

2011-2012 Main Proposal

PROJECT TITLE: Genetic Diversity, Conservation and Threats to Wild Rice

I. PROJECT STATEMENT

This proposed research seeks to understand the genetic diversity of wild rice populations in Minnesota. As noted by the MN Department of Natural Resources, “no other native species in Minnesota approaches the level of ecological, cultural and economic values embodied by wild rice.”

Wild rice (scientific name, *Zizania palustris*) is a plant with a long and interesting history, especially in the state of Minnesota. It has significance as:

- a food for humans, harvested from native stands and commercial paddies;
- an important source of nutrition for wildlife and a plant that protects shorelines from erosion;
- an agronomic crop that produces 50% of the world’s supply and is worth \$14M to both growers and those that harvest from natural populations in Minnesota;
- a plant with important spiritual meaning to Native Americans.

However, this plant is under state-wide threats that are well documented in the DNR report on wild rice (Natural Wild Rice in Minnesota, 2008, http://files.dnr.state.mn.us/fish/wildlife/legislativereports/20080215_wildricestudy.pdf). A few of these direct threats include:

- changes in hydrology of lakes and stream flows by highway construction and dams
- changes in seasonal housing on lakes that has jumped 500% in the last 20 years
- competition from both native and exotic species including plants and fish species such as carp
- loss of natural genetic diversity as the habitat declines, competition increases and global climate change accelerates

It is generally agreed, that to preserve wild rice in Minnesota, we must maintain its genetic diversity to ensure the best chance for its future survival. However, the genetic variability among and between regional populations is not known (Figure 1 shows the distribution of wild rice in Minnesota). Thus, the goals of this project are:

- 1) *to use the latest tools of molecular genetics and bioinformatics to genetically characterize the different populations of wild rice in Minnesota; and*
- 2) *to develop the knowledge to preserve and, in the future, restore wild rice populations in a scientifically-sound manner*

This study will have several outcomes. We will gain further specific knowledge about the basic biology of wild rice, *i.e.*, determine the genetic diversity of the “types” of wild rice in Minnesota. Using the powerful techniques of molecular biology and bioinformatics (very powerful computer programs), the populations present in Minnesota can be specifically “finger-printed” and their ranges across the State carefully mapped. Based on this knowledge, we will be able to make intelligent decisions for restoration efforts as needed on a statewide basis in Minnesota. Additionally, we may discover unique wild-rice populations that would need conservation and protection to preserve them for future generations.

This study will employ the latest methods currently used to understand genetic diversity in plants. DNA-based molecular markers will be used to study the populations of wild rice in the State. The most useful types of molecular markers for genetic diversity studies are restriction fragment length polymorphism (RFLP) markers and simple sequence repeat (SSR) markers (also called microsatellites). However, SSR markers are preferred because they are rapid and use a very small amount of leaf material from each sampled plant. To date, about 40 SSR markers have been developed from wild rice *per se*.

Beginning in 2006, a wild rice genetic diversity study was undertaken by Drs. Kahler (U of M) and Tony Kern (Northland College, Wisconsin). This study is ongoing. Additionally, an initial SSR-based wild rice genetic diversity study by Dr. Kahler was just completed as part of a doctoral dissertation study. His preliminary results show the utility of this approach. Most notably, some natural wild rice populations are genetically diverse from each other and one cultivated wild rice variety is genetically diverse from the wild populations.

A final and important note: We are sensitive to the fact that the Native American community might suggest that a study of this type will lead to genetic engineering of wild rice, or that it will allow wild rice breeders to further capitalize on the breeding of wild rice. It will not lead to nor contribute to genetic engineering and production of transgenic strains of wild rice. The outcomes of this study can only benefit the ecology of wild rice, *i.e.*, to give information that can be used both by tribal, DNR, and private land use managers to further conserve and restore the populations of wild rice in this state.

II. DESCRIPTION OF PROJECT ACTIVITIES

Activity 1: Collection of wild rice in western and northern Minnesota Budget: \$115,000

Outcome	Completion Date
1. Collection of wild rice samples from not well known populations in the western and northern counties of Minnesota from public lands by D. Biesboer; other central and eastern counties sampled by Kern and Kahler; samples from leaves frozen on dry ice and transported to the lab; stored at minus 80C until analysis	1 October 2011
2. Samples processed by grinding in a mortar and pestle by undergraduate assistants or DNA isolated using standard chemical procedures and robot; followed by SSR analysis in the laboratory; placing data into databases.	30 June 2012

Activity 2: Collection of wild rice from select sites across the State Budget: \$80,000

Outcome	Completion Date
1. Collection of wild rice samples not previously collected and deemed important to gather information across the state; procedures the same as above.	1 October 2012
2. Samples processed by grinding in a mortar and pestle by undergraduate assistants or DNA isolated using standard chemical procedures and robot; followed by SSR analysis in the laboratory; placing data into databases. Preparation of final report and publishing the results in a scientific journal.	30 June 2013

III. PROJECT STRATEGY

A. Project Team/Partners

Professor David D. Biesboer, Ph.D., Principal Investigator, Department of Plant Biology, U of M. Overall coordinator of project; also responsible for wild rice collections and continuing analyses.
Dr. Alex Kahler, Ph.D., Research Associate, Department of Agronomy and Plant Genetics, U of M. Expert on SSR-based wild rice research; analyses; and project advisor for analysis and bioinformatics.

