Project Abstract
For the period ending June 30, 2002

FINAL REPORT

TITLE: Biological control of Eurasian watermilfoil and purple loosestrife – *Continuation* (Project E02)

Project Manager: Luke C. Skinner
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Legal Citation: Minnesota Laws 1999, Chapter 231, Section 16, Subdivision 16(a).

Appropriation amount: $150,000

OVERALL PROJECT RESULTS:

Long-term, intensive study of five Minnesota lakes documented declines in Eurasian watermilfoil in two lakes that were clearly attributable to weevils. Declines occurred in lakes that appear to have low predation on weevils by sunfish. Populations of weevils reach maximum levels in milfoil growing in large expanses or in shallow sites. Short-term survey of an additional five bays or lakes discovered no declines in milfoil that could be attributed to potential control agents.

Field observations and controlled experiments indicated that predation by sunfish can limit populations of weevils and other herbivores. Populations of weevils did not appear to be limited by plant genotype, sediment on which plants were grown, over-winter mortality, over-winter habitat, parasites, or parasitoids. Modeling of weevil populations suggest that longevity of adults and female reproduction are key determinants of both density of populations and their potential to suppress milfoil.

To facilitate biological control purple loosestrife (*Lythrum salicaria*) we undertook a mass rearing program of the root weevil, *Hylobius transversovittatus*. The root weevil proved challenging to rear and although several hundred adults were successfully reared. The effort required to rear this insect is excessive and we conclude that resources could be better spent on other aspects of the purple loosestrife biological control program. *Hylobius* larvae alone are able with stress crowns of purple loosestrife after two years of feeding. Concurrent *Galerucella* spp. feeding did not reduce *Hylobius* larval activity, as measured by root and crown starch levels. Number of seed capsules was consistently reduced on plants with *N. marmoratus* activity compared with control plants at one of two field sites. Results indicate that *N. marmoratus* is established at both study sites and is consistently reducing purple loosestrife seed production at one site.

PROJECT RESULTS USE AND DISSEMINATION:

The results will be published in peer-reviewed scientific journals,in special publications and newsletters. Results also will be presented at national, regional and state scientific meetings, as well as to resource managers who will use the results of this project.

Date of Report: July1, 2002
Date of Next Status Report:
Date of Work Program Approval: June 16, 1999
Project Completion Date: June 30, 2002

LCMR Final Work Program Report

I. PROJECT TITLE: Biological control of Eurasian watermilfoil and purple loosestrife - *Continuation* (Project E02)

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Total Biennial Project Budget:

LCMR:	$150,000
LCMR Amount Spent:	$ 150,000
$Match:	(see section VII on cooperation)
$Total	$ 150,000
=LCMR Balance:	$ 0

A. Legal Citation: Minnesota Laws 1999, Chapter 231, Section 16, Subdivision 16(a). Appropriation Language: A $75,000 the first year and $75,000 the second year are from the trust fund to the commissioner of natural resources for the fourth biennium of a five-biennium project to develop and implement biological controls for Eurasian water milfoil and purple loosestrife. This appropriation is available until June 30, 2002, at which time the project must be completed and final products delivered, unless an earlier date is specified in the work program.@

B. Status of Match Requirement: Not Applicable.

II. FINAL PROJECT SUMMARY:

Long-term, intensive study of five Minnesota lakes documented declines in Eurasian watermilfoil in two lakes that were clearly attributable to weevils. Declines occurred in lakes that appear to have low predation on weevils by sunfish. Populations of weevils reach maximum levels in milfoil growing in large expanses or in shallow sites. Short-term survey of an additional five bays or lakes discovered no declines in milfoil that could be attributed to potential control agents.

Field observations and controlled experiments indicated that predation by sunfish can limit populations of weevils and other herbivores. Populations of weevils did not appear to be limited by plant genotype, sediment on which plants were grown, over-winter mortality, over-winter habitat, parasites, or parasitoids. Modeling of weevil populations suggest that longevity of adults and female reproduction are key determinants of both density of populations and their potential to suppress milfoil (**see final milfoil report attached**).

To facilitate biological control purple loosestrife (*Lythrum salicaria*) we undertook a mass rearing program of the root weevil, *Hylobius transversovittatus*. The root weevil proved challenging to rear and although several hundred adults were successfully reared. The effort required to rear this insect is excessive and we conclude that resources could be better spent on other aspects of the purple loosestrife biological control program. *Hylobius* larvae alone are able with stress crowns of purple loosestrife after two years of feeding. Concurrent *Galerucella* spp. feeding did not reduce *Hylobius* larval activity, as measured by root and crown starch levels. Number of seed capsules was consistently reduced on plants with *N. marmoratus* activity compared with control plants at one of two field sites. Results indicate that *N. marmoratus* is established at both study sites and is consistently reducing purple loosestrife seed production at one site (**See final loosestrife report attached**).

