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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Solarz, S.L. and R.M. Newman. 1996. Oviposition specificity and behavior of the watermilfoil specialist *Euhrychiopsis lecontei*. Oecologia 106: 337-344.

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Factors Influencing the Control of Eurasian Watermilfoil With Native Or Naturalized Insects

Final Report for 1999-2001

BY

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TO

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30 June 2002

Introduction

Eurasian watermilfoil (*Myriophyllum spicatum* L.) is an exotic aquatic weed that often interferes with recreation (Smith and Barko 1990), inhibits water flow, impedes navigation, (Grace and Wetzel 1978) and will displace other aquatic macrophytes (Madsen et al. 1991). It was first reported in Minnesota in 1987 and occurred in over 120 Minnesota waterbodies by fall 2000 (Exotic Species Program 2001).

Three native or naturalized species have been considered as potential Eurasian watermilfoil control agents: the moth *Acentria ephemerella* (Denis & Schiffermüller) (= *Acentria nivea* (Olivier)) a naturalized Pyralidae, the indigenous midge *Cricotopus myriophylli* Oliver and the indigenous weevil *Euhrychiopsis lecontei* (Dietz) (= *Eubrychiopsis lecontei*) (e.g., Painter and McCabe 1988, Kangasniemi et al. 1993, Creed and Sheldon 1995, Sheldon 1997a, Johnson et al. 2000). All three taxa are present in the midwest (Newman and Maher 1995, Scholtens and Balogh 1996, Creed 1998). Although all three taxa have potential to control milfoil (e.g., Johnson et al. 1998, 2000, Kangasneimi et al. 1993, Gross et al. 2001), prior research (Creed and Sheldon 1995, Sheldon and Creed 1995, Creed 1998, Newman and Biesboer 2000) suggests that *E. lecontei* is the most promising control agent. The weevil is is highly specific to watermilfoils (Solarz and Newman 2001) and has been associated with numerous milfoil declines (Creed 1998). Sheldon and O'Bryan (1996), Newman et al. (1996, 1997), Mazzie et al. (1999) and Newman et al. (2001b) describe the life history and development times of the weevil and Getsinger et al. (*in press*) provide a good overview of the life history and host specificity. .

Although declines of milfoil in several lakes have been directly related to the occurrence of *E. lecontei* (Sheldon and Creed 1995, Lillie 1996, 2000, Creed 1998, Newman and Biesboer 2000), it is clear that at many sites in Minnesota, weevil densities do not get high enough to effect control (Newman et al. 1996, Newman et al. 1998, Newman and Biesboer 2000). Fish predation may be one factor limiting populations in some lakes (Sutter and Newman 1997, Newman and Biesboer 2000). Identification and amelioration of these limiting factors will be essential to reliable use the weevil for milfoil control (Creed 2000, Newman and Biesboer 2000). Getsinger et al. (*in press*) provide a good overview of the potential use of the weevil for control of milfoil.

The aim of this project was to monitor a set of milfoil populations for potential declines, determine factors that may be limiting control agent densities and their effectiveness in the field, determine the effects of fish on weevil augmentations and determine if chronic effects such as sediment quality or competition with native plants is responsible for declines of milfoil associated with herbivores. This report summarizes our methods and results for 1999-2001 and presents a discussion of our research through 2001. Some results from 2001 were covered by renewal funding and will be further elaborated in future reports for the 2001-2003 funding period.

Acknowledgements

Numerous people assisted with this project, including: Alyson Milles, Kristine Mazzie, Susan Solarz, John Foley, Ray Valley, Andrea Cade, Karl Hammers, Nate Awe, Sally Beach Hersrud, Ganesh Padmanabhan, Erik Heinen, Ramona Johnson, Tanya Adelman, Matt Gove, Darren Ward, Meredith Drebert, Joanna Watson, Kristen Tomaszewski, William Tanberg, Todd Kittel, Kerry Accola, Amanda Bruce, Jennifer Dehn, Meg Lelonek, Donell Swenson, Margaret Tilman, Chris Lemmon, Aaron Berger, Jon German, Ruth Isakson, Chris Kolasinski, Nick Lehnertz and Jack Lund. Aaron Berger, Chris Lemmon and Darren Ward were instrumental in sample collection, sample processing and data tabulation for this report. Darren Ward conducted much of the 2000-2001 work and analysis of fish effects in Cedar, Cenaiko and Otter Lakes as part of his MS thesis. Michelle Marko and Julie Kruger conducted the fecundity experiments.

Methods

Semi-permanent Transect Sites:

During the summers of 1993 and 1994, we initiated selection of semi-permanent sampling sites, which can be repeatedly sampled at fixed locations (Newman and Ragsdale 1995). The sites were Lake Auburn (Carver Co.; T116N; R24W; S10), Otter Lake (Anoka and Ramsey Co.; T30-31N; R22W; S3-4, S35-36), Cedar Lake (Hennepin Co.; T29N; R24W; S29) and Smith's Bay of Lake Minnetonka (Hennepin Co.; T117N; R23W; S10,11). At each site, 5 transects, 30 m apart, were run from near shore (0.5 m depth) toward the plant limit. At Lake Auburn and Cedar Lake, the transects were extended to 50 m from the shoreward starting point, in approximately 2.5 m depth at Auburn and 5 m depth in Cedar. Semipermanent stations were marked along the transect at 10 m intervals with fluorescent floats that were attached to bricks and suspended 0.5-1m beneath the surface. At Otter Lake, the transects were extended 100 m from shore, in approximately 2 m depth. At Smith's Bay, transects were started 100 m from shore and run to 4.5 m depth, approximately 0.8 km from shore, with 5 sampling stations along each transect approximately geometrically spaced. Distances from shore determined from GPS data were: 100m, 200m, 370m, 585m and 805m. These stations were marked with floating milfoil buoys.

In summer 1996, we noticed a dense population of weevils at Cenaiko Lake (Anoka Co.; T31N; R24W; S26). We therefore sampled this lake in July and September as a new site to be regularly sampled. We ran 3 or 4 transects, west to east across the north end of the lake, with sampling stations every 30 m. This resulted in 25-32 samples on each date (21-30 with plants; deep stations were deleted from the analysis). At Lake Auburn transects were sampled at 10 m intervals (stations) , resulting in 6 samples per transect, or 30 samples. At Otter Lake samples were taken at each 20m sampling station, resulting in 5-6 samples per transect or 27 samples. At Cedar (30) and Smiths Bay (25), all stations were sampled, however, several stations in Cedar Lake were deeper than the plant limit (>7m) and these are excluded if no plants occurred there during the season. In 1997 sampling occurred twice: in late June to early July and in mid-September. In 1998, three lakes (Auburn, Cenaiko and Smith's Bay) were sampled thrice, in June, late-July or early August and in September. Otter and Cedar were sampled in June and September. Samples were alternately taken 2m from each side of each station on successive sampling dates to minimize sampling disturbance.

In 1999, two lakes (Cenaiko, and Smith's Bay) were sampled thrice, in June, late-July or early August and in late August. Auburn and Cedar were sampled in June and late August and Otter was sampled in June and early August. In 2000, four lakes were sampled three times (Auburn, Cenaiko, Otter and Smith's Bay), in June, July and August and Cedar Lake was sampled twice, in June and August. Twenty-four to thirty samples were collected at each lake on each date. In 2001, four lakes (Auburn, Cenaiko, Otter and Smith's Bay) were sampled three times, in June, late July and late August. Cedar was sampled in June and August. Twenty-five to thirty samples were collected at each lake on each date.

At each sampling station, plant biomass and invertebrate samples were taken from 0.1 m^2 quadrats (all plant material was clipped at sediment interface and immediately placed in a sealable bag underwater). Sediment cores were also collected at shallow, medium and deep stations along 3 transects (transects 1, 3 and 5 at all but Cenaiko, where 1-3 were sampled) at each site.

A set of water column parameters were measured in the open water (>5.5m depth and >100 m from the bed) at each site on each sampling date. Secchi depth and surface conductivity were measured and a water sample (combined surface and Secchi depth sample) was collected for pH, alkalinity and chlorophyll a determination. A light (Photosynthetically active radiation = PAR, Li-Cor LI-189 with LI-192SA quantum sensor), temperature and oxygen (YSI 50B) profile was taken at 0.5 m depth increments from surface to bottom.

Alkalinity was determined by titration in the field. For chlorophyll, 500 ml of water were filtered through a 1.2 mm glass fiber filter, the filter was placed on dry ice and returned to the

laboratory and frozen until analysis. Chlorophyll was extracted and measured spectrophotometrically (APHA 1989). Sediment cores were stored on ice and returned to the laboratory. Within 48 hr the top 15 cm of sediment was homogenized. A 5 ml sediment subsample was dried at 105 °C for 24-48 hrs and then weighed to obtain bulk density (g dry mass ml^{-1}). The dried sediment was then ashed at 550 °C for 4 hrs to obtain percent organic matter ([AFDM dry $mass^{-1}$] X 100). Pore water was extracted from the remaining sediment by centrifugation, acidified to < pH 2 and stored in the refrigerator. Within seven days, the NH_3 concentration was determined by selective electrode (APHA, 1989).

Biomass samples were rinsed of invertebrates and invertebrates were picked (endophytic and external on milfoil and from the wash water) from all samples; weevils and Lepidoptera were enumerated. Milfoil stems were counted and the average maximum stem length determined. Plants were separated, identified to species, spun for 15 sec in a salad spinner and wet mass was recorded. These samples were dried (105 °C for 48h) and weighed or were frozen for later dry mass determination.

Because the relatively infrequent sampling of these sites (2 or 3 times per summer) does not provide very good resolution of weevil population dynamics, we initiated a biweekly weevil survey in Lake Auburn in 1998, and in 1999 added Cenaiko and Smiths Bay to our weevil surveys. In 2000 we added Otter to our survey sites and we conducted bi-weekly surveys at Auburn, Cenaiko, Otter and Smith's Bay in 2000 and 2001. For each survey, 5-8 stems (top 50 cm) of milfoil were collected at each of 15-18 stations every other week (at Cenaiko we often were unable to find milfoil at some stations). At sites with lower densities of weevils we have been collecting 7 or 8 stems to increase our power to detect weevils. Weevils and Lepidoptera were removed from the samples, which were scanned at 8X magnification, and enumerated by life stage. Results were expressed as numbers per basal stem.

Weevils collected from the surveys in 1999 were examined for pathogens (Oien and Ragsdale 1993). Samples were put in PBS with azide and squashed. A 10 microliter sample of each squashed tissue was then placed on a slide with a coverslip and examined under a compound microscope in phase contrast. Infection was defined as protozoan, microsoridia, or saprophytic fungi present in individuals of each stage. Those results are presented in Newman et al. (2001b) and are not repeated here.

Survey Sites:

We conducted broader scale (whole lake or bay) surveys in August at 5 sites: Lake Calhoun Hennepin Co.; T28-29N; R24W; S4,5,32,33), Lake Harriet (Hennepin Co.; T28N; R24W; S8,9,16,17), Lake of the Isles (Hennepin Co.; T29N; R24W; S32,33) and Shady Island (Hennepin Co.; T117N; R23W; S26) and Grays Bay (Hennepin Co.; T117N; R22W; S8) in Lake Minnetonka. At each lake, plant community structure was determined with plant hook surveys along 12-15 transects, water quality was recorded and a set of biomass samples was collected.

Localized sites in each of these lakes were sampled quantitatively for milfoil, invertebrates and site characteristics. At two of these sites (Gray's Bay and Shady Island), 3 transects were run perpendicular to shore and 3 stations, based on depth (e.g., 2, 3 and 4 m), were sampled along each transect in August. At Calhoun, Lake of the Isles and Harriet, 5 transects with 5 stations on each transect were sampled in June and August. At each station $0.1 m^2$ quadrat samples were taken for plants and invertebrates. Sediment cores were sampled at the intermediate depth station along each transect. Open-water water quality samples were taken and processed in the same manner as the permanent transect sites. Samples were processed as above for plant mass by species and sediment characteristics.

To quantitatively determine the extent of milfoil coverage, a set of 10-15 transects, perpendicular to shore, was located around the lake or bay in a stratified random manner (i.e., 1 transect located within each 1/10 of the lake shoreline circumference). Along each transect, observations were made from shore (0.5 m depth) to the plant limit at 5 to 6 stations, at 7.5, 15, 30, 60, or 90m intervals to the depth of the plant limit. At steeper transects the shorter

intervals were used, at long and gently sloping transects, the longer intervals were used. Transects were laid with a measuring rope and marked with jugs attached to bricks; the shoreward and offshore positions were recorded with a GPS unit. At each observation point, visible milfoil (% coverage) and other plant occurrence was recorded, plant height determined and plant disk (depth at which a Secchi disk disappears; Crowell et al. 1994) was measured within a $1m^2$ area around the marker jug. Depth was recorded by dropping a plant hook vertically; plant species found on the plant hook or the jug rope and brick were also recorded and milfoil was examined for weevils and given a weevil damage rating (0-5). These data provide an estimate of milfoil and other plant coverage and frequency of occurrence around the lake as well as a relative estimate of weevil damage or occurrence.

Semi-quantitative estimates of plant density and weevil abundance were determined along a stratified subset of 5 of the transects with modification of a grapple hook method of Jessen and Lound (1962). At each sampling point 3 or 4 grapple throws were collected and rated for plant occurrence (Jessen and Lound 1962); these data provide species occurrence and relative density estimates for each species. The milfoil collected on each throw was scanned for the presence of weevils and visually assigned a damage rating (0-5). Thus for these 5 transects, we have both visual estimates of plant occurrence and density as well as the semiquantitative plant hook estimates.

Weevil Introduction/Manipulation:

Our aim was to determine the effects of artificial introduction of weevils, *Euhrychiopsis lecontei*, on the density and condition of Eurasian watermilfoil and other macrophytes during a single growing season by introductions of weevils at replicated sites in fish exclosures and open areas. This should allow us to determine if fish predation may be limiting the success of prior introductions to open areas. To exclude fish, 3m X 3m cages were constructed with PVC pipe and fitted with 1/2" bar nylon mesh netting. The netting was attached to 1m high cross supports and was connected to cylinder floats that allowed the netting to extend to the surface from 1m to 2.25m maximum depth; the tops and bottoms of the cages were open. Ten cages were fitted with mesh on all four sides (complete enclosures) and 10 cages were fitted with two mesh panels that each covered 1.5 sides (i.e., a total of 3m or 1/4 of the cage was open); the open cages served as controls by permitting fish entry.

In July 1998, 20 sites were located in milfoil beds in the NE bay of Cedar Lake in water $\leq$ 2.2m deep and marked with floats. The cages were placed over each float such that the float was in the center of each cage; the frames dropped straight to the bottom and the cylinder floats keep the mesh taut to the surface). Cage bottoms were pushed into the sediment and weighted with bricks. Two plant biomass samples ($0.1m^2$ quadrat samples) were collected from each cage prior to stocking. Cages were then fished to remove fish trapped within the cages. Cages (open or closed) and treatment (stocked or not stocked with weevils) were assigned to the sites in a stratified random block design. One hundred and fifty adult weevils (adults and the apical tips they were collected from, which contained some larvae and eggs), collected from Cenaiko Lake, were stocked into each cage designated to receive weevils (5 closed and 5 open cages). Care was taken to ensure that adults moved onto the live milfoil and the meristems were attached to milfoil plants to ensure that associated larvae and eggs also had access to the live plants. In August, the cages were resampled for biomass and weevils. In 1999 the cages were sampled for plants and weevils (2 samples per cage) in June and were stocked with 150 weevils in July; biomass was sampled again in late August. The samples within each cage (for pre and post stocking samples) were averaged and statistical analyses were performed treating each cage as a true replicate. The experiment was repeated in summer 2000. More effort was placed at removing fish and the weevils were collected from Smith's Bay of Lake Minnetonka.

At approximately biweekly intervals, cages were examined and counts of visible weevils (eggs, larvae, pupae and adults) were made by examining 100 to 150 stems during a 15 min period. Larval occurrence was estimated based on recent stem damage. Any fish observed in the closed cages were enumerated and angling and minnow traps were used to remove these fish. In 2000 we regularly removed any fish that invaded the cages.

We conducted the experiment a final time in 2001, and were able to start much earlier than previous years. Cages were sampled for plant biomass (duplicate 0.1m^2 quadrat samples) in late May and stocked with 175 adult weevils collected from Otter Lake (larvae and eggs associated were also stocked but not enumerated) between 5 June and 15 July (2 stockings). At approximately biweekly intervals, cages were examined and counts of visible weevils (eggs, larvae, pupae and adults) were made by examining 100 to 150 stems during a 15 min period. Larval occurrence was estimated based on recent stem damage. Any fish observed in the closed cages were enumerated and angling and minnow traps were used to remove these fish. Biomass was sampled (2 samples per cage) in early September. In this report we summarize the results of these experiments; additional details and results are presented in our previous reports and in Ward (2002).

