Amount Spent	Use of Other Funds
Non-state			
Sabbatical leave salary of Professor Anthony Kern	$31,513	$31,513	**In-kind Services During Project Period**: Professor Anthony Kern will be on sabbatical leave from Northland College during 2011-2012 (one year). These funds represent an in-kind ½ salary contribution to the project from Northland College.
State			
Professor, U of M, Biesboer	$13,548	$13,548	**In-kind Services During Project Period:** Professor Biesboer is on an 11-month appointment at the U of M and ineligible for salary; he will work at a non-mandatory cost share as indicated for two months of time.
TOTAL OTHER FUNDS:	**$45,061**	**$0**	

VII. PROJECT STRATEGY: Project Partners:

A. Dr. Alex Kahler, Research Associate, Department of Agronomy and Plant Genetics, University of Minnesota (Funds received: $26,660)

B. Dr. Anthony Kern, Associate Professor, Northland College, Ashland, Wisconsin (Funds received: $14,006.00)

B. Project Impact and Long-term Strategy: The importance of this project lies in preserving one of Minnesota's most valuable natural assets. It has been documented for decades that wild rice in the State of Minnesota has been diminishing in abundance and declining in genetic fitness. The direct purpose of this project is to identify the "types" of wild rice that occur in Minnesota, asking the questions "How many unique genetic types of wild rice exist and where do they exist?

The results of this project will be extremely valuable in the decades to come as this unique and valuable species comes under ever increasing detrimental environmental pressures. Having a large subset of Minnesota wild rice populations genetically characterized will allow resource managers to utilize an appropriately specific genetic type to restore or supplement wild rice populations throughout the state. This approach will increase the success of conservation and restoration efforts.

Perhaps most importantly, we fullly expect to identify populations that might be called unique or even rare that will deserve ultimate protection and conservation efforts. Finally, the methods and techniques used and developed in this study may become models for understanding genetic diversity of non-Minnesota wild rice populations and other naturally-occuring plant species in future studies.

Tribal natural resource managers will be able to use the data and methods from this project to scientifically monitor the genetic diversity of wild rice populations on the reservations. The final report will provide the necessary information to allow the tribes to do the use the scientific tools themselves. Of course, if they wish to discuss the technology with the project investigators, they will make themselves available for consultation. It is expected that using genetic diversity data for managing wild rice restoration and population enhancement will result is healthier, more robust natural wild rice populations that will allow for increased and more consistent rice harvests.

C. Spending History: N/A

VIII. ACQUISITION/RESTORATION LIST: N/A

IX. MAP(S): N/A

X. RESEARCH ADDENDUM: See research addendum

XI. REPORTING REQUIREMENTS:
Periodic work plan status update reports will be submitted not later than 30 December 2011, 29 June 2012 and 28 December 2012. A final report and associated products will be submitted between June 30 and August 1, 2013 as requested by the LCCMR.

Correction: A final report will be forthcoming near the end of March, beginning of April, 2014.

XII. SERVICE CONTRACT WITH BIOGENETICS SERVICES, INC: See research contract on the last page.

BIOGENETIC SERVICES, INC.

801 32ND AVENUE • BROOKINGS, SOUTH DAKOTA 57006
BUSINESS: (605)697-8500 • 1-800-423-4163
FAX (605)697-8507
Email: biogene@brookings.net
www.biogeneticservices.com

May 4, 2011

Service Contract

This services contract is to be for 2 years and to conduct genotyping on 70 wild rice populations using 15 SSR markers.

Year 1:

Genotype 48 individuals each from the first 35 populations using the 15 SSR markers.

Cost: 48 individuals X 35 populations X 15 SSR markers X $2.25 per data point = **$56,700**

Year 2:

Genotype 48 individuals each from the remaining 35 populations using the same 15 SSR markers. The entire data set will be completed by May 1, 2013.