The majority of these research results will be published in appropriate scientific journals.

IV. OUTLINE OF RESULTS OF THE PROJECT

Detailed descriptions of the background for each objective listed below, as well as proposed methods to accomplish these objectives, are provided in two proposals written by the researchers who will do this work. The proposals are included as attachments A and B to the workprogram.

A. Eurasian watermilfoil

Result A-1. Identify factors that limit populations of potential biological control agents, particularly the weevil, *Euhrychiopsis lecontei,* and their effectiveness at reducing the abundance of Eurasian watermilfoil by continued long-term sampling in five intensive study sites in different Minnesota lakes.

LCMR Budget: **$35,000** Other: $35,000

Balance: $0 Other Balance: $0

Completion Date: December 31, 2001

Result A-2. Determine the relative importance of factors that limit the populations of potential biological control agents, particularly the weevil, *Euhrychiopsis lecontei,* with frequent field

observations on weevil densities at several lakes and a series of controlled experiments to determine the relative importance of fish predation and plant quality on weevil population parameters.

LCMR Budget:: **$15,000** Other: $15,000
Balance: $ 0 Other Balance:$ 0
Completion Date: December 31, 2001

Result A-3. Determine the competitive interactions between the native macrophytes and the exotic Eurasian watermilfoil and how this influences the potential for longer term control, with manipulations of plant community structure in two lakes.

LCMR Budget:: **$11,500** Other: $11,500
Balance: $0 Other Balance:$ 0
Completion Date: December 31, 2001

Result A-4. Attempt to detect additional lake-wide declines of Eurasian watermilfoil that may be related to the presence of potential biological control agents, and identify environmental variables associated with any identified declines by short-term sampling in approximately five (5) whole lakes or bays in Minnesota.

LCMR Budget: **$6,000** Other: $6,000
Balance: $0 Other Balance:$0
Completion Date: December 31, 2001

Result A-5. Continue development of a mechanistic model of weevil population dynamics in relation to density of Eurasian watermilfoil. Development of this model will be based on comparison of control agent densities and limiting factors, site characteristics, and plant quality in field environments with results predicted from laboratory and simulation studies.

LCMR Budget: **$7,500** Other: $7,500
Balance: $0 Other Balance:$0
Completion Date: December 31, 2001

B. Purple loosestrife

Result B-1. Rearing *H. transversovittatus* on artificial diet. We will continue to refine rearing of *H. transversovittatus* using the artifical diet developed by Blossey *et al.* at Cornell University. In its most simple form, undefined artificial diets have been developed where pulverized plant material is mixed with vitamins, trace elements, antimicrobial agents and agar, sterilized, and fed to immature stages of the root-feeding weevil. The weevils then develops to adult stages in the artificial diet.

The first lab reared weevils will be released into loosestrife infestations in the summer of 1999. This result will increase insect numbers and futher accelerate the biological control effort.

LCMR Budget: **$17,500** Other: $17,500
Balance: $0 Other balance: $ 0
Completion Date: June 30, 2001

Result B-2. Criteria for establishing *Hylobius transversovittatus* in *Galerucella* spp. stressed and non-stressed loosestrife plants. Study the interaction of between *Galerucella* spp.and *H. transversovittatus* in their ability to control purple loosestrife. Since *Galerucella* spp. are well established in many Minnesota wetlands, it will be important to ascertain how *H. transversovittatus* perform on purple loosestrife plants in the presence of *Galerucella* spp. and on plants previously stressed by *Galerucella* spp. leaf defoliation. The most critical phase that will determine the success or failure of *H. transversovittatus* is the initial establishment of sustainable populations. Since *H. transversovittatus* has such a long generation time, it will be important to know whether the establishment of *H. transversovittatus* on purple loosestrife crowns is impeded by the presence of *Galerucella* spp. If *H. transversovittatus* does not perform well in wetlands previously infested with *Galerucella* spp., natural resource managers will need to release *H. transversovittatus* into wetlands where *Galerucella* spp. has not yet become established or has never been released.

LCMR Budget: **$20,000** Other: $20,000
Balance: $0 Other balance: $0
Completion Date: December 30, 2001

Result B-3. Release of *H. transversovittatus* in cages in Minnesota wetlands. If lab-rearing of *H. transversovittatus* using artificial diet is successful, then we propose to erect at least three large (12m x 12 m x 6 m) screen cages in wetlands, releasing weevils at various densities (0.5, 2 and 4 adults per root crown). Root crowns will be destructively sampled in each fall for presence of larvae. If larvae are not found in Fall 1999, we will add more lab reared adults in Spring 2000. Larvae may be detected using X-ray analysis if preliminary studies show this non-destructive method is suitable for identifying presence or absence of *H. transversovittatus* larvae. If sufficient numbers of *H. transversovittatus* can be reared, some open releases of weevils may be made in separate wetlands.