In addition to the manipulation in Cedar, we also conducted fish manipulations in Cenaiko Lake and in Otter Lake in 2001 For these experiments we added sunfish (ca., 10-15 cm) to enclosed (2mX2m) cages. In Cenaiko Lake unstocked closed and open cages were used as controls; in Otter Lake closed controls were used. There were 4 reps of each treatment at each lake. Due to high and fluctuating water levels and the low density of milfoil, the results from Cenaiko were questionable and are not reported. The results of the Otter manipulations are reported below and in Ward (2002).

Effects of plant community:

To test the hypothesis that plant competition may be important in the reestablishment of Eurasian watermilfoil after a decline (or reduction due to weevil damage) we established 16 plots in Otter Lake (good water clarity and healthy native plant community) and 16 plots in Lake Auburn (poor water clarity with community dominated by Eurasian watermilfoil and coontail) in 1998. Plots were marked with a center float and spaced at least 10 m apart. Biomass was determined in each plot (two 0.1m^2 samples) and each plot was assigned to one of four treatments: no manipulation, removal of all Eurasian watermilfoil, removal of all native plants or removal of all plants. After initial sampling, the randomly assigned manipulation was applied to the plot by divers using SCUBA who manually removed vegetation within the area delineated by a 2x2 PVC quadrat. Harvested vegetation was not retained but allowed to float away. The plots were resampled for biomass (two 0.1m^2 samples per plot) five weeks later, at the end of the summer in 1998. These results were reported in our 1997-1999 Final Report (Newman et al. 1999); due to the late start with the removal few interesting significant effects were seen. In summer 1999 we resampled these plots in early and late summer; the results of this sampling are presented below.

We established a new set of plant manipulation plots in Otter Lake and Lake Auburn in 2001. At each lake we established 20 plots marked by 2mx2m pvc quadrats. The plots were sampled in early June for plant biomass (2 0.1-m^2 quadrat samples per plot) prior to manipulation. In five plots no plants were removed, in 5 plots all plants were removed and in the other plots either all native plants or all Eurasian watermilfoil was removed. Approximately every three weeks visual surveys (means of 16 0.5x0.5 cells) of plant coverage were conducted, and in September two biomass samples were taken from each plot. We also collected sediment cores from each plot in Otter Lake. Samples within plots were averaged and statistical analyses were conducted on the replicate plots. Results for initial and final wet biomass are presented here. Additional analyses will be covered in our future 2001-2004 reports.

Influence of milfoil genotype and rearing sediment on weevil performance:

Because previous work indicated that weevils perform better on different milfoil species (Newman et al. 1997a), other studies have shown that plant genotype and nutritional status can vary (Spencer et al. 1999) and affect biocontrol agent performance (Newman et al. 1998), and because we have seen substantial variation in weevil densities amongst lakes, we conducted an experiment to determine the effects of milfoil genotype (lake source) and milfoil rearing

sediment on weevil performance. This experiment, which was a modification of the one conducted in 1998 by Ramona Johnson (see Newman et al. 1999), was conducted by Joanna Watson.

The experiment was set up as a 2 sediment by 2 genotype factorial. Milfoil plants and lake sediment were collected from two Minnesota lakes which have contrasting weevil populations; Cedar Lake, Hennepin Co. and Otter Lake, Ramsey Co. Fifteen cm long cuttings were placed in stock tanks containing either Cedar or Otter sediment, resulting in the two plant genotypes being reared on both lake sediments. The plants were allowed to root and grow for 2-3 weeks until they reached 35 cm. One of eight female weevils, collected from Lake Auburn, was introduced to the meristem of a plant and allowed to oviposit. The egg and plant were then transplanted to a 45 cm tall clear plastic tube containing 5 cm of the original growing sediment. The tubes were placed in a 27 °C environmental chamber (16h daylength) and observed daily for weevil development. Development to each stage was recorded based on criteria of Newman et al. (1997a). Newly eclosed weevils were removed and weighed and stem diameters were measured above and below the pupal case holes.

Plants and sediment were also analyzed for nutrient content. Individual plants were sectioned into top 20 cm, bottom stem, leaves, and roots and then frozen with liquid nitrogen. The plants were then sent to Dr. David Spencer the Exotic & Invasive Weed Research Unit at UC Davis for analysis of carbon and nitrogen. The sediment was analyzed for $NH4^+$, organic content and bulk density with previously described methods.

Weevil development with temperature and modelling:

Previous research determined the number of degree days required for milfoil weevil development (Mazzei et al. 1999). Temperature monitoring in several lakes has since been used to assess potential for weevil population development and for additional modelling.

Degree days above 10 °C (DD) were determined for two lakes (Auburn and Smith's Bay) that were monitored with temperature data loggers (Optic StowAway, Onset Computer, Pocasset, MA) from April or May through October 1996, 1998-2001. Temperatures were recorded every 0.5 hr at 0.75m depth and the surface. These results were used to estimate number of generations and potential population growth at the field sites. These data are summarized in Newman et al. (2001a) and are not presented here.

A stage structured model of weevil development with temperature was developed by grad student Darren Ward. The model is a stage structured model with plausible values for egg-adult survival (Newman et al., 1997; Mazzei et al., 1999), development time (Mazzei et al., 1999), and daily fecundity (Sheldon and O'Bryan 1996, Sheldon and Jones 2001, and the above mentioned experiments). The length of the pre-reproductive adult stage was estimated by an experiment in 2000 and the influence of adult life span and juvenile mortality on summer-long relative densities was explored in various model runs. A model relating to milfoil density and control was linked to this stage structured weevil model to explore the effects of initial population size and different mortality rates on likely milfoil control. These results are summarized here; additional details are reported in Ward (2002).

Results and Discussion

Semi-permanent transect sites:

Milfoil biomass in Cedar Lake remained high (1600-2000 g wet/m^2) during 1999-2001, similar to 1997-1998 (Table 1). Milfoil biomass at Lake Auburn continued to increase from the low densities of 1998-1999 (Fig. 1) to around 1600 g wet/m^2 during 2001. Nevertheless, milfoil remained suppressed well below densities found from 1994-1997.

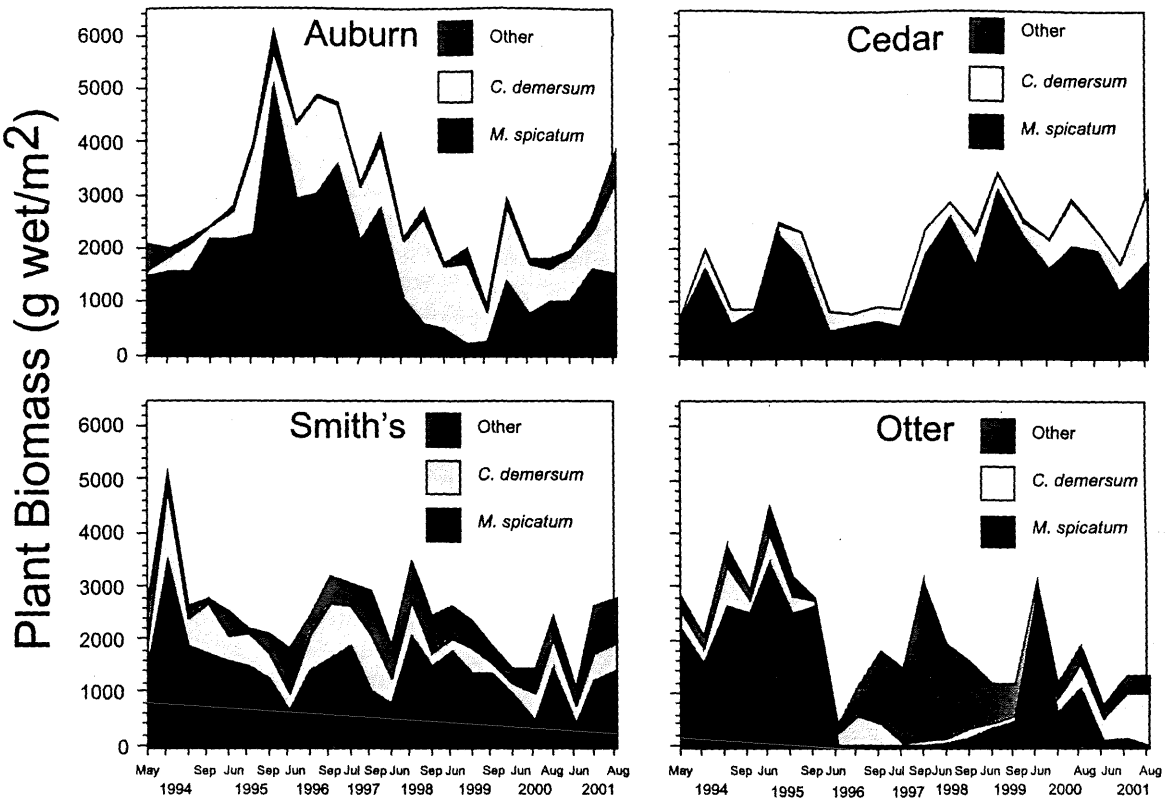

Fig. 1. Total plant biomass (Eurasian watermilfoil, coontail and other non-milfoil biomass; g wet/m^2) at the four permanent transect sites from May 1994 - August 2001.

Milfoil in Smith's Bay fluctuated from 400 g wet/m^2 to 1440 g wet/m^2; with the exception of 1999, milfoil typically increased over the summer from lower June densities (Fig. 1). The higher milfoil density later in the summer was mainly due to a high densities at the deepest three stations (>1400 g wet/m^2); density at the two shallowest stations remained low even in August. Milfoil increased at Otter Lake during 1998 to June 2000, finally recovering from the winter kill in 1995-1996. In June 2000, milfoil reached a density of 2650 g/m^2, the highest density recorded at Otter Lake since 1995. Milfoil declined however during 2000 to 1100 g/m^2 in August and the decline continued dramatically at Otter Lake from 116 g wet/m^2 in June to 24 g/m^2 in August 2001. This two-year decline is clearly associated with weevils (see below). Changes in milfoil biomass at our various sites (Fig. 1) are not due to regional changes; there was little concordance among the sites.

Non-milfoil biomass was generally lower in 1999 and 2000 compared to previous years, but increased at all lakes in summer 2001, and by August was similar or higher than previous years (Table 2). The contribution of the non-milfoil plant community remained moderate at Smith's Bay and Lake Auburn; at Smith's Bay, Eurasian watermilfoil contribution dropped from 60% of plant biomass in 1999 to ≤45% in 2001 (Table 3). It should be noted that in 2000 and 2001, at the shallowest station at Smith's Bay, northern watermilfoil and other native plants dominated Eurasian watermilfoil (<10% of biomass there). With the increase in milfoil at Lake Auburn from very low levels in 1999, the contribution of milfoil increased from 15% in 1999 to over 50% in 2001, however, it remained well below mid-1990 values of 70-90%.

With the decline of milfoil at Otter Lake in 2000-2001, it's contribution dropped from 80% of plant biomass in June 2000 to 5% in August 2001. Eurasian watermilfoil biomass remained high at Cedar Lake and contributed 60-80% of the plant biomass and coontail typically composed over 90% of the non-milfoil biomass.

Table 1. Biomass ± 1SE (g wet/m^2) of Eurasian watermilfoil at the four sampling sites in 1994-2001. n = number of samples. Dry biomass (g/m^2 ± 1SE) is presented for 1995-2001.

Sampling Date	Auburn	n	Cedar	n	Otter	n	Smith's Bay	n
5/19-6/3/94	1474 ± 326	10	610 ± 289	18	2208 ± 332	21	1470 ± 320	14
7/1-7/11/94	1570 ± 297	16	1642 ± 523	18	1589 ± 231	27	3478 ± 399	16
8/12-8/19/94	1581 ± 224	15	601 ± 207	15	2626 ± 472	14	1886 ± 328	16
9/14-9/21/94	2205 ± 350	19	824 ± 188	24	2510 ± 557	9	1767 ± 386	14
6/07-6/27/95	1999 ± 324	30	2307 ± 631	23	3444 ± 336	27	1618 ± 289	25
dry	280 ± 43		245 ± 67		312 ± 33		158 ± 28	
7/31-8/15/95	2277 ± 417	19	1821 ± 797	10	2526 ± 385	15	1481 ± 245	25
dry	267 ± 46		172 ± 79		171 ± 29		149 ± 28	
9/18-9/29/95	5044 ± 752	17	479 ± 173	17	2629 ± 323	18	1281 ± 178	25
dry	551 ± 94		37 ± 13		194 ± 23		113 ± 15	
6/12-6/24/96	2959 ± 402	30	568 ± 200	30	21 ± 8	27	665 ± 144	25
dry	306 ± 40		59 ± 24		2 ± 1		46 ± 10	
7/30-8/9/96	3035 ± 619	27	665 ± 219	30	1 ± 1	27	1415 ± 256	25
dry	390 ± 82		62 ± 20		0 ± 0		176 ± 36	
9/12-9/19/96	3622 ± 469	30	574 ± 174	30	0 ± 0	27	1656 ± 393	25
dry	361 ± 49		50 ± 14		0 ± 0		156 ± 40	
6/27-7/17/97	2134 ± 321	30	1906 ± 341	28	24 ± 22	26	1880 ± 327	25
dry	294 ± 46		210 ± 40		3 ± 3		296 ± 55	
9/8-9/18/97	2786 ± 400	30	2646 ± 502	29	4 ± 4	27	1055 ± 170	25
dry	321 ± 49		271 ± 55		0 ± 0		100 ± 18	
6/8-6/18/98	1080 ± 168	30	1690 ± 360	31	79 ± 52	27	815 ± 164	25
dry	130 ± 18	30	213 ± 52	31	7 ± 4	27	105 ± 21	25
7/27-8/3/98	581 ± 133	30					2103 ± 475	25
dry	67 ± 16	30					286 ± 65	25
9/8-9/16/98	530 ± 76	30	3146 ± 514	29	181 ± 44	27	1487 ± 338	25
dry	48 ± 7	30	367 ± 63	29	15 ± 4	27	172 ± 40	25
6/15-6/22/99	202 ± 50	30	2238 ± 393	28	355 ± 113	27	1806 ± 289	25
dry	24 ± 7	30	252 ± 50	28	25 ± 8	27	155 ± 32	25
7/29-8/3/99					483 ± 101	27	1358 ± 289	25
dry					36 ± 8	27	189 ± 44	25
8/23-8/25/99	253 ± 83	30	1632 ± 237	30			1362 ± 320	25
dry	25 ± 9	30	105 ± 15	30			106 ± 26	25
6/6-6/23/00	1392 ± 263	30	2045 ± 321	29	2652 ± 340	27	981 ± 318	25
dry	208 ± 39	30	219 ± 38	29	331 ± 42	27	109 ± 37	25
7/11-7/19/00	783 ± 200	30			607 ± 82	27	501 ± 150	25
dry	115 ± 32	30			45 ± 7	27	77 ± 22	25
8/23-8/29/00	1007 ± 152	30	1988 ± 305	29	1098 ± 136	27	1474 ± 346	25
dry	91 ± 14	30	175 ± 28	29	90 ± 14	27	162 ± 40	25
6/18-6/25/01	1022 ± 199	30	1213 ± 267	29	116 ± 34	27	408 ± 107	25
dry	109 ± 21	30	108 ± 26	30	9 ± 3	27	31 ± 8	25
7/17/-7/30/01	1641 ± 279	30			138 ± 58	25	1211 ± 290	25
dry	232 ± 45	30			6 ± 3	27	168 ± 43	25
8/23-8/30/01	1549 ± 289	30	1798 ± 398	25	24 ± 11	27	1438 ± 381	25
dry	158 ± 33	30	145 ± 38	28	2 ± 1	27	160 ± 43	25

Table 2. Mean number of species per sample (Spp/S) ± 1SE and non-milfoil biomass (B; g wet /m^2) at the 4 sampling sites in 1994-2001. Number of samples is given in Table 1.

