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

Date of Status Update:

Date of Next Status Update: 12/1/2011

Date of Work Plan Approval: 6/23/2011

Project Completion Date: 6/30/2014 **Is this an amendment request?** _____

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:

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 $:	0
	Balance $:	195,000

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 30 December 2011:

Project Status as of 29 June 2012:

Project Status as of 30 December 2012:

Project Status as of 28 June 2013:

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: $ 0
Balance: $97,000

Activity Completion Date: 29 June 2012

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: $ 0
Balance: $98,000

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 Status as of 1 March 2012:

Activity Status as of 1 October 2012:

Activity Status as of 1 March 2013

Final Report Summary:

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:

Status as of* 29 June 2012*:

Status as of 30 December 2012:

Status as of 28 June 2013

Final Report Summary:

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	$0	**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	$0	**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.

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

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 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:
Project Manager: David D. Biesboer
M.L. 2011 (FY 2012-13) ENRTF Appropriation: $ *195,000*
Project Length and Completion Date: 30 June 2013
Date of Update: 4 May 2011

ENVIRONMENT AND NATURAL RESOURCES TRUST FUND BUDGET	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	0		13,330	0		26,660	
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	0		7,003	0		14,006	
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	0		56,700	0		113,400	
Equipment/Tools/Supplies								
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	0		0	0		500	
2) Chemicals and Reagents - DNA extraction and PCR reagents including *Taq* enzyme, PCR primers and comsumables kit for automated DNA extraction.	3,620	0		8,150	0		11,770	
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	0		6,300	0		8,400	
4) Postage - mailing costs for shipping PCR reactions to Biogenetic Services, Inc. for genotyping service.	210	0		481	0		691	
Capital equipment over $3,500								
1) Sealing machine - for sealing PCR plates for genetic analysis	4,000	0		0	0		4,000	
2) Computer software - for genetic analaysis	4,537	0		463	0		5,000	
Printing		0		573	0		573	
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	0		5,000	0		10,000	
COLUMN TOTAL	**$97,000**			**$98,000**			**$195,000**	**TRUE**