LCMR Budget: **$17,500** Other: $ 17,500
Balance: $0 Other Balance:$ 0
Completion Date: June 30, 2001

Result B-4. Effect of wetland type on successful establishment of purple loosestrife biocontrol agents. This study will determine the effect of wetland type on the potential for successful establishment of biological control agents of purple loosestrife in Minnesota. For classification of wetland type, we will use the National Wetlands Inventory System. We will explore the correlation

between success of *Galerucella* spp. establishment and wetland type. The success of *Galerucella* spp. establishment has been monitored in up to 120 releases sites to date by DNR personnel (Luke Skinner, personal communication) with more sites to be monitored next season . The success of *Galerucella* spp. establishment and defoliation will be correlated with the digitized National Wetland Inventory data for Minnesota with GIS to determine wetland type. We can then determine whether there are correlations between wetland type and success of *Galerucella* spp. populations. It may be possible to make predictions on site preferences for future *Galerucella* spp. releases on the basis of wetland type.

LCMR Budget: **$8,750** Other: $8,750
Balance: $0 Other Balance:$0
Completion Date: December 30, 2001

Result B-5. Impact of previously released *Nanophyes marmoratus* on purple loosestrife seed production. *N. marmoratus* feeds on developing buds of purple loosestrife. The result is a reduction in number of seed capsules and decrease in seed production. A biological control agent, such as *N. marmoratus,* can reduce the numbers of seed in the seedbank by reducing seed production. Work by this project on the impact of *N. marmoratus* on purple loosestrife seed production will be continued as populations of *N. marmoratus* increase at release sites.

LCMR Budget: **$5,000** Other: $5,000
Balance: $0 Other Balance: $0
Completion Date: December 30, 2001

Result B-6. Development of the plant pathogen, Microsphaeropsis. Studies will be designed to better understand the effect of *Microsphaeropsis* on plant growth the year of inoculation, as well as in succeeding years. Plants will be sprayed with *Microsphaeropsis* inoculate in combination with water and surfactant. Plants will be rated for presence of disease lesions and *Microsphaeropsis* will be re-isolated from lesions if present.

A field study will be conducted to determine the efficacy of *Microsphaeropsis* in a wetland environment. *Microsphaeropsis* will be sprayed and individual plants with disease lesions present will be tagged and tracked the following year. Crown survival and shoot regrowth will be noted. If present, *Microsphaeropsis* will be re-isolated from disease lesions the year of spraying and in succeeding years.

LCMR Budget: **$5,000** Other: $5,000
Balance: $0 Other Balance: $0
Completion Date: December 30, 2001

Result 7. Final report provided.

LCMR Budget:	**$1,250**	Other:	$1,250
Balance:	$0	Other Balance:	$0

Completion Date: December 30, 2001

V. DISSEMINATION: It is expected that the results of this project will be published in peer-reviewed scientific journals and also in special publications and newsletters. Results also will be presented at national, regional and state scientific meetings to peers in the field, as well as to resource managers and planners who will use the results of this project.

VI. CONTEXT

A. Significance: Eurasian watermilfoil is a significant problem in Minnesota because it can produce dense mats at the water's surface. Mats of milfoil can severely limit water recreation and also reduce the biodiversity of aquatic ecosystems.

Drastic declines in populations of milfoil in North America have been documented. Though the precise causes of these declines are often unknown, herbivory by three insect species has contributed to at least some of them. Recent research in Minnesota (see VI-C-1. Funding History) determined that 1.) all three of these insects are present in the state, 2.) one of these insects, a weevil, can severely damage milfoil under controlled experimental conditions, 3.) these insects, particularly the weevil, have caused declines of milfoil in some Minnesota Lakes but not in others, 4.) factors that limit densities of weevils in Minnesota lakes (e.g., fish predation, plant quality and resistance) and factors that enhance the competitive abilities of milfoil (light availability, native plant community and sediment conditions) have been found to be important determinates of the degree of control, 5) the relative importance of these factors is unknown and likely varies among lakes. Proposed research will continue and extend the evaluation of factors that limit the potential of insects to control milfoil under a variety of field conditions in Minnesota lakes, to determine if ways to alleviate these factors are feasible and to be able to predict under what circumstances these insects may be expected to be useful and not useful.

The Minnesota Legislature has directed the DNR to initiate research on biological control of milfoil (M.S. 84D.02, subdivision (2), item (3)).