Sampling Date	Auburn		Cedar		Otter		Smith's Bay	
	Spp/S	B	Spp/S	B	Spp/S	B	Spp/S	B
5/19-6/3/94	3.80±0.47	670	1.33±0.28	75	4.76±0.19	600	3.29±0.22	1231
7/1-7/11/94	3.63±0.29	444	1.83±0.28	370	4.37±0.29	520	3.75±0.35	1604
8/12-8/19/94	3.00±0.28	647	1.53±0.26	282	5.57±0.39	1126	3.13±0.42	765
9/14-9/21/94	3.11±0.37	268	1.46±0.19	54	4.89±0.61	431	3.50±0.39	975
6/07-6/27/95	2.23±0.22	822	1.43±0.20	214	4.70±0.21	1065	3.64±0.30	877
7/31-8/15/95	3.37±0.26	1789	1.70±0.15	516	4.27±0.30	642	2.68±0.24	703
9/18-9/29/95	2.18±0.18	1058	1.41±0.17	337	2.44±0.34	135	2.80±0.20	856
6/12-6/24/96	2.93±0.24	1450	2.10±0.22	248	5.19±0.25	434	4.32±0.36	1159
7/30-8/9/96	2.78±0.31	1186	1.43±0.18	270	4.19±0.20	1171	3.88±0.41	1017
9/12-9/19/96	2.50±0.20	1166	1.57±0.16	307	3.93±0.28	1798	3.88±0.32	1531
6/27-7/17/97	2.97±0.14	1435	1.82±0.14	460	4.31±0.29	1516	4.16±0.39	1162
9/8-9/18/97	2.63±0.17	1500	1.59±0.09	235	4.81±0.26	3180	3.64±0.27	1863
6/8-6/18/98	2.43±0.18	1158	1.74±0.81	637	5.37±0.24	1835	5.32±0.43	1038
7/27-8/3/98	2.97±0.23	2197					5.00±0.44	1385
9/8-9/16/98	2.40±0.12	1258	1.62±0.12	296	4.74±0.39	1423	4.32±0.38	969
6/15-6/22/99	3.07±0.16	1806	1.86±0.13	326	4.52±0.31	825	4.60±0.37	810
7/29-8/3/99					5.33±0.30	720	3.72± 0.31	973
8/23-8/25/99	1.93±0.13	679	1.37±0.09	570			2.92± 0.33	534
6/6-6/23/00	3.17±0.19	1597	1.62±0.10	919	4.33±0.28	471	3.44±0.39	458
7/11-7/19/00	2.70±0.20	1090			4.59±0.24	595	4.48±0.45	949
8/23-8/29/00	2.30±0.12	852	1.62±0.10	354	4.33±0.21	778	4.00±0.36	979
6/18-6/25/01	2.77±0.21	971	1.52±0.11	495	4.44±0.23	628	4.00±0.35	663
7/17/-7/30/01	2.40±0.11	996			3.04±0.24	1189	3.96±0.32	1387
8/23-8/30/01	2.80±0.16	2314	1.80±0.08	1303	3.81±0.27	1293	3.60±0.28	1342

Table 3. Percentages of total plant wet biomass that was Eurasian watermilfoil (±1SE) and number of species (N) collected at each site. These are the average percentage found in the samples and are thus not equal to total mean milfoil biomass/plant biomass.

Sampling Date	Auburn	N	Cedar	N	Otter	N	Smith's Bay	N
5/19-6/3/94	65% ±10%	9	67% ±11%	4	80% ± 6%	9	64% ±10%	8
7/1-7/11/94	79% ± 6%	9	67% ± 9%	4	75% ± 5%	9	72% ± 6%	11
8/12-8/19/94	74% ± 6%	9	61% ±13%	3	75% ± 6%	11	81% ± 5%	11
9/14-9/21/94	91% ± 6%	9	87% ± 5%	4	83% ± 6%	11	71% ± 8%	9
6/07-6/27/95	72% ± 7%	7	82% ± 7%	3	79% ± 4%	9	61% ± 5%	10
7/31-8/15/95	58% ± 7%	7	58% ± 6%	2	80% ± 7%	9	63% ± 6%	11
9/18-9/29/95	81% ± 7%	5	38% ± 5%	2	95% ± 1%	6	63% ± 7%	10
6/12-6/24/96	70% ± 7%	7	57% ± 7%	5	7% ± 5%	9	33% ± 6%	10
7/30-8/9/96	56% ± 8%	7	59% ± 9%	5	0.1% ± 0.1%	10	56% ± 7%	11
9/12-9/19/96	69% ± 6%	8	73% ± 6%	4	0% ± 0%	9	49% ± 7%	10
6/27-7/17/97	53% ± 13%	10	82% ± 9%	3	1.2% ± 2.3%	12	54% ± 14%	12
9/8-9/18/97	60% ± 13%	8	88% ± 9%	2	0.2% ± 0.3%	13	40% ± 14%	11
6/8-6/18/98	42% ± 5%	11	79% ± 5%	4	4% ± 2%	15	37% ± 6%	15
7/27-8/3/98	24% ± 4%	12					49% ± 8%	16
9/8-9/16/98	34% ± 4%	7	82% ± 6%	4	20% ± 5%	13	50% ± 8%	13
6/15-6/22/99	14% ± 4%	7	82% ± 6%	3	30% ± 6%	13	61% ± 7%	12
7/29-8/3/99					40% ± 5%	14	53% ± 8%	13
8/23-8/25/99	36% ± 7%	6	85% ± 6%	2			61% ± 8%	12
6/6-6/23/00	43% ± 6%	9	75% ± 7%	5	81% ± 5%	12	49% ± 9%	13
7/11-7/19/00	37% ± 6%	9			53% ± 4%	15	40% ± 8%	15
8/23-8/29/00	55% ± 6%	6	77% ± 6%	3	63% ± 5%	9	50% ± 8%	13
6/18-6/25/01	52% ± 6%	10	77% ± 6%	2	20% ± 5%	15	35% ± 8%	14
7/17/-7/30/01	56% ± 6%	5			9% ± 4%	11	42% ± 7%	14
8/23-8/30/01	40% ± 6%	5	59% ± 8%	2	5% ± 3%	12	42% ± 8%	12

The number of species in each lake followed trends similar to previous years. The total and mean number of species remained low at Cedar Lake, with 2-5 species seen and an average of only 1.4-1.8 species per sample (Table 2 and 3). Coontail is the other dominant species. There was some suggestion of a decrease in number of species found at Ceder in 2000-2001 relative to the year or two following alum treatment in 1996. The number of species appeared to decreased in Auburn with the increase in percentage milfoil during 2001 but Auburn retained a moderate diversity (5-10 total species, 2-3 per sample). Diversity remained relatively high at Otter and Smith's Bay (10-15 species, 3.0 to 4.5 per sample) and diversity does not appear to be as affected by Eurasian watermilfoil in these lakes (Table 3).

Water clarity improved at Lake Auburn in 2000-2001 from poor levels in 1997-1999 (Table 4). This may in part be related to the low milfoil biomass in 1997-1999 and its subsequent increase in 2000-2001.

Table 4. Sediment characteristics (bulk density, percent organic matter, sediment pore water ammonium and water column characteristics in 1995-2001 at the four permanent transect sites. Sediment samples were collected from shallow, moderate and deep stations along transects 1, 3 and 5 (n=9). Secchi depth (SD), chlorophyll a (Chl-a; pooled surface and SD sample) and light and temperature profiles were taken in deep water > 100 m from the plant bed. Temperature is at 1m depth and 10% PAR depth is the depth at which light intensity was 10% of surface light (presented as the range which encompassed the 10% value).

Lake/Date	Bulk Dens. (g dm/ml)	NH_4 (mg/L)	% Organic	Chl-a (mg/m^3)	SD (m)	Temp (°C 1m)	10% PAR Depth (m)	Plant Limit (m)
Auburn								
6/15/95	0.60	3.96	11.34	9.5	2.3	20.7	2.5-3.0	3.0
2se	0.15	0.91	3.73					
8/1/95	0.49	4.00	10.69	13.9	1.4	26.0	1.5-2.0	3.0
2se	0.18	1.24	4.39					
9/26/95	0.45	4.40	12.67	8.0	2.0	14.8	2.5	3.0
2se	0.13	1.96	4.05					
6/13/96	0.41	3.08	16.0	2.9	4.2	25.1	3	3.0
2se	0.11	1.66	8.6					
7/31/96	0.42	5.81	13.6	12.8	2.4	23.3	1-1.5	3.0
2se	0.17	1.52	4.7					
9/12/96	0.38	2.68	13.7	8.8	2.4	21.2	2.5-3.0	3.0
2se	0.14	0.95	4.3					
6/23/97	0.59	1.93	25.64	11.2	1.2	24.5	2.0	3.4
2se	0.22	0.56	16.79					
9/8/97	0.48	4.42	12.30	16.6	1.4	22.4	1.5-2.0	3.4
2se	0.14	1.46	3.27					
6/8/98	0.23	11.82	11.91	14.4	1.9	18.8	1.5-2.0	
2se	0.08	4.07	4.43					
7/28/98	0.45	20.09	9.52	41.2	0.7	25.7	0.5-1.0	
2se	0.27	3.68	4.25					
9/9/98	0.44	37.72	11.86	36.4	1.1	21.9	1.0-1.5	
2se	0.15	12.57	4.59					
6/22/99	0.50	2.79	13.62	9.4	1.8	22.4	2.0	
2SE	0.16	1.06	3.80					
8/23/99	0.44	10.98	11.64	11.0	1.5	23.1	1.0-1.5	
2SE	0.12	1.81	4.23					
6/19/00	0.51	2.36	11.14	5.9	2.1	20.4	2.5-3.0	
2se	0.14	0.51	4.00					
7/17/00	0.57	4.61	10.15	5.3	2.5	25.3	2.5-3.0	
2se	0.22	1.54	3.63					
8/28/00	0.53	7.75	11.78	5.3	2.3	24.3	3.0	
2se	0.14	1.58	3.93					
6/15/01	0.50	0.98	11.23	6.7	2.9	21.5	3	
2se	0.18	0.38	4.23					
7/17/01	0.57	3.72	25.69	7.2	1.8	27.9	2.5	
2se	0.26	1.92	30.49					
8/29/01	0.47	5.46	10.90	0.8	1.7	24.3	2-2.5	
2se	0.18	1.11	3.77					

Table 4 Continued

Cedar								
6/28/95	0.62	3.90	13.73	10.2	4.5	24.0	4.5	4.0
2se	0.36	1.63	6.00					
8/3/95	0.45	7.27	16.41	16.3	1.2	26.7	1.0-1.5	3.1
2se	0.33	1.39	7.40					
9/28/95	0.43	6.06	21.56	27.5	0.8	14.8	1.0-1.5	3.1
2se	0.36	1.98	7.38					
6/18/96	0.57	3.78	13.3	1.1	5.5	24.6	3.5-4.0	6.5
2se	0.38	1.34	6.3					
8/1/96	0.42	3.86	19.0	4.5	1.9	23.8	2.5-3.0	3.1
2se	0.38	1.59	7.5					
9/16/96	0.41	5.12	18.5	5.3	2.8	20.1	2-2.5	3.1
2se	0.37	1.63	6.9					
7/8/97	0.54	3.97	12.89	9.6	2.5	21.0	3.0-4.0	6.0
2se	0.40	2.87	5.97					
9/11/97	0.42	5.69	15.76	0.8	3.7	22.0	3.0-3.5	6.4
2se	0.33	2.26	6.31					
6/18/98	0.31	4.01	18.35	2.1	4.7	22.6	4.5-5.0	
2se	0.30	1.99	5.27					
7/24/98*	N.A.	N.A.	N.A.	1.3	4.7	26.0	4.5-5.0	
9/16/98	0.29	34.77	18.68	6.9	2.6	23.4	2.5-3.0	
2se	0.30	18.72	4.78					
6/23/99	0.51	4.68	16.15	5.3	2.6	25.6	3.5	
2SE	0.36	1.68	8.79					
8/24/99	0.36	12.35	12.14	17.6	1.6	22.9	2.0-2.5	
2SE	0.34	3.87	3.37					
6/23/00	0.32	2.29	18.28	5.1	3.3	23.1	3.0-3.5	
2se	0.25	1.42	4.77					
8/8/00	0.52	4.15	16.89	4.3	1.6	25.9	3.5-4.0	
2se	0.40	3.91	8.43					
6/19/01	0.60	3.83	22.49	15.0	1.9	22.9	3	
2se	0.43	2.14	16.81					
8/30/01	0.45	2.87	14.92	15.8	1.8	24.7	3-3.5	
2se	0.40	0.74	5.99					
Otter								
6/26/95	0.42	3.27	20.26	5.6	3.0	30.0	3.5-4.0	4.0
2se	0.18	1.43	7.23					
8/10/95	0.39	4.66	24.44	12.5	2.5	24.7	1.5-2.0	4.0
2se	0.26	1.77	9.49					
9/30/95	0.38	2.76	25.07	3.7	1.1	14.5	1.0-1.5	4.0
2se	0.26	1.34	11.34					
6/20/96	0.47	4.86	23.5	8.5	1.9	21.1	1.5-2.0	3.5
2se	0.34	1.67	10.2					
8/6/96	0.27	3.54	27.5	4.8	2	26	2-2.5	4.0
2se	0.16	0.88	8.6					
9/17/96	0.33	3.77	24.9	8.0	1.5	17.9	1.5-2.0	4.0
2se	0.24	1.76	9.5					
7/2/97	0.33	1.89	26.42	9.9	1.3	21.1	2.0-2.5	3.5
2se	0.21	1.09	8.17					
9/15/97	0.29	5.88	27.47	4.8	2.1	21.0	2.0-2.5	3.5
2se	0.16	2.61	9.52					
6/10/98	0.18	10.51	24.24	2.9	2.6	17.8	4.5-5.0	
2se	0.11	3.55	8.54					
9/10/98	0.24	27.47	24.36	1.6	4.0	21.1	3.5-4.0	
2se	0.11	9.40	7.55					
6/21/99	0.24	3.37	27.31	15.5	2.7	24.5	2.5	
2SE	0.07	0.83	8.34					
7/29/99	0.22	9.58	25.37	13.4	2.1	26.4	2.0	
2SE	0.12	3.02	8.61					
7/11/00	0.47	2.69	21.36	6.9	2.5	26.7	1.5-2.0	
2se	0.32	1.63	9.13					
8/29/00	0.25	3.16	29.84	4.5	2.9	23.7	2.0-2.5	
2se	0.13	1.69	9.13					

Table 4 Continued
Otter continued

6/21/01	0.34	2.55	25.25	3.2	2.9	22.5	2.5	
2se	0.20	1.07	10.83					
7/18/01	0.36	3.64	27.71	3.2	2.1	27.8	2.0-2.5	
2se	0.21	1.38	9.70					
8/28/01	0.35	2.77	23.05	5.1	2	24.9	2.5-3.0	
	0.19	1.13	8.12					
Smith's								
6/29/95	0.59	5.18	11.81	4.0	3.9	23.7	5.0	5.0
2se	0.25	3.40	4.62					
8/16/95	0.28	4.06	12.86	7.5	2.1	24.9	3.5-4.0	5.0
2se	0.14	0.97	3.71					
9/18/95	0.31	4.25	12.50	10.7	2.1	14.7	2.5	5.0
2se	0.15	0.77	3.98					
6/24/96	0.36	1.13	13.9	3.7	3.7	20.6	3.5-4.0	5.0
2se	0.22	0.32	4.7					
8/8/96	0.37	2.61	17.6	1.3	3.4	24.4	4.5-5.0	5.0
2se	0.21	1.01	5.3					
9/19/96	0.32	2.43	19.1	3.2	3.5	20.1	3.0-3.5	5.0
2se	0.18	0.90	14.3					
7/15/97	0.34	2.44	9.29	1.6	3.5	22.2	4.5-5.0	5.0
2se	0.17	0.80	3.48					
9/18/97	0.31	2.94	14.10	5.3	2.4	20.9	2.5-3.0	5.0
2se	0.17	1.21	4.74					
6/15/98	0.35	3.35	11.50	1.6	3.6	21.0	4.0-4.5	
2se	0.19	1.98	4.22					
8/4/98	0.34	9.32	11.76	4.0	2.9	23.6	3.5-4.0	
2se	0.16	3.27	3.59					
9/15/98	0.30	26.00	13.55	4.3	2.7	22.5	3.0-3.5	
2se	0.14	5.87	3.40					
6/16/99	0.34	2.21	12.71	4.3	3.7	20.8	4.0	
2SE	0.18	0.40	4.08					
8/4/99	0.37	11.54	10.32	4.8	2.6	26.1	4.5-5	
2SE	0.22	8.83	3.84					
8/25/99	0.30	9.71	10.63	7.2	2.9	24.7	4.0	
2SE	0.16	3.24	3.52					
6/20/00	0.39	2.03	11.06	4.3	3.2	19.9	4.0-4.5	
2se	0.16	0.62	3.17					
7/18/00	0.38	4.00	9.91	4.5	1.9	24.3	4.5-5.0	
2se	0.20	1.13	4.71					
8/23/00	0.42	3.02	12.90	4.3	3.2	23.9	4.0	
2se	0.24	0.82	4.69					
6/22/01	0.33	1.93	12.52	2.1	2.9	20.8	4.0-4.5	
2se	0.19	0.81	4.47					
7/24/01	0.38	2.42	13.57	14.4	2.3	26.9	4	
2se	0.24	1.37	5.15					
8/23/01	0.37	3.30	12.93	3.5	3.4	24.7	4.0-4.5	
2se	0.24	1.16	4.29					

In Cedar Lake, the opposite was happening; water clarity declined from high levels following alum treatment in 1996 to <2m during most of 2000 and 2001. Chlorophyl levels mirrored Secchi depth at both lakes. Trends in clarity were less evident at Otter Lake, but clarity was better (>2m) than following the plant decline in 1996-1997. Clarity at Smith's Bay was similar to previous years (2-3.5m). Sediment pore water ammonium levels in 2000-2001 were lower than 1998-1999 in most lakes (Table 4), but were relatively similar among lakes Sediment pore water ammonium levels were not noticeably lower in Otter Lake during or after the milfoil decline and nutrient depletion would not appear to be the cause of the milfoil decline there. As in previous years, Otter had the most organic sediment, followed by Cedar, Smith's Bay and Lake Auburn.