Cost: 48 individuals X 35 populations X 15 SSR markers X $2.25 per data point = **$56,700**

The total amount to complete the 2 year project will be **$113,400.**

Sincerely,

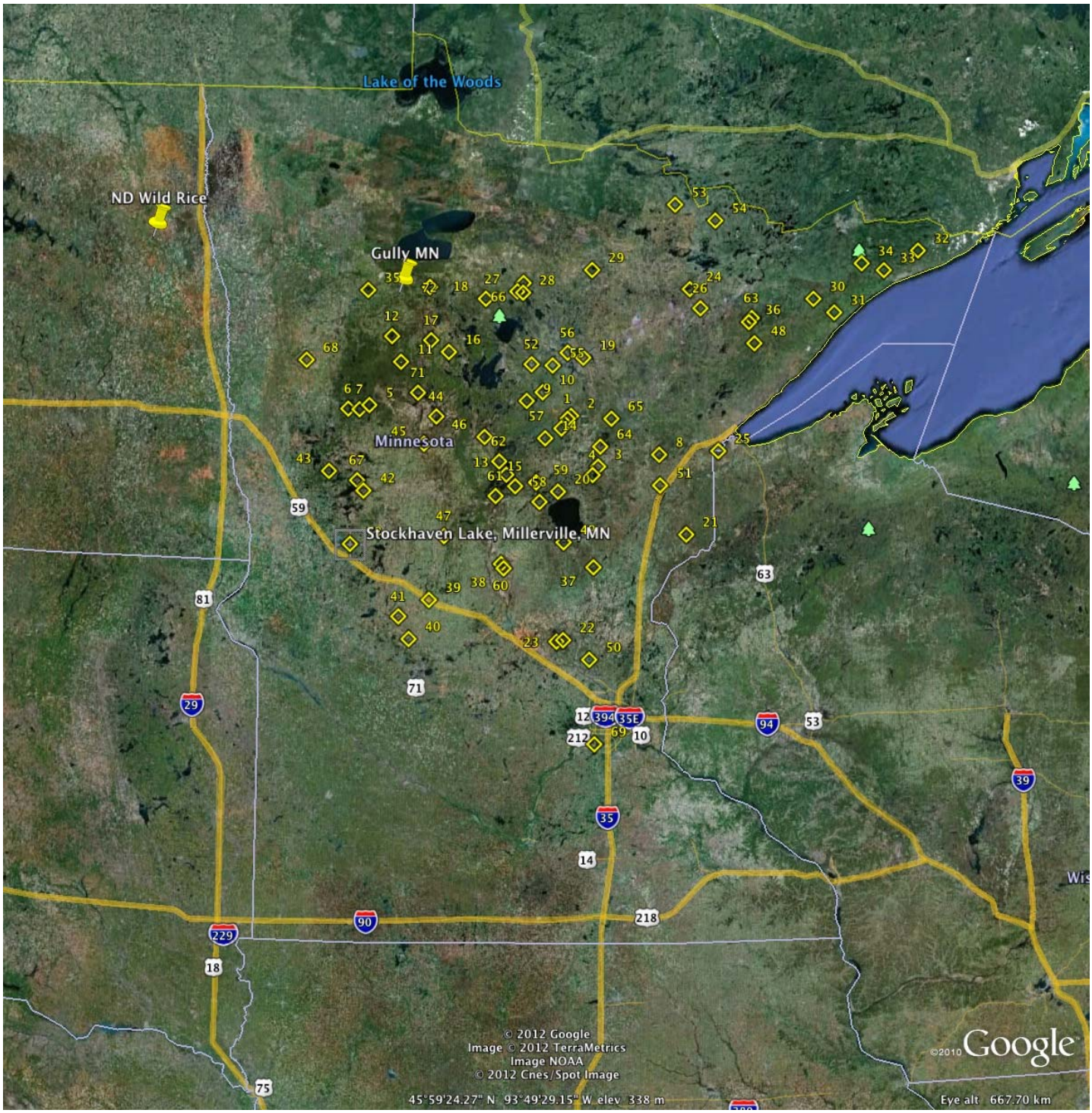
J. Kahler, Lab Director

Attachment I. Wild Rice Populations Collected Between 1 August 2011 and 15 September 2011; and 1 August 2012 to 15 September 2012.