Professor Anthony Kern, Ph.D., Northland College, Ashland, Wisconsin. Expert on SSR-based wild rice analysis, responsible for wild rice collections and project advisement.

B. Timeline Requirements

This project is straight forward in terms of timeline requirements. Two field seasons are needed to adequately visit and sample populations across Minnesota. In the first field season (summer 2011), Biesboer will focus on western counties of Minnesota that have historically been poorly sample and surveyed with some sampling of the western and central populations by Drs. Kahler and Kern. After our initial survey, the second field season (summer 2013) will be spent identifying and sampling critical or rare/distinct populations to complete the diversity survey.

C. Long-term Strategy and Future Funding Needs

This study will be a seminal study for the genus *Zizania*. It is long overdue because understanding the genetic biodiversity of this ecologically and economically important plant is central to its preservation for future generations...especially as it is threatened state-wide by global climate change (Figure 2). We anticipate no other funding to complete this project.

IV. PROJECT OUTREACH PLANS

After meeting with the LCCMR concerning outreach, the project team has been making very progress in informing the various bands of this research. The primary method of outreach will be telephone, e-mail correspondence and face-to-face discussions with directors of resource management or tribal councils for the tribes. These initial communications will focus on informing the directors of the proposed project and asking them for guidance in discussing the proposed project with the tribe. If significant questions arise or if a need arises for more in-depth discussion with a broader group, a meeting time and place will be arranged for such discussion to take place.

Outreach activities have begun with: The Leech Lake Band, White Earth Band, Red Lake Band, Grand Portage Band and Fondulac Band. Already, we have been told that the Portage Band will support this project. Meetings are being scheduled with the Leech Lake Band. And, at a wild rice camp at Lake Itasca on September 10, band leaders were contacted directly by Dr. Biesboer with the promise that the matter might already be discussed by tribal council the week of 13 September. Additionally, others with close contacts in the Indian community at the U of M have come on board to add more lines of communication to the tribes.

Other bands of Ojibwe will be included in outreach activities as guided by the aforementioned bands. The desired outcome of these outreach activities is that the majority of the Native American community of Minnesota will not be in opposition of this proposed project. We suggest the majority of bands will be in favor of this project once it is understood that it is aimed at conservation and restoration of natural wild rice communities.

V. ENRTF EQUIPMENT STATEMENT

All equipment purchased for use in this research will be made available for any research project studying wild rice or in ecological projects that can use it to study the genetic diversity of organisms present in the State of Minnesota. The equipment will be housed either at the main campus of the University of Minnesota or on the U of M Itasca Biological Station and Laboratories.

2011-2012 Detailed Project Budget

IV. TOTAL TRUST FUND REQUEST BUDGET: 2 years

BUDGET ITEM (See list of Eligible & Non-Eligible Costs, p. 13)	AMOUNT
Dr. Anthony Kern (one month summer salary - 2 summers) - \$9850 Graduate student, PhD (salary + tuition + fringe rate (24 months) - \$76034 Undergraduate students (isolate DNA; \$9/hr + 8.16% FICA x 1000 hrs) - \$9740	\$96,000
Equipment/Tools/Supplies: Supplies - DNA isolation chemicals, mortars, collecting supplies (2 years) - \$17,000 SSR genetic analysis (3000 plants x ca. \$5/sample x 2 years) - \$30,000 Canoe (plastic, car top for wild rice collections in shallow, rice choked lakes and rivers; this unit will be heavily used; it must be an easily portable unit; the canoe will be used after the study for similar future wild rice research and kept at the U of M Itasca Biology Station for rice research) -\$1000 Invitrogen DNA iPrep Purification Instrument (or similar) - This machine rapidly isolates and purifies DNA in a very efficient and cost effective manner. It will save many hundreds of hours of hand labor and is state-of-the art in genetic diversity studies. This machine will be part of undergrad and grad training. It will remain in the laboratory of D. Biesboer, U of M & used in future genetic diversity studies - \$45,000	\$93,000
Travel: Vehicle rental(Fleet services; U of M; cargo van; pickup) 6 wks x \$265/wk - 1590 Fuel prices: ESTIMATED (6000 miles/yr x 2 years) - \$3760	\$6,000
TOTAL ENVIRONMENT & NATURAL RESOURCES TRUST FUND \$ REQUEST	\$195,000

V. OTHER FUNDS

SOURCE OF FUNDS	AMOUNT	Status
Other Non-State \$ Being Applied to Project During Project Period:	NA	
Other State \$ Being Applied to Project During Project Period:	NA	
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.	\$6,774	
Remaining \$ from Current ENRTF Appropriation (if applicable):	NA	
Funding History: Indicate funding secured prior to July 1, 2011 for activities directly relevant to this specific funding request. State specific source(s) of	NA	