Weevil densities (N/m^2) were generally lower in 2001 than in 2000, but with the exception of Smith's Bay, were higher than in 1998-1999 (Table 5). Weevils disappeared from Auburn in July 1998 and did not return to our samples until 2000. More weevils were seen in Cedar Lake in 1999-2001 compared to previous years, but densities never exceeded 9/m^2.

Table 5. Density (N/m^2 ± 2 SE and N per stem ± 2SE) of *Euhrychiopsis lecontei* larvae, pupae and adults, *Acentria ephemerella* and *Parapoynx* at the four permanent transect sites, 1994-2001. *Parapoynx* were not enumerated before 1996. A stem is a basal milfoil stem emerging from the sediment; estimates per stem do not include samples without milfoil and because caterpillars occurred often without milfoil, per stem estimates are not reported for them.

Cedar

	Weevil	Larvae	Pupae	Adults	Total *E.l.*	*Acentria*	*Parapoynx*
Date	n	N/m^2	N/m^2	N/m^2	N/m^2	N/m^2	
May-94	11	5.5± 10.9	0.0± 0.0	0.9± 1.8	6.4± 10.9	0.0± 0.0	
per stem	0	–	–	–	–		
Jul-94	14	4.3± 8.6	1.4± 2.9	1.4± 2.9	7.1± 14.3	0.0± 0.0	
	0	–	–	–	–		
Aug-94	11	0.0± 0.0	0.0± 0.0	0.0± 0.0	0.0± 0.0	0.0± 0.0	
Sep-94	17	0.0± 0.0	0.0± 0.0	0.0± 0.0	0.0± 0.0	0.0± 0.0	
Jun-95	18	0.0± 0.0	0.0± 0.0	0.0± 0.0	0.0± 0.0	0.0± 0.0	
Aug-95	10	0.0± 0.0	0.0± 0.0	0.0± 0.0	0.0± 0.0	0.0± 0.0	
Sep-95	17	0.0± 0.0	0.0± 0.0	0.0± 0.0	0.0± 0.0	0.0± 0.0	
Jun-96	29	0.3± 0.7	0.0± 0.0	0.0± 0.0	0.3± 0.7	0.0± 0.0	0.0±0.0
per stem	25	0.010±0.020	0.000±0.000	0.000±0.000	0.010±0.020		
Aug-96	21	0.0± 0.0	0.5± 1.0	0.5± 1.0	1.0± 1.9	0.0± 0.0	0.0±0.0
per stem	21	0.000±0.000	0.002±0.004	0.002±0.004	0.004±0.008		
Sep-96	23	0.0± 0.0	0.0± 0.0	0.0± 0.0	0.0± 0.0	0.0± 0.0	0.0±0.0
per stem	24	0.000±0.000	0.000±0.000	0.000±0.000	0.000±0.000		
Jul-97	28	0.0±0.0	0.0±0.0	0.4±0.7	0.4±0.7	0.4±0.7	0.0±0.0
per stem	28	0.000±0.000	0.000±0.000	0.002±0.003	0.002±0.003		
Sep-97	26	0.8±1.1	0.0±0.0	0.4±0.8	1.2±1.3	0.0±0.0	0.0±0.0
per stem	26	0.012±0.016	0.000±0.000	0.002±0.003	0.013±0.019		
Jun-98	31	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
per stem	30	0.000±0.000	0.000±0.000	0.000±0.000	0.000±0.000		
Sep-98	28	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.4±0.7	0.0±0.0
per stem	24	0.000±0.000	0.000±0.000	0.000±0.000	0.000±0.000		
Jun-99	26	1.9±2.5	0.0±0.0	0.38±0.77	2.3±2.6	0.0±0.0	0.0±0.0
per stem	24	0.011±0.013	0.000±0.000	0.003±0.006	0.013±0.013		
Aug-99	27	0.7±1.5	0.0±0.0	0.0±0.0	0.7±1.5	0.0±0.0	0.0±0.0
per stem	26	0.002±0.004	0.000±0.000	0.000±0.000	0.002±0.004		
Jun-00	26	7.7±6.8	0.8±1.5	0.4±0.8	8.8±7.8	0.0±0.0	0.0±0.0
per stem	25	0.035±0.031	0.003±0.005	0.001±0.002	0.039±0.034		
Aug-00	27	3.3±3.2	0.0±0.0	0.0±0.0	3.3±3.2	0.7±1.0	0.0±0.0
per stem	25	0.023±0.023	0.000±0.000	0.000±0.000	0.023±0.023		
Jun-01	28	0.0±0.0	1.1±2.1	2.1±4.3	3.2±6.4	0.0±0.0	0.0±0.0
per stem	20	0.000±0.000	0.017±0.033	0.033±0.067	0.050±0.100		
Aug-01	24	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
per stem	12	0.000±0.000	0.000±0.000	0.000±0.000	0.000±0.000		

Table 5. Continued.

Auburn Weevil		Larvae	Pupae	Adults	Total *E.l.*	*Acentria*	*Parapoynx*
Date	n	N/m^2	N/m^2	N/m^2	N/m^2	N/m^2	
May-94	9	27.8 ±27.4	1.1± 2.2	6.7± 8.8	35.6± 36.5	1.1± 2.2	
per stem	9	0.134±0.103	0.002±0.004	0.018±0.020	0.154±0.106		
Jul-94	16	58.8± 21.1	12.5± 9.6	31.3± 14.0	102.5± 36.7	6.3± 7.7	
per stem	16	0.217±0.092	0.034±0.034	0.084±0.036	0.335±0.127		
Aug-94	15	8.7± 7.5	2.0± 2.9	3.3± 3.7	14.0± 9.5	0.7± 1.3	
per stem	15	0.031±0.025	0.003±0.005	0.008±0.008	0.042±0.030		
Sep-94	18	1.7± 3.3	2.2± 2.6	7.8± 7.8	11.7± 11.8	3.9± 3.3	
per stem	18	0.002±0.004	0.006±0.008	0.014±0.012	0.022±0.019		
Jun-95	30	6.0± 4.0	0.7± 0.9	1.0± 1.1	7.7± 2.7	0.3± 0.7	
per stem	21	0.070±0.043	0.003±0.006	0.011±0.015	0.085±0.056		
Jul-95	15	2.0± 2.1	0.7± 1.3	5.3± 5.5	8.0± 3.8	0.0± 0.0	
per stem	14	0.006±0.009	0.000±0.000	0.032±0.039	0.038±0.042		
Sep-95	16	2.5± 2.2	3.1± 3.5	3.8± 4.0	9.4± 3.4	1.3± 1.7	
per stem	11	0.140±0.194	0.049±0.090	0.103±0.180	0.292±0.385		
Jun-96	30	31.0± 17.8	2.0± 2.0	0.0± 0.0	33.0± 19.5	0.3± 0.7	0.0± 0.0
per stem	27	0.729±1.179	0.080±0.148	0.000±0.000	0.809±1.326		
Jul-96	25	9.2± 15.2	3.6± 2.6	12.8± 6.3	25.6± 17.9	1.6±1.5	0.8±1.1
per stem	23	0.029±0.043	0.020±0.021	0.048±0.027	0.096±0.061		
Sep-96	30	6.7± 4.3	2.3± 1.6	3.0± 2.7	12.0± 6.5	0.7±0.9	5.7± 4.4
per stem	29	0.048±0.053	0.007±0.005	0.011±0.010	0.065±0.055		
Jun-97	30	35.7±19.6	0.3±0.7	4.3±5.9	40.3±24.3	0.7±1.3	0.0±0.0
per stem	27	0.201±0.126	0.001±0.003	0.022±0.027	0.224±0.144		
Sep-97	30	0.3±0.7	0.0±0.0	1.7±1.4	2.0±1.5	1.7±2.7	2.3±2.8
per stem	29	0.001±0.001	0.000±0.000	0.007±0.007	0.008±0.008		
Jun-98	27	1.0±1.1	0.0±0.0	0.3±0.7	1.3±1.3	1.0±2.0	0.0±0.0
per stem	27	0.005±0.005	0.000±0.000	0.001±0.003	0.006±0.006		
Jul-98	28	0.0±0.0	0.0±0.0	0.0±0.0	0.±0.0	0.7±1.0	0.0±0.0
per stem	24	0.000±0.000	0.000±0.000	0.000±0.000	0.000±0.000		
Sep-98	30	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.3±0.7
per stem	28	0.000±0.000	0.000±0.000	0.000±0.000	0.000±0.000		
Jun-99	27	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.3±0.7	0.0±0.0
per stem	19	0.000±0.000	0.000±0.000	0.000±0.000	0.000±0.000		
Aug-99	27	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
per stem	19	0.000±0.000	0.000±0.000	0.000±0.000	0.000±0.000		
Jun-00	26	0.8±1.1	0.0±0.0	1.5±1.4	2.3±2.0	0.0±0.0	0.0±0.0
per stem	23	0.004±0.005	0.000±0.000	0.007±0.007	0.010±0.009		
Jul-00	28	1.6±2.5	0.4±0.8	3.6±3.6	5.4±5.5	0.0±0.0	0.0±0.0
per stem	21	0.009±0.014	0.004±0.008	0.027±0.025	0.039±0.038		
Aug-00	28	1.1±2.1	0.0±0.0	2.1±2.4	3.2±4.4	0.0±0.0	2.1±3.1
per stem	27	0.011±0.022	0.000±0.000	0.024±0.028	0.035±0.047		
Jun-01	29	0.3±0.7	2.1±2.5	0.7±1.0	3.1±2.6	0.0±0.0	0.0±0.0
per stem	24	0.003±0.006	0.020±0.028	0.008±0.012	0.031±0.030		
Jul-01	30	0.7±0.9	0.3±0.7	1.0±1.1	2.0±1.5	0.0±0.0	0.0±0.0
per stem	25	0.011±0.015	0.002±0.003	0.007±0.008	0.019±0.016		
Aug-01	30	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.3±0.7	0.7±0.9
per stem	19	0.000±0.000	0.000±0.000	0.000±0.000	0.000±0.000		

Table 5. Continued.
Otter

Weevil Date	n	Larvae N/m^2	Pupae N/m^2	Adults N/m^2	Total *E.l.* N/m^2	*Acentria* N/m^2	*Parapoynx*
May-94	20	12.5± 10.2	0.0± 0.0	0.0± 0.0	12.5± 10.2	0.5± 1.0	
per stem	20	0.047±0.038	0.000±0.000	0.000±0.000	0.047±0.038		
Jul-94	24	0.4± 0.9	0.0± 0.0	0.4± 0.9	0.8± 1.2	0.0± 0.0	
	24	0.001±0.002	0.000±0.000	0.001±0.003	0.002±0.003		
Aug-94	14	0.0± 0.0	0.0± 0.0	0.0± 0.0	0.0± 0.0	1.4± 2.9	
	14	0.000±0.000	0.000±0.000	0.000±0.000	0.000±0.000		
Sep-94	8	0.0± 0.0	1.3± 2.5	2.5± 3.3	3.8± 3.7	6.3± 5.3	
	7	0.000±0.000	0.003±0.007	0.013±0.022	0.016±0.021		
Jun-95	27	5.9± 5.1	2.6± 3.3	3.3± 3.4	11.9± 9.0	0.4± 0.7	
	26	0.033±0.030	0.021±0.034	0.022±0.020	0.076±0.071		
Aug-95	15	0.0± 0.0	0.0± 0.0	0.7± 1.3	0.7± 1.3	0.0± 0.0	
	1	0.000±0.000	0.000±0.000	0.000±0.000	0.000±0.000		
Sep-95	18	0.6± 1.1	0.0± 0.0	1.1± 2.2	1.7± 2.4	0.0± 0.0	
	1	0.000±0.000	0.000±0.000	0.000±0.000	0.000±0.000		
Jun-96	25	0.0± 0.0	0.0± 0.0	0.0± 0.0	0.0± 0.0	0.8± 1.6	0.8±1.6
	5	0.000±0.000	0.000±0.000	0.000±0.000	0.000±0.000		
Aug-96	26	0.0± 0.0	0.0± 0.0	0.0± 0.0	0.0± 0.0	0.8± 1.1	2.3± 2.0
	2	0.000±0.000	0.000±0.000	0.000±0.000	0.000±0.000		
Sep-96	27	0.0± 0.0	0.0± 0.0	0.0± 0.0	0.0± 0.0	4.4± 3.6	100.4±24.5
	0	–	–	–	–		
Jul-97	26	0.4±0.8	0.0±0.0	0.0±0.0	0.4±0.8	6.2± 3.9	20.8±20.5
	3	0.083±0.167	0.000±0.000	0.000±0.000	0.083±0.167		
Sep-97	27	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	1.5±1.8	30.0±13.8
	1	0.000±0.000	0.000±0.000	0.000±0.000	0.000±0.000		
Jun-98	27	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	1.1±1.6	0.4±0.7
	13	0.000±0.000	0.000±0.000	0.000±0.000	0.000±0.000		
Sep-98	27	4.1±4.3	0.0±0.0	1.9±3.0	5.9±5.1	0.0±0.0	4.4±5.4
	16	0.206±0.219	0.000±0.000	0.049±0.084	0.255±0.223		
Jun-99	22	1.4±2.0	0.0±0.0	0.0±0.0	1.4±2.0	0.0±0.0	0.0±0.0
	20	0.030±0.050	0.000±0.000	0.000±0.000	0.030±0.050		
Jul-99	26	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
	26	0.000±0.000	0.000±0.000	0.000±0.000	0.000±0.000		
Jun-00	27	14.4±14.8	4.8±4.3	4.8±3.9	24.1±20.4	0.0±0.0	0.4±0.7
	27	0.092±0.093	0.029±0.037	0.028±0.027	0.150±0.131		
Jul-00	27	1.1±1.6	0.0±0.0	0.7±1.5	1.9±3.0	0.0±0.0	0.0±0.0
	27	0.019±0.030	0.000±0.000	0.015±0.030	0.033±0.059		
Aug-00	27	4.1±4.8	0.0±0.0	1.5±1.4	5.6±5.7	1.9±1.5	3.3±2.4
	27	0.064±0.074	0.000±0.000	0.011±0.012	0.076±0.083		
Jun-01	27	1.1±2.2	0.4±0.7	2.2±3.3	3.7±4.3	4.1±3.6	0.7±1.5
per stem	21	0.014±0.029	0.005±0.010	0.083±0.131	0.102±0.134		
Jul-01	25	0.0±0.0	0.0±0.0	0.8±1.6	0.8±1.6	0.4±0.8	12.4±9.4
per stem	3	0.000±0.000	0.000±0.000	0.333±0.667	0.333±0.667		
Aug-01	19	6.8±8.0	0.0±0.0	0.5±1.1	7.4±8.9	3.2±4.6	26.3±14.0
per stem	0	.-	.-	.-.	.-		

Table 5. Continued.