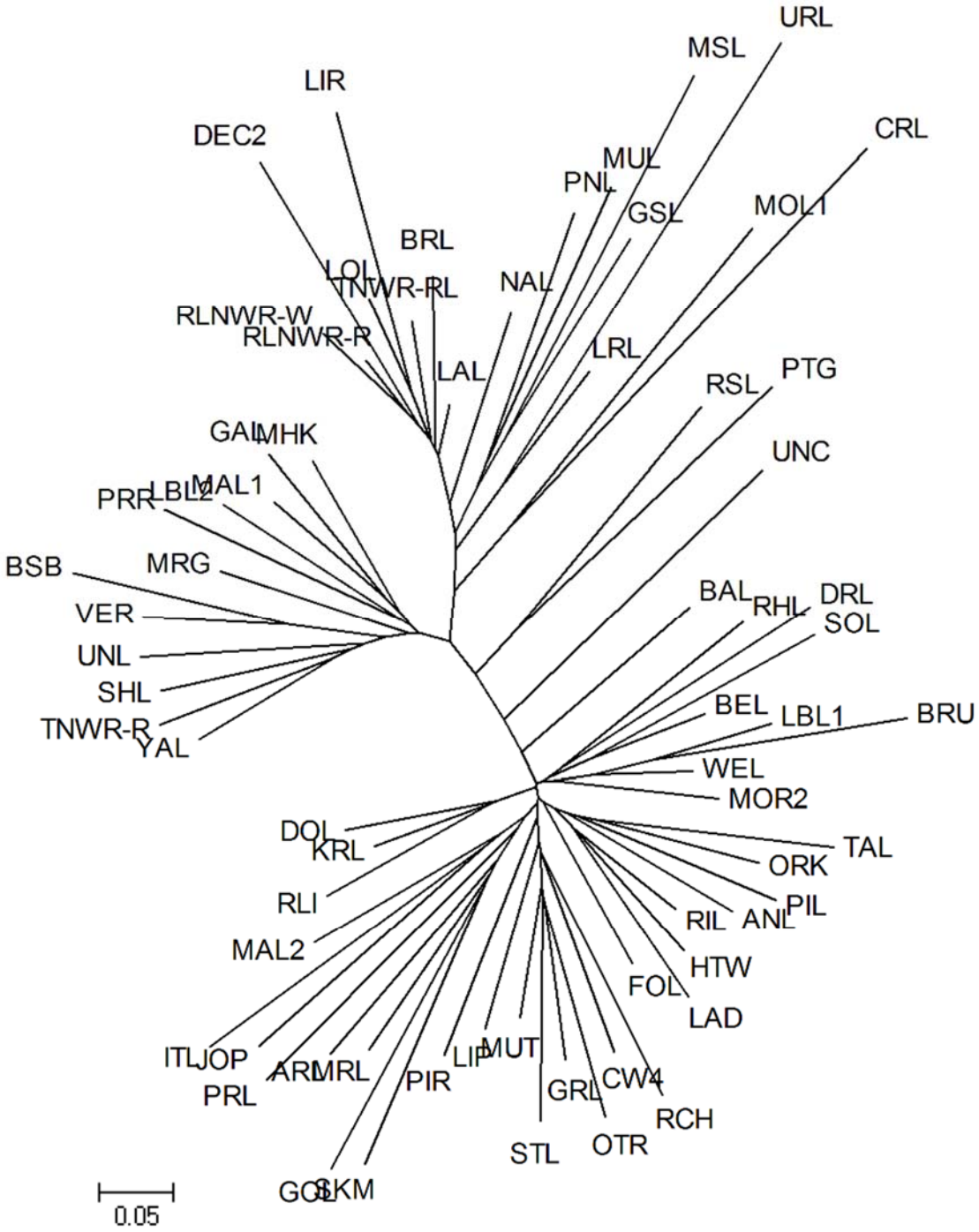
This is a key for the following collection map.
This is a list of all wild rice
collections made in 2011 and 2012.

Map Designation	Name
1	Moose Lake
2	Moose River
3	Rice River
4	Rice Lake
5	Shell Lake
6	Tamarack Lake
7	Rice Lake
8	Kettle Lake
9	Laura Lake
10	Little Birch Lake
11	Itasca Lake
12	Upper Rice Lake
13	Garden Lake
14	Lows Lake
15	Mississippi River
16	Necktie River
17	Plantagenet Lake
18	Decker Lake
19	Prairie River
20	Mallard Lake
21	Crooked Lake
22	Josephine Pool
23	Orrock Lake
24	Big Rice Lake
25	St. Louis River
26	Pike River
27	Nature's Lake
28	Rice Lake
29	Deer Lake
30	Round Island Lake
31	Cramer Lake
32	Brule River
33	Mark Lake
34	Marsh Lake
35	Pine Lake
36	St. Louis River