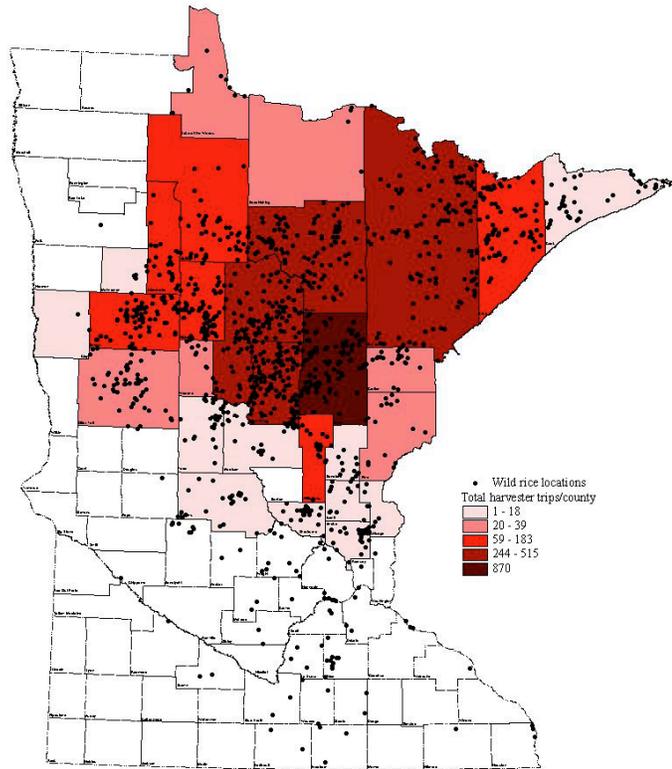


Figure 1. Wild rice lakes and harvesting pressure in Minnesota – DNR Natural Wild Rice in Minnesota, 2008

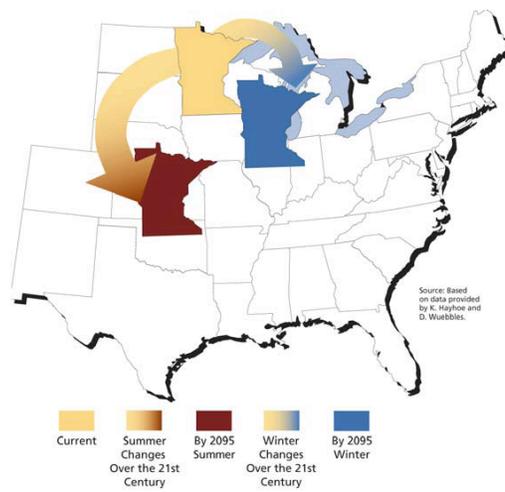


Figure 2. Predicted climate changes for Minnesota due to global climate change - DNR Natural Wild Rice in Minnesota, 2008

David D. Biesboer, Ph.D.

Professor of Plant Biology

Director, Itasca Biological Station and Laboratories

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EDUCATION

- Post-doctoral in Organic Chemistry, Indiana University, 1979-80.
- Ph.D. in Plant Biology, Indiana University, 1975-79.
- M.S. in Plant Systematics, Illinois State University, 1975.
- B.S. in Biology-Chemistry, Calvin College, 1973.

PROFESSIONAL EXPERIENCE

- Director, Itasca Biological Station and Laboratories, 1990 to present.
- Professor of Plant Biology, University of Minnesota, 1980 to present.
- Morse-Alumni Distinguished Professor of Teaching, 1995.

AWARDS AND HONORS

- Fulbright Research/Lectureship to Brazil, 2001
- Research Partnership Award, Center for Transportation Studies, Minnesota Department of Transportation, 2000.
- Appointed to the President's "Academy of Distinguished Teachers", 1999.
- Award of Merit, The Honor Society of Agriculture, 1999.
- Minnesota Erosion Control Association. Research Award for "Outstanding Contribution Advancing the State of the Art of Erosion Control, 1998.
- Morse-Alumni Award for Outstanding Contributions to Undergraduate Education; Distinguished Professor of Undergraduate Teaching, 1995.

EXPERTISE

- Ecosystem biology especially in wetlands biology with experience in the U.S., Bolivia, Brazil, South Africa, and Russia.
- Biology of wild rice

PUBLICATIONS (only a few pertinent to this request out of 70+ scientific publications)

- Duvall, M. and D.D. Biesboer. 1988. Nonreciprocal hybridization failure in crosses between annual species of wild-rice (*Zizania palustris* L. x *Zizania aquatica* L. : Poaceae). *Systematic Botany* 13:229-334.
- Duvall, M. and D.D. Biesboer. 1989. Comparisons of electrophoretic seed protein profiles among North American populations of *Zizania*. *Biochemical Systematics and Ecology* 17: 39-43.
- Cregan, J. 2004. Aspects of seeds storage, pollen travel, and populations dynamics of wild rice (*Zizania palustris*). M.S. Thesis, U of M.
- Eule-Natosha, A. 2010. Seed size of wild rice in riverine vs. lacustrine environments in Minnesota. M.S. study in progress.

Organization Description: University of Minnesota, Department of Plant Biology, St. Paul Campus Itasca Biological Station and Laboratories, located in Itasca State Park.