Smith's Bay	Weevil	Larvae	Pupae	Adults	Total *E.l.*	*Acentria*	*Parapoynx*
Date	n	N/m^2	N/m^2	N/m^2	N/m^2	N/m^2	
Jun-94	13	3.8± 5.3	0.0± 0.0	0.8± 1.5	4.6± 6.6	0.0± 0.0	
per stem	12	0.020±0.030	0.000±0.000	0.005±0.010	0.025±0.040		
Jul-94	11	12.3± 13.0	6.9± 8.0	1.5± 2.1	20.8± 20.9	0.8± 1.5	
	13	0.064±0.083	0.038±0.052	0.006±0.009	0.108±0.137		
Aug-94	16	18.0± 15.0	3.1± 4.0	1.9± 2.7	23.1± 20.2	0.6± 1.3	
	15	0.104±0.079	0.019±0.022	0.010±0.015	0.133±0.109		
Sep-94	14	0.0± 0.0	1.4± 2.9	2.1± 2.3	3.6± 4.5	0.0± 0.0	
	14	0.000±0.000	0.003±0.006	0.013±0.020	0.016±0.022		
Jun-95	25	0.4± 0.8	0.0± 0.0	0.8± 1.1	1.2± 1.3	0.0± 0.0	
	14	0.001±0.003	0.000±0.000	0.027±0.048	0.028±0.047		
Aug-95	25	4.0± 4.3	1.2± 1.8	0.4± 0.8	5.6± 5.3	0.0± 0.0	
	9	0.080±0.096	0.000±0.000	0.007±0.015	0.087±0.107		
Sep-95	25	0.8± 1.1	2.0± 3.3	0.8± 1.1	3.6± 5.0	0.0± 0.0	
	15	0.010±0.014	0.025±0.039	0.013±0.019	0.048±0.061		
Jun-96	25	4.8± 5.8	0.0± 0.0	0.0± 0.0	4.8± 5.8	5.2± 8.8	0.0± 0.0
	20	0.037±0.043	0.000±0.000	0.000±0.000	0.037±0.043		
Aug-96	25	12.4± 10.0	1.2± 1.8	2.0± 2.0	15.6± 10.5	0.0± 0.0	1.6± 2.5
	24	0.107±0.084	0.006±0.008	0.015±0.015	0.127±0.087		
Sep-96	25	1.2± 1.8	2.0± 2.0	2.8± 3.4	6.0± 5.3	0.8± 1.1	0.0± 0.0
	24	0.005±0.007	0.009±0.009	0.014±0.015	0.028±0.022		
Jul-97	25	5.2±4.3	0.4±0.8	4.0±3.7	9.6±6.9	0.0± 0.0	0.8±1.6
	21	0.049±0.053	0.003±0.005	0.043±0.049	0.094±0.094		
Sep-97	25	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.4±0.8	0.0± 0.0
	21	0.000±0.000	0.000±0.000	0.000±0.000	0.000±0.000		
Jun-98	25	7.2±7.2	0.4±0.8	0.0±0.0	7.6±7.6	1.2±1.8	0.0±0.0
	21	0.052±0.054	0.002±0.005	0.000±0.000	0.054±0.055		
Aug-98	25	1.2±1.8	0.0±0.0	0.8±1.1	2.0±2.0	0.0±0.0	0.0±0.0
	20	0.017±0.023	0.000±0.000	0.002±0.005	0.019±0.023		
Sep-98	25	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.4±0.8
	19	0.000±0.000	0.000±0.000	0.000±0.000	0.000±0.000		
Jun-99	22	0.9±1.3	0.0±0.0	0.9±1.3	1.8±2.1	0.9±1.3	0.0±0.0
	22	0.047±0.091	0.000±0.000	0.047±0.091	0.094±0.182		
Jul-99	25	2.4±4.8	0.8±1.1	1.2±1.3	4.4±4.9	0.0±0.0	1.2±1.5
	21	0.000±0.000	0.002±0.003	0.014±0.024	0.017±0.024		
Aug-99	23	0.9±1.2	0.0±0.0	0.9±1.2	1.7±2.0	0.0±0.0	0.0±0.0
	22	0.005±0.007	0.000±0.000	0.007±0.010	0.012±0.015		
Jun-00	22	3.6±4.1	0.9±1.8	1.8±1.7	6.4±5.5	1.4±2.0	0.0±0.0
	20	0.027±0.035	0.007±0.014	0.008±0.009	0.042±0.042		
Jul-00	24	0.0±0.0	0.0±0.0	0.8±1.7	0.8±1.7	0.0±0.0	0.0±0.0
	19	0.000±0.000	0.000±0.000	0.009±0.018	0.009±0.018		
Aug-00	23	1.3±1.4	0.0±0.0	0.0±0.0	1.3±1.4	0.0±0.0	1.7±2.4
	21	0.009±0.010	0.000±0.000	0.000±0.000	0.009±0.010		
Jun-01	25	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.4±0.8	0.0±0.0
per stem	13	0.000±0.000	0.000±0.000	0.000±0.000	0.000±0.000		
Jul-01	24	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
per stem	17	0.000±0.000	0.000±0.000	0.000±0.000	0.000±0.000		
Aug-01	20	0.0±0.0	0.0±0.0	0.5±1.0	0.5±1.0	0.0±0.0	0.0±0.0
per stem	14	0.000±0.000	0.000±0.000	0.002±0.005	0.002±0.005		

Weevil densities increased at Otter Lake from low levels after the milfoil crash to over 20/m^2 in June 2000. *Acentria* and *Parapoynx* were not found at Cedar and were found at very low densities in Auburn and Smith's Bay, but increased at Otter Lake in 2000-2001 as the milfoil reached very low densities and was replaced by native plants. The milfoil decline at Otter does not appear related to these herbivores whose densities increase when milfoil declines and is replaced by native plants. *Parapoynx* especially appears to increase on the native plants following a milfoil decline.

Cenaiko Lake

The suppression of milfoil biomass at Cenaiko Lake (Fig. 2) continued from 1999 through 2001 (Table 6). Milfoil biomass did not exceed 10 g dry/m^2 or 8% of total plant biomass during this time. Native plant biomass remained dominant (50-200 g dry/m^2) and the number of species remained moderate. The native plant community was reduced somewhat in 2001 with lower biomass and fewer species than in previous years, likely due to the extremely high early and mid-summer water levels (up to 2m above normal) which appeared to suppress plant growth of all species except coontail. It is unclear what the implications are for 2002.

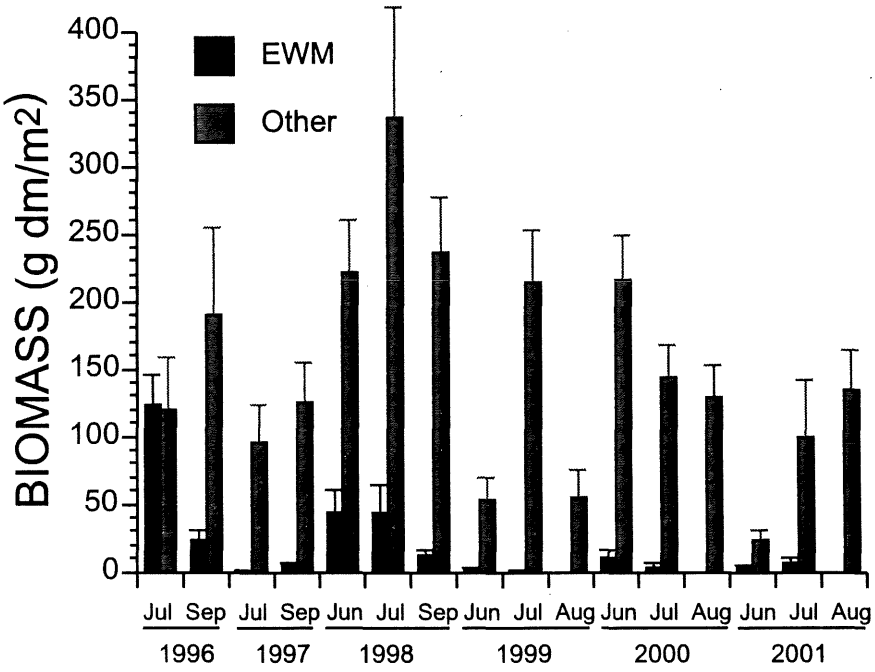

Fig. 2. Dry biomass of milfoil (EWM) and non-milfoil plants at Cenaiko Lake, 1996-2001. Milfoil was present but not found in August 1999 samples and was 0.01g/m^2 in August 2001. N > 20 samples on each date.

Weevils were not detected in the biomass samples during most dates in 1999-2001 (Table 7), partly due to the low density of milfoil (0 to 7 samples typically contained enough intact milfoil for stem counts). Weevils were found in the biweekly surveys (see next section) although at low densities, and we did notice more sunfish in 2001 than in previous years. *Acentria* was present at high density in 2000 but decreased to detectible but low density (< 5/m^2) in June and July 2001. *Parapoynx* was rarely present and at low density. It is possible that *Acentria* is assisting with maintaining the milfoil decline (e.g., high density in June 2000), however, it is often also found on other plants and does not appear to be the main suppressant of Eurasian watermilfoil at Cenaiko. We should note that if fish predation are important for weevil densities and fewer fish also likely

allow higher densities of *Acentria*; Cenaiko has the highest density of *Acentria* among our study lakes. The possible increase in sunfish during 2001 might also have resulted in the low *Acentria* densities found in 2001. The high water levels in 2001 noted above may have adversely affected both the milfoil and herbivore populations, but the low levels of herbivores in 2001 might allow a resurgence of milfoil in 2002 if they do not increase.

Table 6. Biomass (g dry/m2) of all plants (Total), Eurasian watermilfoil (MSP), the dominant plants (coontail (CRT), *Zosterella* (= *Heteranthera*) *dubia* (ZOS), *Potamogeton zosteriformis* (PZS), *Chara* (CHA) and *Potamogeton amplifolius* (PAM)), non-milfoil biomass (NAT), total (TN) and mean number of species (N Sp) and mean percentage of biomass that was Eurasian watermilfoil in Cenaiko Lake 1999-2001. N=22-27 samples per date. In July and August 2001, *Potamogeton nodosus* was present at densities of 36 and 19 g dry/m^2.

Date	Total	MSP	CRT	PZS	ZOS	CHA	PAM	TN	N Sp.	NAT	%MSP
6/24/99	53.7	1.3	32.2	0.2	3.0	0.5	12.3	11	1.9	52.4	7.9%
1 S.E.	17.0	0.9	12.0	0.2	2.5	0.4	10.7		0.2	17.1	5.2%
8/2/99	214.6	1.1	124.5	0.0	26.7	0.0	34.1	10	2.6	213.5	1.0%
1 S.E.	40.1	0.8	37.5	0.0	9.7	0.0	23.6		0.2	40.2	0.7%
8/26/99	55.0	0.0	30.2	0.1	5.0	0.0	6.7	5	1.5	55.0	0.0%
1 S.E.	20.1	0.0	20.1	0.1	3.4	0.0	4.4		0.1	20.1	0.0%
6/29/00	225.9	10.0	123.9	0.0	16.3	46.0	19.8	9	2.1	215.9	3.1%
1 SE	34.1	5.2	31.2	0.0	8.2	21.1	14.3		0.2	33.1	1.7%
7/20/00	146.8	3.7	86.4	0.0	19.5	14.5	18.3	8	2.4	143.2	8.4%
1 SE	23.6	2.2	22.5	0.0	10.1	9.4	11.8		0.3	24.1	5.1%
8/30/00	134.5	0.1	89.4	34.5	0.0	8.0	1.7	8	1.8	129.4	0.1%
1 SE	22.0	0.1	23.5	14.9	0.0	7.3	1.5		0.2	22.8	0.1%
6/26/01	25.5	2.8	17.2	0.6	0.0	0.0	0.6	7	1.4	22.7	3.5%
1 SE	8.5	2.8	7.9	0.3	0.0	0.0	0.6		0.4	8.0	3.3%
7/30/01	105.4	6.8	59.5	0.0	0.0	0.0	0.0	7	1.1	98.6	7.1%
1 SE	43.1	4.0	26.1	0.0	0.0	0.0	0.0		0.3	42.6	4.4%
8/27/01	133.6	0.0	98.8	1.0	0.0	0.0	8.8	6	1.0	133.6	4.0%
1 SE	29.6	0.0	27.3	0.5	0.0	0.0	6.4		0.1	29.6	4.0%

Water clarity in 2000 and 2001 was lower than previous years, but this was primarily due to rainfall events and the associated suspended clay rather than algae (Table 8). Sediment nutrients and organic matter remained low and similar to previous years, but were higher than the levels in 1996 before milfoil had totally declined (Table 8). Bulk density remains much higher and organic matter lower than any of our other lakes.

Table 7. Density ($N/m^2 \pm 2$ SE and N per stem) of *Euhrychiopsis lecontei* (*E.l.*) larvae, pupae and adults, and *Acentria ephemerella* and *Parapoynx* sp. at Cenaiko Lake in 1996-2001.
Densities per stem were only calculated for samples with Eurasian watermilfoil and because the caterpillars often occurred in samples with no milfoil their densities per stem were not calculated. A stem is a basal milfoil stem emerging from the sediment. Samples with no plants were not included in herbivore density estimates.

Date	Weevil n	Larvae N/m^2	Pupae N/m^2	Adults N/m^2	Total *E.l.* N/m^2	*Acentria* N/m^2	*Parapoynx* N/m^2
7/22/96	29	48.6± 25.2	22.8± 10.8	31.7± 13.6	103.1± 41.9	18.3± 7.7	1.0± 1.5
per stem	26	0.923±1.292	0.337±0.458	0.381±0.280	1.640±1.972		
9/5/96	21	2.9± 2.4	1.0± 1.3	4.3± 4.3	8.1± 5.6	31.9± 20.2	0.0± 0.0
per stem	8	0.229±0.259	0.008±0.017	0.417±0.516	0.654±0.721		
7/16/97	26	1.5±1.8	0.0±0.0	0.0±0.0	1.5±1.8	8.8±5.8	0.0±0.0
per stem	3	0.389±0.401	0.000±0.000	0.000±0.000	0.389±0.401		
9/17/97	24	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	32.1±19.6	1.7±2.0
per stem	6	0.000±0.000	0.000±0.000	0.000±0.000	0.000±0.000		
6/16/98	25	0.4±0.8	0.0±0.0	0.0±0.0	0.4±0.8	17.6±9.1	0.4±0.8
per stem	15	0.004±0.009	0.000±0.000	0.000±0.000	0.004±0.009		
7/29/98	25	0.0±0.0	0.0±0.0	0.8±1.6	0.8±1.6	1.6±1.5	0.4±0.8
per stem	12	0.000±0.000	0.000±0.000	0.019±0.037	0.019±0.037		
9/14/98	25	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	6.4±4.5	21.6±19.8
per stem	3	0.000±0.000	0.000±0.000	0.000±0.000	0.000±0.000		
6/24/99	26	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	16.9±10.3	0.0±0.0
per stem	3	0.000±0.000	0.000±0.000	0.000±0.000	0.000±0.000		
8/2/99	24	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	2.0±1.1	0.0±0.1
per stem	3	0.000±0.000	0.000±0.000	0.000±0.000	0.000±0.000		
8/26/99	23	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	6.5±5.4	0.0±0.0
per stem	0	-	-	-	-		
06/29/00	22	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	69.1±43.2	0.0±0.0
per stem	6	0.000±0.000	0.000±0.000	0.000±0.000	0.000±0.000		
07/20/00	22	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	32.0±16.1	3.0±5.0
per stem	7	0.000±0.000	0.000±0.000	0.000±0.000	0.000±0.000		
08/30/00	21	0.5±1.0	0.0±0.0	0.0±0.0	0.5±1.0	12.9±9.4	4.3±8.6
per stem	7	0.000±0.000	0.000±0.000	0.000±0.000	0.000±0.000		
6/26/01	20	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	3.5±4.9	0.0±0.0
per stem	1	0.000±.	0.000±.	0.000±.	0.000±.		
7/30/01	21	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	4.8±4.3	0.0±0.0
per stem	3	0.000±0.000	0.000±0.000	0.000±0.000	0.000±0.000		
8/27/01	19	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
per stem	0	-	-	-	-		

Table 8. Sediment characteristics (bulk density, percent organic matter, sediment pore water ammonium and water column characteristics in 1996-2001 at Cenaiko Lake. Sediment samples were collected from shallow, moderate and deep stations along transects 1, 2 and 3 (n=9).