37	**Ann Lake**
38	**Mud Lake**
39	**Unnamed lake**
40	**Tamarac Lake**
41	**Grove Lake**
42	**Grass Lake**
43	**Star Lake**
44	**4th Crow Wing Lake**
45	**Yaeger Lake**
46	**Pine River**
47	**Mud Lake**
48	**Breda Lake**
49	**Lake Onemia**
50	**Pickerel Lake**
51	**Moose Horn River**
52	**Goose Lake**
53	**Vermillion River**
54	**Little Indian Sioux River**
55	**Unnamed Lake**
56	**Bass Lake**
57	**White Elk Lake**
58	**Hesitation WMA**
59	**Rice Lake**
60	**Platte River**
61	**Red Sand Lake**
62	**Rice Lake**
63	**Turtle River**
64	**Stone Lake**
65	**Flowage Lake**
66	**Rat House Lake**
67	**Otter Tail River**
68	**Unnamed lake**
69	**Fisher Lake**
70	**Stockhaven Lake**
71	**Rice Lake**
72	**Little Puposky**


Lake of the Woods
ND Wild Rice
Gully MN
Minnesota
Stockhaven Lake, Millerville, MN
© 2012 Google
Image © 2012 TerraMetrics
Image NOAA
© 2012 Cnes/Spot Image
45°59'24.27" N 93°49'29.15" W elev 338 m
Eye alt 667.70 km
©2010 Google

Attachment II. First cut of data gathered from the analysis of 70 lake and river populations across Minnesota. Unrooted dendrogram using Nei 83 genetic distance values for all wild rice population. The radiation tree was generated using pair-wise Nei 83 values. It is not a final dendrogram but is used here to illustrate the nature of the information that we will provide in the final report. A key is provided in Attachment III that corresponds to these populations.