Research efforts suggest that biological control of purple loosestrife is very feasible. Extensive research conducted on loosestrife in Europe has demonstrated that the plant is successfully controlled by insect herbivores. Research completed in the United States has demonstrated that these European insects pose no known threat to native plants. Four European insects, one root-feeding weevil, one flower-feeding weevil, and two leaf-eating beetles, have been identified as promising candidate biological control agents for introduction into the U.S. and have received

federal and state approval for release in the United States and Minnesota as potential natural enemies of purple loosestrife.

Biological control offers the most suitable and environmentally safe technique to manage loosestrife long term, especially in nature reserves. Many times a combination of insects is more effective than one species by itself. The idea is to increase stress on purple loosestrife by introducing predators that feed on leaves, flowers and roots of the plant. The two beetles in particular can cause high plant mortality, reduce shoot growth, suppress flowering and reduce seed output. Testing combinations of these insects will be an important part of the research. All four species have been released in stands of purple loosestrife in Minnesota. Currently 1,000,000 leaf-eating beetles have been released on 200 sites statewide. All four insect species have survived the winter in Minnesota and are reproducing. This is a big step forward towards finding a successful biological control.

B. Time: Development of biological controls for milfoil in Minnesota has been underway for six years and may well require four or more years of additional effort. Development of biological controls for loosestrife in Minnesota began eight years ago. Achieving successful control may well require 10 or more years of effort. The project proposed for the 1999 Biennium should be extended to 30 June 2002 in order to allow researchers to work in the field during the whole of the summer of 2001.

C. Budget Context: Information to describe the project context and budget history is presented as follows: 1) funding history which summarizes expenditures for the previous four biennia; 2) proposed and Anticipated Expenditures for the FY00-01 and FY02-03 biennia; and 3.) Detailed budget.

1. Funding History

	Jul 91-Jun 93	Jul 93-Jun (Dec)95	Jul 95-Jun (Dec)97	Jul 97-Jun (Dec)99
LCMR	$160,000	$400,000	$300,000	$150,000
Other state	--	--	--	$150,000
Non-State match	--	--	--	--
In-kind	--	$200,000	--	--
Total	$160,000	$400,000	$300,000	$300,000

2. Proposed and Anticipated Expenditures

	July 99-June(Dec) 01	July 01-June (Dec)02

	Proposed Expenditures	Future Expenditures
LCMR	$ 150,000	$ 150,000
Other State	$ 150,000	$ 150,000
Non State Match	$ -	$ -
In-Kind	$ -	$ -
Total	**$ 300,000**	**$ 300,000**

3. **Detailed Budget**: This work will be done by the University of Minnesota under contract to the DNR.

A. Eurasian watermilfoil - Budget

1. Salaries and fringe	
Technicians	124,750
2. Supplies	13,500
3. Travel	3,000
4. Vehicle rental	8,750
Total	150,000

B. Purple loosestrife

1. Salaries and fringe	
Technicians	144,500
2. Supplies	3,000
3. Travel	2,500
Total	150,000

VII. Cooperation: The DNR=s Exotic Species Program will apply $150,000 from the Water Recreation Account, designated as >other= in this work program, towards this project over a two year period. This support in conjunction with funding that we hope the legislature will appropriate at the recommendation of the LCMR will provide $300,000 for this research. This project will be directed by Luke Skinner with assistance from Chip Welling and Wendy Crowell, both of the DNR.

A. Eurasian watermilfoil

Cooperators at the University of Minnesota include: Drs. Raymond Newman, David Ragsdale, and David Biesboer. Technical expertise on milfoil will be provided by the Army Corps of Engineers.

Cooperator	Dollars received	Percent time spent on project
R. Newman*	$150,000	20%

B. Purple loosestrife

Cooperators at the University of Minnesota include: Drs. Roger Becker, David Ragsdale, and Elizabeth Stamm Katovich. Technical expertise on loosestrife will be provided by Dr. Bernd Blossey of Cornell University, and Dr. Dharma Sreenivasam, Minnesota Department of Agriculture

Cooperators	Dollars received	Percent time spent on project
R. Becker and D. Ragsdale*	$150,000	15% each

*Includes DNR Funding contribution

VIII. Location: Big Woods, St. Croix Moraines & Outwash Plains, Anoka Sand Plain, Mille Lacs Uplands, Pine Moraines & Outwash Plains, Twin Cities Metro Lakes

IX. **Reporting** Requirements: Periodic workprogram progress reports will be submitted at six-month intervals beginning on 31 December 1999. A final workprogram report and associated products will be submitted by 30 June 2002.

X. **Research Projects**: Refer to the attached abstracts from the two proposals that were attached to the previous work program as addenda. If you would like to receive additional copies of the complete proposals, please contact Welling.

Literature Cited

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