Date	Bulk Dens. (g dm/ml)	NH_4 (mg/L)	% Organic	Chl-a (mg/m^3)	SD (m)	Temp (°C 1m)	10% PAR Depth (m)	Plant Limit (m)
7/22/96	1.23	0.60	1.5%	1.34	5.0	25.4	4.5-5.0	3.4
2se	0.22	0.54	0.5%					
9/5/96	1.22	0.67	2.4%	5.61	4.0	25.7	5.0	3.4
2se	0.23	0.40	1.1%					
7/16/97	1.10	1.63	2.5%	4.54	2.3	27.6	3.5	3.0
2se	0.20	0.67	0.6%					
9/17/97	0.96	2.87	2.5%	1.60	2.3	21.3	2.0-2.5	3.0
2se	0.18	1.65	0.5%					
6/16/98	0.98	2.37	2.2%	2.41	3.8	23.7	5.5-6.0	3.4
2se	0.18	0.66	0.5%					
7/29/98	0.97	4.98	2.3%	2.41	4.4	25.9	4.5-5.0	3.4
2se	0.16	2.31	0.7%					
9/14/98	1.12	6.08	1.7%	3.21	3.0	23.8	3.5-4.0	3.2
2se	0.12	4.90	0.5%					
6/24/99	1.12	1.12	1.76%	1.3	2.7	24.3	3.5-4.0	
2SE	0.24	0.24	0.82%					
8/2/99	1.14	2.09	1.29%	3.5	2.7	27.4	3.0-3.5	
2SE	0.17	0.78	0.40%					
8/26/99	1.22	4.20	1.30%	2.1	3.1	24.3	3.0-3.5.0	
2SE	0.14	1.27	0.45%					
6/29/00	1.08	1.11	2.31%	2.14	2.3	23.5	3.5	
2se	0.27	0.73	0.41%					
7/20/00	1.13	4.09	3.01%	3.47	1.6	23.2	2.0-2.5	
2se	0.35	.	1.57%					
8/30/00	1.25	3.27	2.43%	2.94	1.4	23.1	4.5-5.0	
2se	0.26	2.41	0.70%					
6/26/01	1.05	1.45	3.69%	4.3	1.3	25.2	2.5	
2se	0.28	0.75	3.66					
7/30/01	1.27	2.07	1.80%	4.5	0.9	26.9	1.5	
2se	0.23	0.65	0.59					
8/27/01	1.26	3.92	1.70%	17.6	2.3	25.6	4.5	
2se	0.21	2.08	0.60					

Bi-weekly weevil surveys

The bi-weekly weevil surveys initiated at Lake Auburn in 1998, Smith's Bay and Cenaiko in 1999 and Otter Lake in 2000 have been quite instructive. Weevil densities declined in Lake Auburn from May to July 1998 and no weevils were found there from the end of July 1998 until May 2000. Moderate densities (0.05-0.2/stem) were maintained from May 2000 until the end of July 2001 when weevils again dropped below our detection limits (Table 9). At Smith's Bay and

Cenaiko Lake, populations persisted throughout the summer (Fig. 3). Furthermore, these populations persisted across years (e.g., Fig. 4).

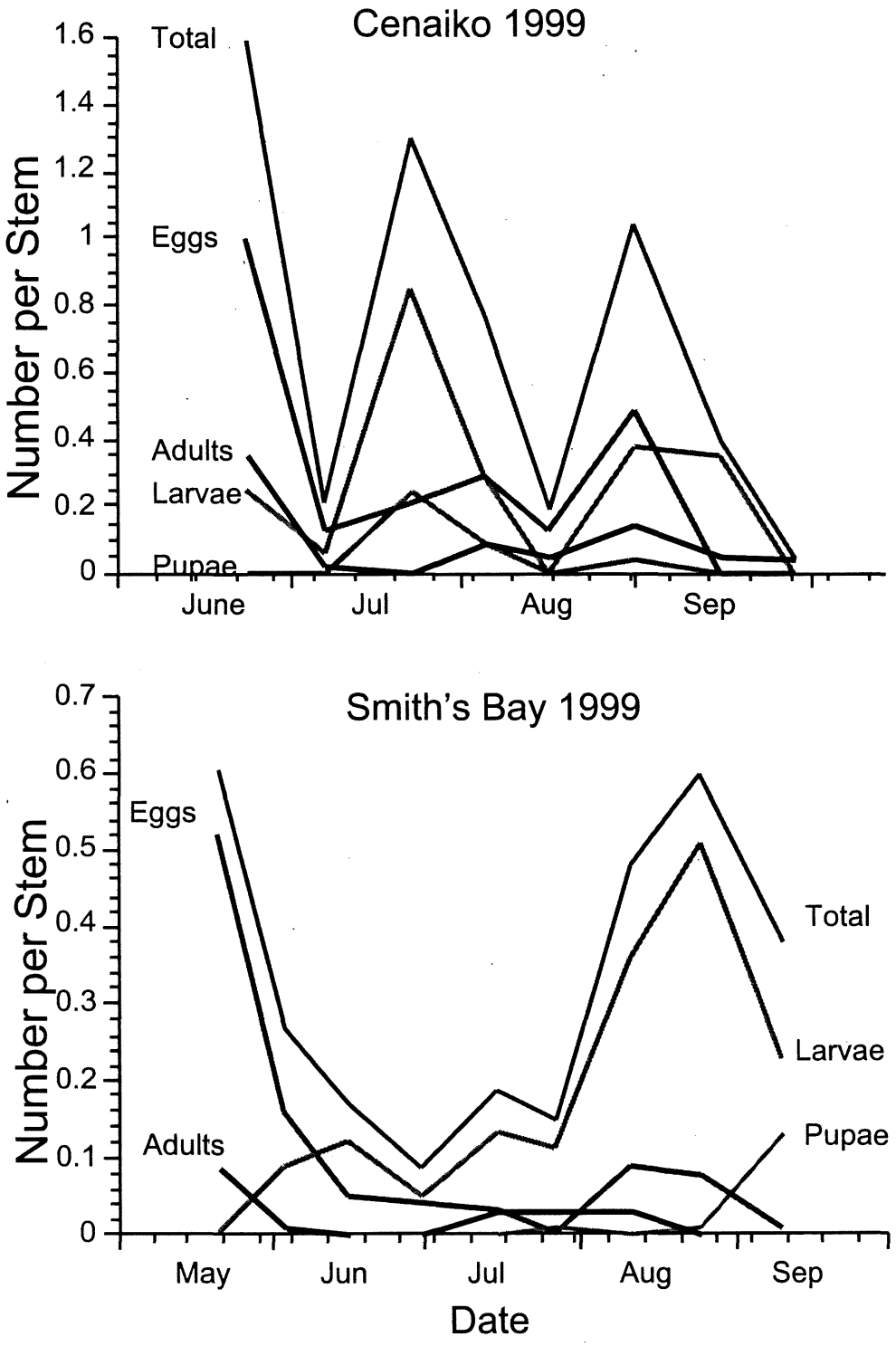

Fig. 3. Number of weevils per stem from bi-weekly surveys at Smith's Bay and Cenaiko, 1999.

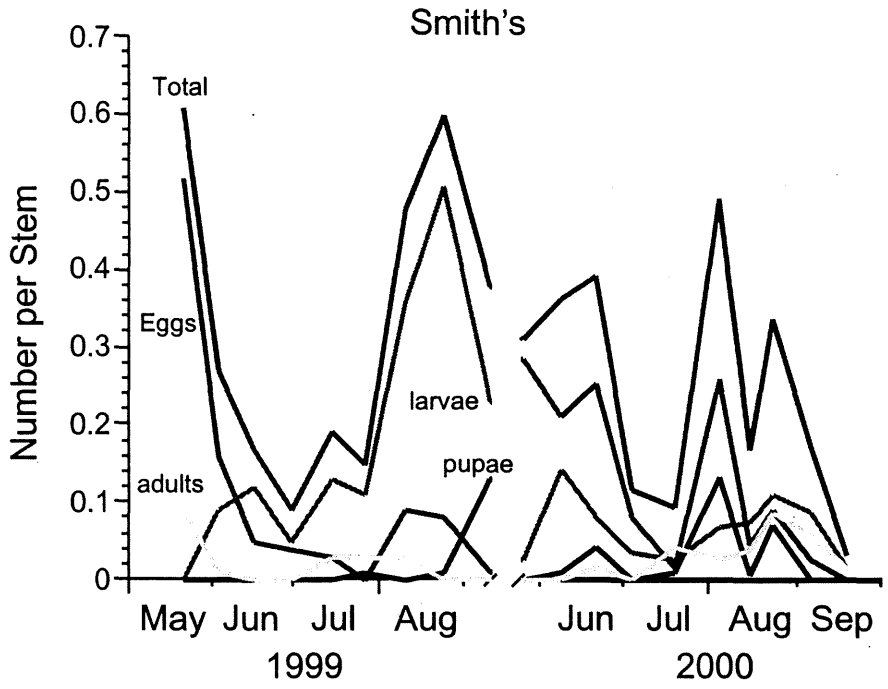

Fig. 4. Number of weevils per stem from bi-weekly surveys at Smith's Bay, 1999-2000.

Densities at Cenaiko Lake decreased from 1999-2001. Densities after July 2001 were quite low (Table 9) and this may have been due to the high June and July water levels, however, we also noted more sunfish at Cenaiko in 2001. With the exception of May, *Acentria* densities were also lower than in 2000 and *Parapoynx* was not found in 2001 (Tables 7 and 9). Weevil densities at Smith's Bay were also lower in 2001 than in 1999-2000, but weevils persisted throughout the summer. Weevil densities increased from 2000 to 2001 at Otter Lake and persisted at high density throughout summer 2001 (Fig. 5). Total densities ranged from 0.2 to 0.8 per stem and adults were present throughout the summer suggesting low adult mortality at Otter in 2001. The persistently higher adult populations (per stem) rival those seen at Cenaiko during the major decline (Newman and Biesboer 2000). Weevil damage was clearly responsible for the milfoil decline at Otter during 2000-2001.

Parapoynx was present only at Otter Lake (and Auburn in 2000) and appeared primarily in the late summer and fall when densities approached 0.2/stem (Table 9). None were found in July and August. *Acentria* was also absent from Lake Auburn and Smith's Bay in 2001 and was generally most abundant early and late in the season but not in mid summer (probably in egg and undetected early instar stages during mid-summer). Densities at Otter never exceeded 0.05/stem but high densities (> 0.1/stem) were found at Cenaiko in late May and mid September 2001. Similar patterns were seen in 2000. The low densities of caterpillars at Otter in 2000 and in May through August (<0.05/stem) 2001 suggests they were not instrumental in the milfoil decline. Although the number of observations is low, it appears that the caterpillars are not common in lakes or years with low weevil densities (e.g., Table 9; Auburn and Cedar), further suggesting that sunfish may be limiting populations of potential control agents.

Table 9. Density of weevil life stages (per stem), total weevils per stem and density of the caterpillars *Acentria* (Acent) and *Parapoynx* (Parap) from the bi-weekly weevil surveys.

Lake Date	Eggs	Larvae	Pupae	Adults	Total	Acent	Parap
Cenaiko							
5/16/00	0.1952	0.0229	0.0000	0.0000	0.2181	0.2762	0.0000
5/30/00	0.0397	0.0159	0.0069	0.0000	0.0625	0.1905	0.0000
6/13/00	0.1190	0.0883	0.0488	0.0756	0.3318	0.1584	0.0000
6/29/00	0.2476	0.0556	0.0397	0.0238	0.3667	0.0508	0.0000
7/11/00	0.3214	0.0347	0.0208	0.1141	0.4911	0.1141	0.0000
7/24/00	0.7393	0.0208	0.0069	0.1181	0.8851	0.0417	0.0000
8/10/00	0.5417	0.0917	0.0000	0.0167	0.5667	0.0083	0.0000
8/24/00	0.0822	0.0519	0.0065	0.0652	0.2058	0.0465	0.0000
9/7/00	0.0278	0.0324	0.0379	0.0866	0.1847	0.1554	0.0000
9/20/00	0.0000	0.0694	0.0000	0.0478	0.1173	0.0556	0.0000
10/3/00	0.0000	0.0368	0.0000	0.0083	0.0451	0.0000	0.0000
5/21/01	0.0833	0.0000	0.0000	0.0000	0.0833	0.8068	0.0000
6/6/01	0.6893	0.0000	0.0000	0.1857	0.8750	0.1250	0.0000
6/18/01	0.0500	0.0000	0.0000	0.0000	0.0500	0.0000	0.0000
7/3/01	0.0343	0.0000	0.0000	0.0000	0.0343	0.0100	0.0000
7/19/01	0.0000	0.1268	0.0000	0.0000	0.1268	0.0250	0.0000
7/30/01	0.0000	0.0000	0.0000	0.0125	0.0125	0.0250	0.0000
8/15/01	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
8/27/01	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
9/5/01	0.0104	0.0000	0.0000	0.0000	0.0104	0.0625	0.0000
9/18/01	0.0000	0.0000	0.0000	0.0000	0.0000	0.1472	0.0000
Auburn							
5/19/00	0.0267	0.0267	0.0000	0.0000	0.0533	0.0000	0.0000
6/1/00	0.0000	0.0218	0.0000	0.0079	0.0298	0.0000	0.0000
6/15/00	0.0139	0.0278	0.0000	0.0000	0.0417	0.0000	0.0000
6/27/00	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
7/10/00	0.0000	0.0000	0.0069	0.0347	0.0417	0.0000	0.0000
7/25/00	0.1528	0.0000	0.0069	0.0556	0.2153	0.0000	0.0000
8/9/00	0.0368	0.0515	0.0515	0.0294	0.1691	0.0000	0.0000
8/28/00	0.0000	0.0000	0.0000	0.0074	0.0074	0.0000	0.0000
9/12/00	0.0000	0.0208	0.0062	0.0123	0.0394	0.0000	0.0149
9/28/00	0.0000	0.0000	0.0000	0.0139	0.0139	0.0000	0.0000
5/10/01	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
5/24/01	0.2562	0.0139	0.0000	0.0309	0.3009	0.0000	0.0000
5/30/01	0.1847	0.0000	0.0000	0.0000	0.1847	0.0000	0.0000
6/13/01	0.0069	0.0139	0.0139	0.0308	0.0655	0.0000	0.0000
6/28/01	0.0278	0.0139	0.0000	0.0000	0.0417	0.0000	0.0000
7/9/01	0.0278	0.1389	0.0139	0.0139	0.1944	0.0000	0.0000
7/23/01	0.0000	0.0123	0.0270	0.0139	0.0532	0.0000	0.0000
8/8/01	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
8/20/01	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
9/11/01	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
9/27/01	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000

Table 9. Continued.

Lake	Date	Eggs	Larvae	Pupae	Adults	Total	Acent	Parap
Otter								
	6/5/00	0.1940	0.1321	0.0500	0.0821	0.4583	0.0250	0.0000
	6/22/00	0.1395	0.2027	0.0580	0.0804	0.4806	0.0268	0.0089
	7/5/00	0.0000	0.0403	0.0079	0.0079	0.0575	0.0000	0.0000
	7/18/00	0.0000	0.0074	0.0074	0.0000	0.0147	0.0000	0.0000
	8/2/00	0.0218	0.0000	0.0069	0.0218	0.0506	0.0069	0.0000
	8/16/00	0.0074	0.0147	0.0000	0.0000	0.0221	0.0000	0.0000
	8/29/00	0.0000	0.0441	0.0074	0.0515	0.1029	0.0000	0.0000
	9/13/00	0.0000	0.0394	0.0278	0.0231	0.0903	0.0000	0.0000
	9/26/00	0.0000	0.0069	0.0764	0.1042	0.1875	0.0000	0.0000
	5/21/01	0.3268	0.0000	0.0000	0.1250	0.4518	0.0000	0.0000
	6/4/01	0.2225	0.0000	0.0000	0.1789	0.4015	0.0417	0.0147
	6/21/01	0.5345	0.0407	0.0000	0.0663	0.6415	0.0074	0.0000
	7/5/01	0.4117	0.1354	0.0851	0.1634	0.7955	0.0202	0.0000
	7/16/01	0.1119	0.0000	0.0000	0.2608	0.3727	0.0000	0.0000
	8/1/01	0.1027	0.0469	0.0000	0.1007	0.2502	0.0000	0.0000
	8/13/01	0.1507	0.0306	0.0000	0.0512	0.2324	0.0000	0.0000
	8/28/01	0.0515	0.1922	0.0000	0.0221	0.2658	0.0074	0.0000
	9/5/01	0.1128	0.1553	0.0131	0.1063	0.3875	0.0378	0.0069
	9/17/01	0.0278	0.2750	0.0486	0.2935	0.6449	0.0069	0.1918
	10/2/01	0.0193	0.0432	0.0288	0.1211	0.2124	0.0455	0.0481
Smith's								
	5/25/00	0.2867	0.0267	0.0000	0.0000	0.3133	0.0000	0.0000
	6/8/00	0.2095	0.1429	0.0095	0.0000	0.3619	0.0000	0.0000
	6/21/00	0.2519	0.0824	0.0429	0.0167	0.3938	0.0583	0.0000
	7/3/00	0.0810	0.0369	0.0000	0.0000	0.1179	0.0000	0.0000
	7/19/00	0.0167	0.0250	0.0111	0.0417	0.0944	0.0000	0.0000
	8/4/00	0.2604	0.0702	0.1339	0.0274	0.4919	0.0000	0.0000
	8/15/00	0.0472	0.0750	0.0074	0.0389	0.1685	0.0000	0.0000
	8/23/00	0.0919	0.1100	0.0726	0.0871	0.3361	0.0085	0.0000
	9/6/00	0.0250	0.0880	0.0000	0.0591	0.1721	0.0000	0.0000
	9/19/00	0.0000	0.0167	0.0000	0.0167	0.0333	0.0000	0.0000
	5/15/01	0.0000	0.0000	0.0000	0.0083	0.0083	0.0000	0.0000
	5/31/01	0.0241	0.0000	0.0000	0.0333	0.0574	0.0000	0.0000
	6/11/01	0.2287	0.0083	0.0000	0.0095	0.2466	0.0000	0.0000
	6/25/01	0.0222	0.0000	0.0000	0.0274	0.0496	0.0000	0.0000
	7/10/01	0.0000	0.0482	0.0240	0.0000	0.0722	0.0000	0.0000
	7/23/01	0.0000	0.0639	0.0307	0.0000	0.0946	0.0000	0.0000
	8/8/01	0.0250	0.1480	0.0194	0.0083	0.2008	0.0000	0.0000
	8/24/01	0.0148	0.0917	0.0083	0.0000	0.1148	0.0000	0.0000
	9/13/01	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000