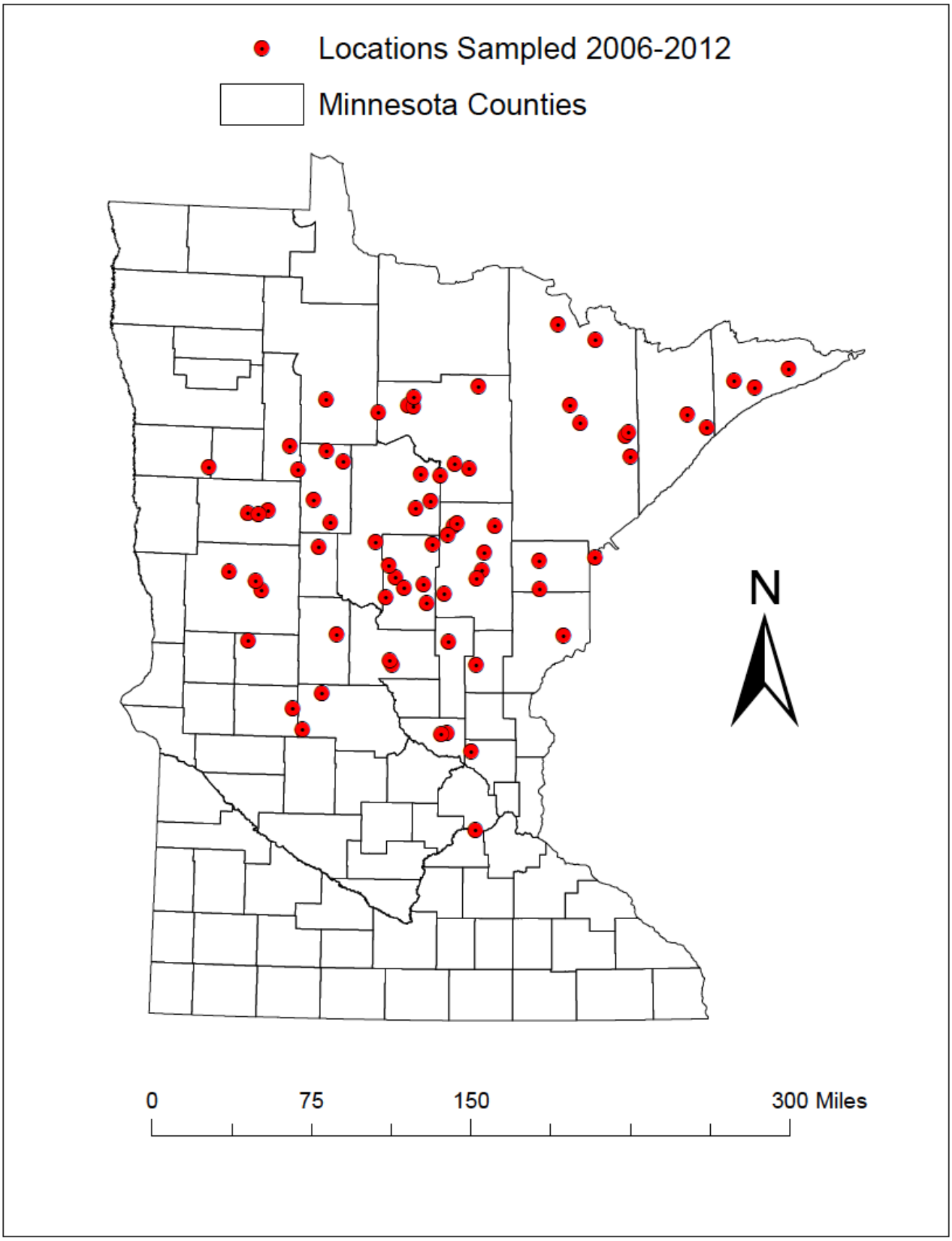


Attachment III. This is a key that matches the populations shown in the dendrogram in Attachment II. This is not complete as we double check each population to ensure the information presented in the table is accurate.

Population Name	ID	County
(another) Rice Lake	ARL	Crow Wing
4th Crow Wing Lake	CW4	Hubbard
Ann Lake	ANL	Kanabec
Bass Lake	BAL	Itasca
Big Rice Lake	BRL	St. Louis
Breda Lake	BEL	St. Louis
Brule River	BRU	Cook
Cramer	KRL	Lake
Crooked Lake	CRL	Pine
Decker Lake	DEL2	Itasca
Deer Lake	DRL	Itasca
Dora Lake	DOL	Itasca
Flowage Lake	FOL	Aitkin
Garden Lake	GAL	Crow Wing
Goose Lake	GSL	Cass
Grass Lake	GRL	Otter Tail
Grove Lake	GOL	Pope
Hart Lake (Necktie River)	HAL	Hubbard
Hesitation WMA	HTW	Crow Wing
Itasca	ITL	Clearwater
Josephine Pool (Sher. NWR)	JOP	Sherburne
Kettle	KEL	Carlton
Lake Onemia	LAO	Mille Lacs
Laura	LAL	Cass
Little Birch	LBL2	Cass
Little Indian Sioux River	LIR	St. Louis
Little Puposky	LIP	Beltrami
Lows Lake	LOL	Crow Wing
Mallard Lake	MAL	Mille Lacs
Mark Lake	MRL	Cook
Marsh Lake	MSL	Cook
Miss. River-Green's Pt.	MRG	Crow Wing
Moose Horn River	MHR	Carlton
Moose Lake	MOL1	Aitkin
Moose River	MOR2	Aitkin
Mud Lake	MUL	Morrison
Mud Lake	MUT	Todd
Nature's Lake	NAL	Itasca
Orrock Lake (Sher. NWR)	ORK	Sherburne
Otter Tail River	OTR	???
Pickerel Lake	PIL	Anoka
Pike River	LRL	St. Louis
Pine Lake	PNL	Clearwater
Pine River	PIR	Cass
Plantagenet	PTG	Hubbard