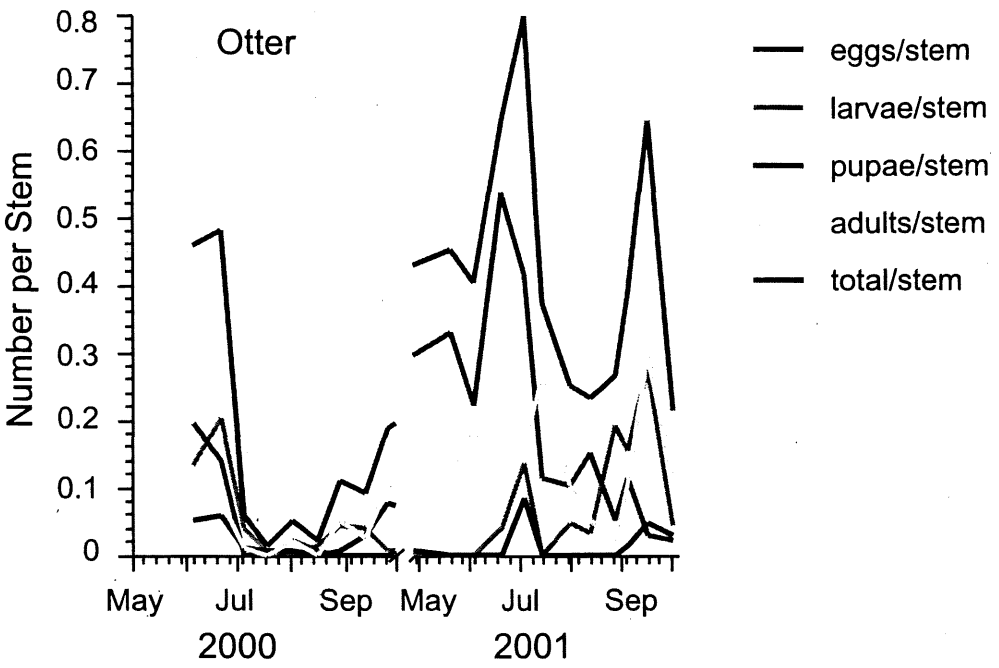

Fig. 5. Biweekly density (number per milfoil stem) of weevil life stages at Otter Lake in 2000-2001.

Relation of herbivore density to milfoil populations:

We have now developed a long enough data set to see trends in weevil and milfoil populations (Fig. 6a and 6b). At Smith's Bay, weevil densities were positively related to milfoil density ($p < 0.05$) but increases in weevil density were generally associated with following declines in milfoil. Similarly, at Auburn, although there was no significant correlation of weevil density with milfoil abundance, increasing weevil abundance was associated with declining milfoil and low weevil populations were eventually followed by increases in milfoil. The decline of milfoil in from 1997-1999 appears to have been initiated by weevils but the continued decline in 1998 and 1999 was more likely due to other factors such as water clarity and competition from plants. Despite the increase of milfoil in 2000-2001, weevil densities remain low.

At Otter Lake (Fig. 6b) weevil populations increased prior to the winterkill decline of milfoil in 1995-1996, but densities do not appear to have been adequate to account for this decline, and certainly were not the cause of the rapid over winter milfoil crash. It is interesting to note that other herbivores (*Acentria* and particularly *Parapoynx*) increased after the milfoil decline when native plants became more abundant. *Parapoynx* in particular is a generalist herbivore and Ward (2002) showed that its suppression by sunfish can result in an increase in *Heteranthera (Zosterella dubia)*. The increase in milfoil weevil abundance in June 2000 was followed by a milfoil decline and weevils appear to continue to now suppress the milfoil. Caterpillars increased with the increase in native plants.

As we noted previously (Newman et al. 1999) and as is discussed by Getsinger et al. (*in press*), depth or proximity to the deep edge of the be may limit weevil populations. Weevil densities and thus milfoil control may be greater in shallow sites or bigger expanses of milfoil. For example, at Smith's Bay, most weevils are found in the first two stations (≤ 200m from shore; Table 10) and weevil density appears to be controlling milfoil at these sites (Fig. 7). In fact, the near elimination of Eurasian watermilfoil at the shallowest station (100 m from shore, ca 1.5 m depth) is evident (Fig. 7). Although weevils were found once at the furthest station (805m from shore) they are uncommon at the deeper sites >200m from shore. At these sites weevils are clearly

having no effect on milfoil. This relationship is not due to distance from shore however. A similar effect is seen at Lake Auburn where most weevils occur in the first four station (≤ 40 m from shore) and fewer are seen at the deepest site only 60m from shore (Table 10). Again, at the shallowest sites weevils appear associated with milfoil declines (Fig. 7) and weevil densities are considerably higher than the two deepest sites. At the deeper sites, the weevils that occur at lower densities do not appear to have an effect on the milfoil.

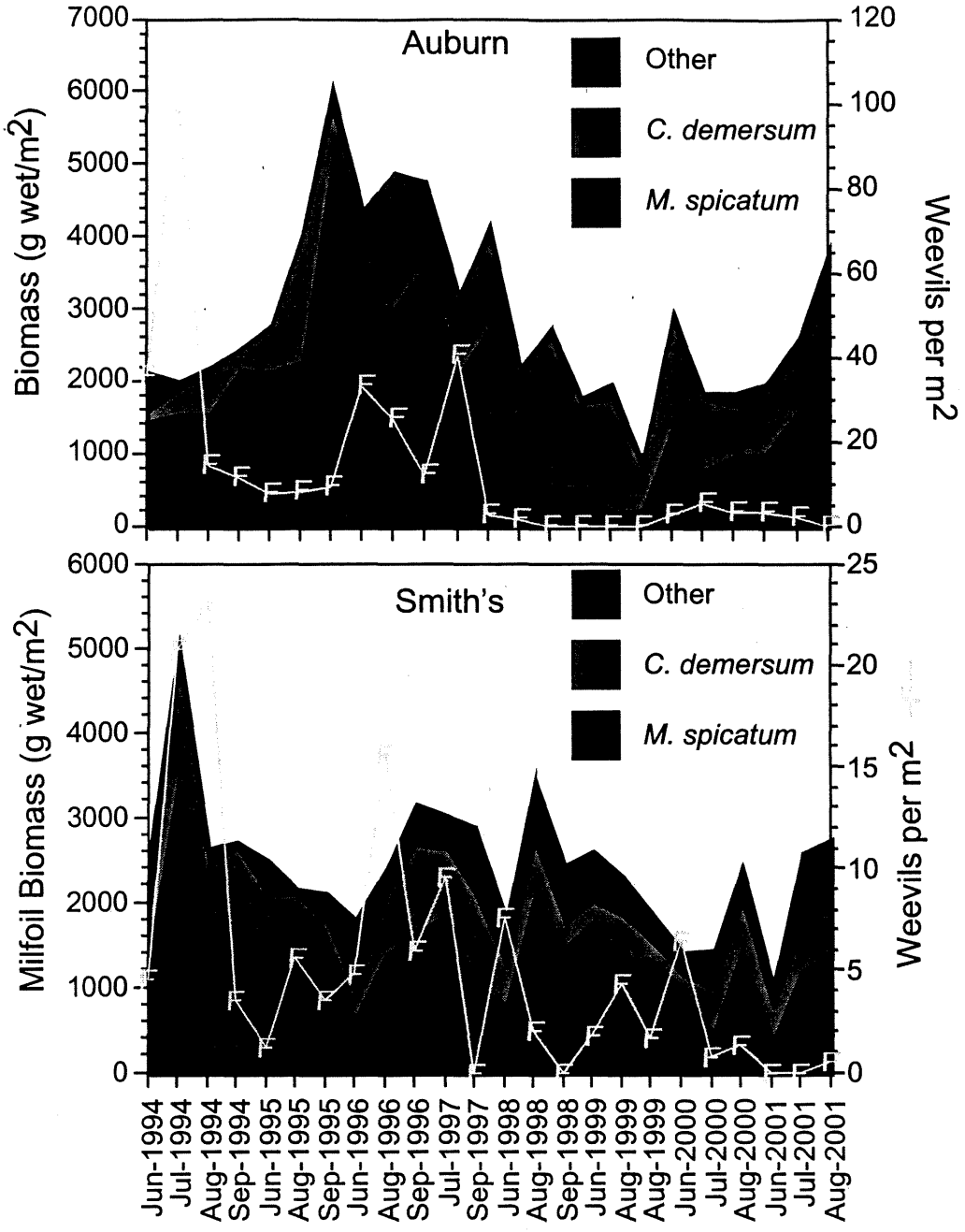

Fig. 6a. Trends in plant and weevil density from 1994-2001 in Lake Auburn and Smith's Bay.

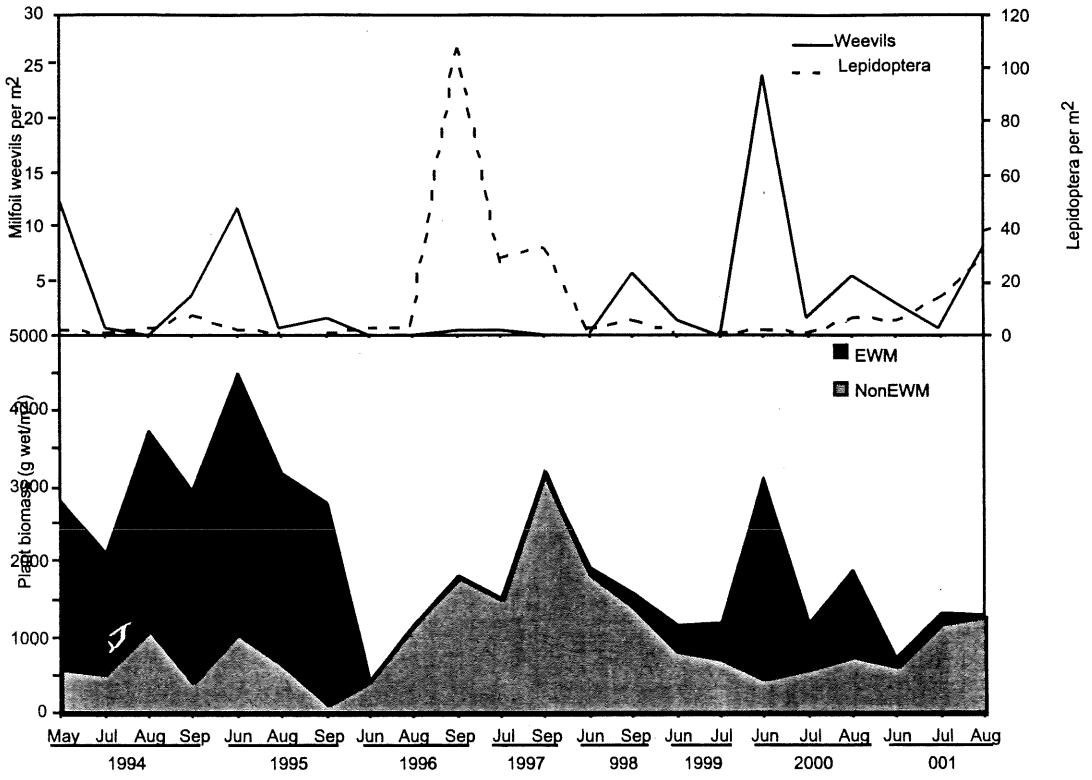

Fig. 6b . Herbivorous invertebrate density (top) and plant density (bottom) in Otter Lake from 1994-2001.

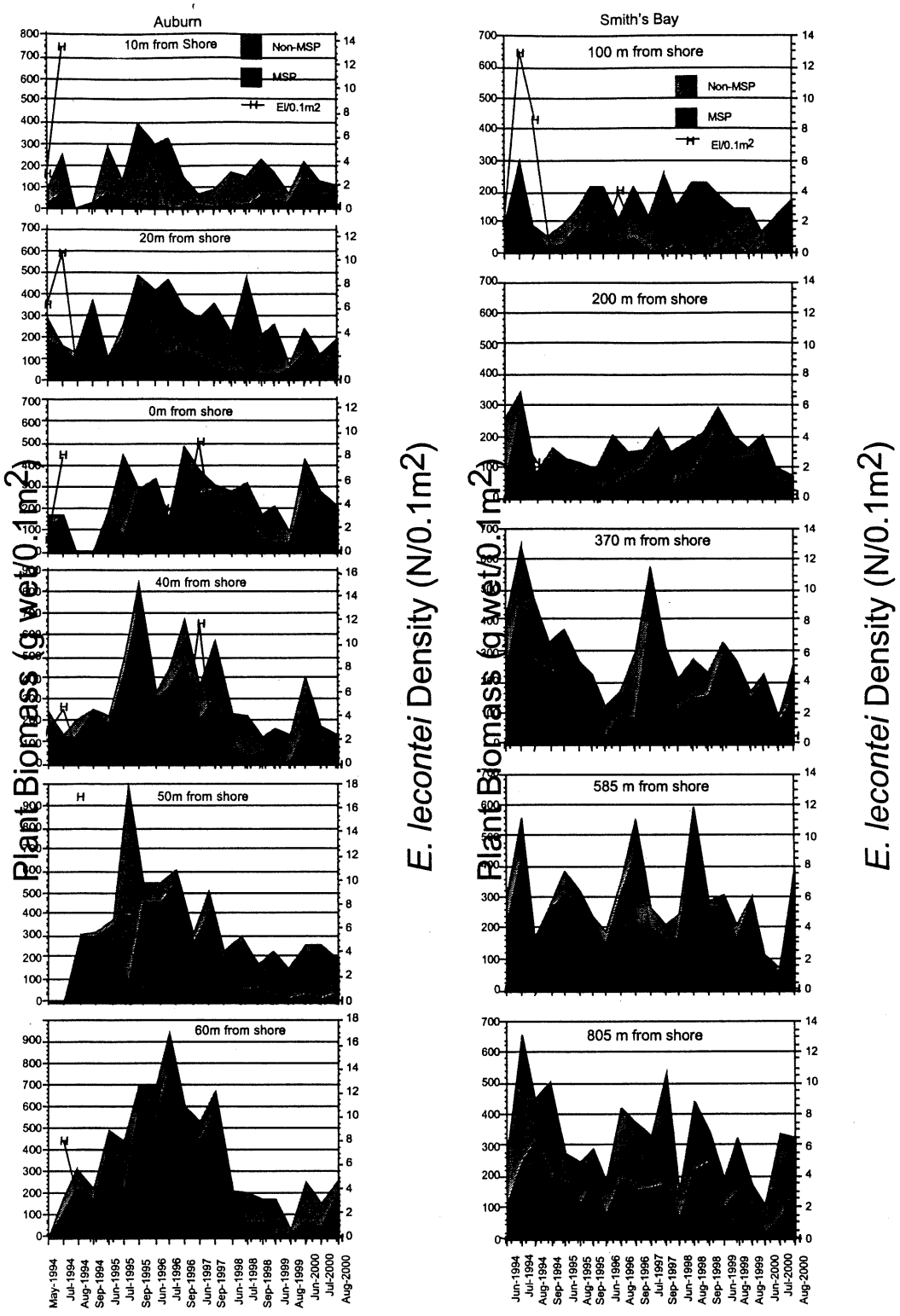

Fig. 7. Distribution of weevil, milfoil and other plant abundance by depth or distance from shore at Lake Auburn and Smith's Bay. Densities are per 0.1 m^2. Gaps in 1994 are missing values.