Platte River	PLR	Morrison
Prairie River	PRR	Itasca
Rat House Lake	RHL	???
Red Sand Lake	RSL	Crow Wing
Rice	RLI	Itasca
Rice Lake	RIC	Crow Wing
Rice Lake	RCH	Hubbard
Rice Lake NWR-River	RLNWR-R	Aitkin
Rice Lake NWR-west	RLNWR-W	Aitkin
Round Island Lake	RIL	Lake
Shell	SHL	Becker
St. Louis River, North Bay	BSB	St. Louis
St. Louis River, Skibo Mill	SKM	St. Louis
Star Lake	STL	Otter Tail
Stone Lake	SOL	St. Louis
Tamarac Lake	TAL	Stearns
Tamarac NWR-Rice Lake	TNWR-RL	Becker
Tamarac NWR-Tamarac Lake	TNWR-TL	Becker
unnamed	UNL	Stearns
unnamed	UNC	Cass
unnamed	UNN	Norman
Upper Rice Lake	URL	Clearwater
Vermilion River	VER	St. Louis
White Elk Lake	WEL	Aitkin
Yaeger Lake	YAL	Wadena

Attachment IV. Locations of wild rice collections in rivers and lake in Minnesota. This map will be ultimately keyed to corresponding population abbreviations as illustrated in Attachment III.



Attachment A: Budget Detail for M.L. 2011 (FY 2012-13) Environment and Natural Resources Trust Fund Projects

Project Title: Understanding Threats, Genetic Diversity, and Conservations Options for Wild Rice
Legal Citation: M.L. 2011, First Special Session, Chp. 2, Art.3, Sec. 2, Subd. 04o
Project Manager: David D. Biesboer
M.L. 2011 (FY 2012-13) ENRTF Appropriation: $ *195,000*
Project Length and Completion Date: 30 June 2014
Date of Update: Final Report

ENVIRONMENT AND NATURAL RESOURCES TRUST FUND BUDGET- Final Report	**Activity 1 Budget**	**Amount Spent**	**Balance**	**Activity 2 Budget**	**Amount Spent**	**Balance**	**TOTAL BUDGET**	**TOTAL BALANCE**
BUDGET ITEM	***Collection/Analysis 1***			***Collection/Analysis 2***				
Personnel (Wages and Benefits)								
1) Dr. Alexander Kahler - salary for 25% time continuously throughout the year. Will collect wild rice leaf samples, extract DNA, set up PCR and analyze genetic data. Includes 33.3% fringe benefits ($3330 for C/A 1 and $3330 for C/A 2)	13,330	13,330	0	13,330	16,412	-3,082	26,660	-3,082
2) Dr. Anthony Kern - 8.33% time between August 2011 and June 2012. The remaining 91.67% time will be donated to this project. Will collect wild rice leaf samples, assist with DNA extraction and PCR set up. Includes 32.33% fringe benefits ($2264 for C/A 1 and $2264 for C/A 2)	7,003	7,003	0	7,003	7,087	-84	14,006	-84
Service contracts **1)** Specific service contract with Biogenetic Services, Inc. (Brookings, SD) to complete genotyping of wild rice populations with 15 SSR markers.	56,700	56,700	0	56,700	28,350	56,700	113,400	0
Equipment/Tools/Supplies								0
1) Equipment - 2 GPS units to record sample collection sites. 2-way radios for communication and safety in remote sampling locations. 1 Kayak for wild rice sampling.	500	184	316	0	1,596	-1,596	500	-1,280
2) Chemicals and Reagents - DNA extraction and PCR reagents including *Taq* enzyme, PCR primers and comsumables kit for automated DNA extraction.	3,620	3,620	0	8,150	11,522	-3,372	11,770	0
3) Cosumable Plastics - DNA extraction and PCR consumables including plastic plates for tissue grinding and PCR set up and pipette tips for liquid sample handling.	2,100	2,100	0	6,300	5,531	769	8,400	769
4) Postage - mailing costs for shipping PCR reactions to Biogenetic Services, Inc. for genotyping service.	210	210	0	481	172	309	691	309
Capital equipment over $3,500								0
1) Sealing machine - for sealing PCR plates for genetic analysis	4,000	3,140	860	0	0	860	4,000	860
2) Computer software - for genetic analaysis	4,537	0	4,537	463	0	463	5,000	3,495
Printing	0	0	0	573	63	510	573	510
Travel expenses in Minnesota- mileage reimbursement (personal vehicle at U of M rate of $0.51 /mile), meals and lodging for sample collection trips in 2011, and 2012	5,000	5,000	0	5,000	3,877	1,123	10,000	1,123
COLUMN TOTAL	**$97,000**	**$91,287**	**$5,713**	**$98,000**	**$74,612**	**$52,598**	**$195,000**	**$0**