Table 10. Distribution of *E. lecontei* (mean N/m^2 and N/Stem at each station) along sample transects for Lake Auburn and Smith's Bay, Lake Minnetonka. Distances are from shore and represent sampling stations and the mean distance is an estimate of the weighted distance from shore. It was estimated by $[\Sigma(N_D * D)]/N_T$ where D = distance, $N=N/m^2$ or N/stem and T=total. There were too few weevils at Cedar and Otter Lakes (prior to 2000) to provide a useful comparison.

Auburn Date	Dist (m)	10	20	30	40	50	60	Mean N	Mean Dist (m)
5/24/94	N/m^2	30.0	63.3	10.0	26.7	0.0	0.0	35.6	22.6
	N/stem	0.43	0.18	0.04	0.11	.	.	0.15	17.7
7/8/94	N/m^2	135.0	106.7	80.0	46.7	170.0	80.0	102.5	34.0
	N/stem	0.60	0.37	0.18	0.20	0.37	0.33	0.33	31.8
8/18/94	N/m^2	.	6.0	.	6.0	.	30.0	14.0	51.4
	N/stem	.	0.02	.	0.04	.	0.08	0.04	49.6
9/20/94	N/m^2	0.0	32.0	.	10.0	.	0.0	11.7	24.8
	N/stem	0.00	0.06	.	0.02	.	0.00	0.02	24.8
6/7/95	N/m^2	0.0	4.0	26.0	10.0	6.0	0.0	7.7	33.9
	N/stem	0.00	0.08	0.30	0.09	0.00	0.00	0.08	30.3
7/31/95	N/m^2	.	18.0	.	4.0	.	2.0	8.0	26.7
	N/stem	.	0.10	.	0.02	.	0.00	0.04	24.6
9/26/95	N/m^2	0.0	6.0	0.0	15.0		12.0	9.4	43.6
	N/stem	.	0.26	0.00	0.69	.	0.04	0.29	35.7
6/13/96	N/m^2	40.0	46.0	46.0	42.0	12.0	12.0	33.0	28.8
	N/stem	6.33	0.29	0.12	0.10	0.04	0.04	0.81	11.8
7/31/96	N/m^2	0.0	67.5	36.7	27.1	20.0	16.0	25.6	32.8
	N/stem	0.00	0.19	0.29	0.12	0.07	0.03	0.10	32.0
9/12/96	N/m^2	0.0	12.0	24.0	22.0	6.0	8.0	12.0	36.4
	N/stem	0.00	0.15	0.14	0.06	0.01	0.02	0.07	29.9
6/27/97	N/m^2	0.0	14.0	92.0	118.0	12.0	6.0	40.3	36.0
	N/stem	0.00	0.05	0.68	0.44	0.04	0.02	0.22	34.3
9/8/97	N/m^2	0.0	0.0	8.0	0.0	2.0	2.0	2.0	38.3
	N/stem	0.00	0.00	0.04	0.00	0.00	0.00	0.01	33.6
6/8/98	N/m^2	0.0	0.0	0.0	4.0	2.0	2.0	1.3	47.5
6/8/98	N/stem	0.00	0.00	0.00	0.02	0.01	0.01	0.01	48.0
7/27/98	N/m^2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	.
	N/stem	0.00	0.00	0.00	0.00	0.00	0.00	0.00	.
9/8/98	N/m^2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	.
	N/stem	0.00	0.00	0.00	0.00	0.00	0.00	0.00	.
6/22/99	N/m^2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	.
	N/stem	0.00	0.00	0.00	0.00	0.00	0.00	0.00	.
8/23/99	N/m^2l	0.0	0.0	0.0	0.0	0.0	0.0	0.0	.
	N/stem	0.00	0.00	0.00	0.00	0.00	0.00	0.00	.
6/15/00	N/m^2	0.2	0.5	0.5	0.2	0.0	0.0	2.8	25.0
	N/stem	0.01	0.02	0.01	0.01	0.00	0.00	0.01	22.1
7/17/00	N/m^2	0.0	1.3	2.5	0.0	0.0	0.0	5.4	26.5
	N/stem	0.00	0.08	0.15	0.00	0.00	0.00	0.04	26.4
8/24/00	N/m^2	1.2	0.4	0.2	0.0	0.0	0.0	3.2	14.4
	N/stem	0.12	0.02	0.05	0.00	0.00	0.00	0.04	16.3

Table 10 continued

Smith's Bay

Date	Dist (m)	100	200	370	585	805	Mean N	Mean Dist (m)
6/3/94	N/m^2	20.0	0.0	0.0	0.0	0.0	4.6	100
	N/stem	0.15	0.00	0.00	0.00	0.00	0.02	100
7/11/94	N/m^2	130.0	40.0	5.0	0.0	0.0	20.8	131
	N/stem	0.87	0.17	0.01	0.00	0.00	0.11	118
8/19/94	N/m^2	86.7	23.3	13.3	0.0	0.0	8.5	148
	N/stem	0.44	0.13	0.09	0.00	0.00	0.13	158
9/21/94	N/m^2	3.3	15.0	3.3	0.0	0.0	3.6	214
	N/stem	0.05	0.03	0.01	0.00	0.00	0.02	158
6/27/95	N/m^2	2.0	0.0	2.0	2.0	0.0	1.2	352
	N/stem	0.07	0.00	0.01	0.02	0.00	0.03	226
8/15/95	N/m^2	4.0	18.0	6.0	0.0	0.0	5.6	222
	N/stem	0.08	0.23	0.00	0.00	0.00	0.09	173
9/18/95	N/m^2	16.0	0.0	0.0	2.0	0.0	3.6	154
	N/stem	0.20	0.00	.	0.03	0.00	0.05	166
6/24/96	N/m^2	8.0	16.0	0.0	0.0	0.0	4.8	167
	N/stem	0.06	0.11	0.00	0.00	0.00	0.04	164
8/9/96	N/m^2	40.0	24.0	4.0	10.0	0.0	15.6	207
	N/stem	0.41	0.15	0.03	0.03	0.00	0.13	158
9/19/96	N/m^2	14.0	12.0	0.0	4.0	0.0	6.0	205
	N/stem	0.07	0.07	0.00	0.01	0.00	0.03	189
7/14/97	N/m^2	8.0	16.0	20.0	4.0	0.0	9.6	286
	N/stem	0.50	0.08	0.10	0.02	0.00	0.09	164
9/18/97	N/m^2	0.0	0.0	0.0	0.0	0.0	0.0	.
	N/stem	0.00	0.00	0.00	0.00	0.00	0.00	.
6/15/98	N/m^2	0.0	34.0	4.0	0.0	0.0	7.6	218
6/15/98	N/stem	0.00	0.21	0.02	0.00	0.00	0.05	214
8/3/98	N/m^2	2.0	8.0	0.0	0.0	0.0	2.0	180
8/3/98	N/stem	0.00	0.08	0.00	0.00	0.00	0.02	200
9/15/98	N/m^2	0.0	0.0	0.0	0.0	0.0	0.0	.
9/15/98	N/stem	0.00	0.00	0.00	0.00	0.00	0.00	.
6/16/99	N/m^2	5.0	2.0	0.0	0.0	2.5	1.8	307
	N/stem	0.67	0.00	0.00	0.00	0.01	0.09	110
8/3/99	N/m^2	12.0	4.0	2.0	4.0	0.0	4.4	231
	N/stem	0.00	0.01	0.00	0.05	0.00	0.02	503
8/25/99	N/m^2	0.0	8.0	0.0	0.0	0.0	1.7	200
	N/stem	0.00	0.05	0.00	0.00	0.00	0.01	200
6/20/00	N/m^2	10.0	20.0	0.0	0.0	0.0	6.4	167
	N/stem	0.07	0.14	0.00	0.00	0.00	0.04	167
7/19/00	N/m^2	0.0	4.0	0.0	0.0	0.0	0.8	200
	N/stem	0.00	0.03	0.00	0.00	0.00	0.01	200
8/23/00	N/m^2	0.0	2.0	4.0	0.0	0.0	1.3	313
	N/stem	0.00	0.01	0.03	0.00	0.00	0.01	313

Survey Sites:

Trends in milfoil biomass varied among sites. Milfoil at Gray's Bay declined from high levels in 1998 to 14g dry/m^2 in 2001. At Shady Island and Lake-of-the-Isles, milfoil biomass peaked in 2000 and decreased in 2001 (Table 11). At Lake Harriet, milfoil fluctuated between 150 and 250 g dry/m^2.

Table 11. Total plant and milfoil biomass (g dry/m^2) and mean percent of plant biomass that was Eurasian watermilfoil at three survey sites in summer 1995-2001. N ≥ 9 samples at all sites. Results for 2 additional sites sampled in 1999-2001 are also presented.

Lake	Date	Total Plant Biomass (g/m^2)	Milfoil Biomass (g/m^2)	% Milfoil (of biomass)	Secchi Depth (m)
Gray's Bay	8/30/95	209.4	194.0	94.0%	2.0
	SE	55.3	53.2	3.8%	
	9/4/96	309.0	49.5	30.9%	1.9
	SE	132.1	21.1	12.7%	
	8/15/97	323.7	99.7	37.3%	3.5
	SE	43.0	29.6	10.6%	
	8/25/98	420.0	294.3	58.5%	2.3
	SE	61.8	40.8	6.9%	
	8/12/99	270.0	117.0	27.2%	3.1
	SE	67.0	37.0	6.7%	
	7/27/00	359.6	103.2	33.0%	2.5
	1 SE	43.6	22.8	7.1%	
	8/6/01	179.6	14.2	14.5%	2.6
	1 SE	49.7	2.5	2.9%	
Shady Island	9/12/95	259.8	215.1	83.6%	1.8
	SE	42.8	37.3	4.8%	
	9/4/96	262.2	158.6	70.5%	2.3
	SE	45.5	30.6	10.8%	
	8/28/97	432.9	175.6	47.4%	2.4
	SE	45.8	47.5	12.5%	
	8/27/98	339.6	139.2	42.6%	1.9
	1 SE	59.4	57.7	15.2%	
	8/6/99	100.4	40.3	41.1%	2.2
	1SE	28.0	19.0	14.2%	
	8/2/00	383.3	201.0	54.6%	2.2
	1 SE	64.5	71.7	17.1%	
	8/6/01	148.1	86.8	39.3%	2.4
		41.2	37.9	13.0%	
Lake of the Isles	9/14/95	62.5	58.3	90.1%	0.5
	SE	20.6	22.6	5.0%	
	8/30/96	199.7	169.2	74.6%	1.1
	SE	74.0	74.1	10.1%	
	8/14/97	31.9	9.9	22.4%	1.4
	SE	10.4	5.3	8.6%	
	8/31/98	28.2	14.0	36.9%	0.3
	1 SE	4.7	6.1	12.2%	
	8/16/99	51.8	49.3	88.3%	0.5
	1SE	14.8	14.5	4.4%	
	6/28/00	265.4	252.9	88.9%	2.3
	1 SE	45.6	46.9	3.7%	
	8/16/00	195.4	192.7	97.7%	2.2
	1 SE	17.6	17.8	1.1%	
	6/27/01	22.0	4.5	30.0%	1.6
	1 SE	7.1	1.8	8.2%	
	9/7/01	16.0	3.0	18.6%	0.8
	1 SE	8.9	2.2	7.9%	

Table 11. Continued.

Lake	Date	Total Plant Biomass (g/m^2)	Milfoil Biomass (g/m^2)	% Milfoil (of biomass)	Secchi Depth (m)
Calhoun	9/16/99	41.6	8.1	10.8%	1.6
	1 SE	10.7	3.9	5.5%	
	6/26/00	22.7	10.8	38.3%	3.1
	1 SE	11.3	5.6	13.5%	
	8/18/00	12.5	10.9	56.5%	1.8
	1 SE	4.0	4.1	10.0%	
	6/28/01	99.8	98.1	81.0%	3.2
	1 SE	24.9	25.0	7.1%	
	9/6/01	142.1	121.9	73.3%	2.3
	1 SE	30.5	31.3	8.4%	
Harriet	9/23/99	180.2	168.3	87.9%	2.6
	1 SE	27.6	26.8	5.2%	
	6/30/00	332.1	215.0	61.5%	1.6
	1 SE	53.2	37.8	5.7%	
	8/22/00	106.0	90.7	78.0%	2.3
	1 SE	18.9	19.5	5.9%	
	7/2/01	311.1	259.4	74.1%	2.5
	1 SE	46.4	45.9	6.9%	
	9/12/01	170.5	149.6	83.7%	3.0
	1 SE	25.7	23.6	5.3%	

The decrease of plants at Lake-of-the-Isles in 2001 was associated with a decrease in water clarity (Table 13) which resulted in low total plant biomass; however, Eurasian watermilfoil appeared more affected and decreased to <20% of the total population. The decline of all plants including milfoil in Calhoun during 1999-2000 and the subsequent recovery in 2001 does not appear to be solely due to changes in water clarity (Table 12) and its cause remains unexplained. Although all plants increased in 2001 most of the increase in plant biomass from 1999 to 2001 was due to increases in milfoil which increased to over 70% of plant biomass. Causes for changes at all but Lake-of-the-Isles were not apparent.

Table 12. Water column characteristics of Lakes Calhoun and Harriet.

Lake/Date		Chl-a (mg/m^3)	SD (m)	Temp (°C 1m)	10% PAR Depth (m)	Milfoil Limit (m)	Plant Limit (m)
Calhoun	9/24/97	7.2	3.1	18.9	2.5-3.0	4.7	4.7
	9/4/98	3.7	3.0	23.7	3.5-4.0	4.1	4.1
	9/21/99	17.1	1.6	18.5	2.0	2.6	3.8
	6/26/00	4.3	3.1	21.4	3.5-4.0		
	8/18/00	8.6	1.8	24.3	3.5-4.0	2.0	2.4
	6/28/01	19.8	3.2	26.1	3.5		
	9/6/01	3.5	2.3	22.9	5.0		
Harriet	10/9/97	4.5	> 5.4	17.3	3.0-3.5	5.2	5.2
	9/23/98	3.7	2.6	20.3	4.0-4.5	5.0	5.0
	9/24/99	7.5	2.6	17.5	3.5	4.0	4.0
	6/30/00	6.1	1.6	22.8	2.5-3.0		
	8/22/00	8.3	2.3	23.1	3.5-4.0	4.1	4.2
	7/2/01	9.1	2.5	23.4	2.5-3.0		
	9/12/01	4.0	3.6	21.5	4.5-5.0		

Table 13. Sediment characteristics (bulk density, percent organic matter, sediment pore water ammonium concentrations) and water column characteristics in 1995-2001 at the three survey sites. Three sediment samples from the intermediate depth were collected at each site (except 9 at Isles in 2000-2001).

Lake/Date	Bulk Dens. (g dm/ml)	NH_4 (mg/L)	% Organic	Chl-a (mg/m^3)	SD (m)	Temp (°C 1m)	10% PAR Depth (m)	Plant Limit (m)
Grays Bay								
8/30/95	0.10	6.75	34.1	6.1	2.0	25.2	3.0-3.5	3.5
2se	0.04	3.39	4.3					
9/4/96	0.12	3.29	21.3	2.1	1.9	26.2	3.0-3.5	3.5
2se	0.04	1.82	1.0					
8/15/97	0.10	4.90	35.4	3.5	3.5	22.6	4.0-4.5	4.1
2se	0.05	3.19	4.9					
8/25/98	0.10	29.13	33.7	3.5	2.3	25.1	3.0-3.5	3.3
2se	0.02	7.08	6.7					
8/12/99	0.07	10.96	27.6	4.3	3.1	25.0	4.0	4.5
2se	0.01	6.24	3.9					
7/27/00	0.10	10.05	27.2	5.1	2.5	24.7	2.5-3.0	5.4
2se	0.03	0.86	6.1					
8/6/01	0.11	5.97	26.3	5.1	2.6	28.1	3.5-4.0	
2se	0.01	2.22	4.6					
Shady Island								
9/12/95	0.14	3.74	23.9	8.8	1.8	21.0	2.0-2.5	4.5
2se	0.05	3.12	2.8					
9/4/96	0.42	1.44	10.1	7.5	2.3	25.1	3.0-3.5	3.5
2se	0.41	0.48	9.0					
8/28/97	0.09	4.49	27.2	2.4	2.4	23.9	3.0-3.5	4.7
2se	0.77	1.87	16.8					
8/27/98	0.69	10.93	10.8	5.9	1.9	24.6	3.0-3.5	4.4
2se	0.93	8.71	10.7					
8/6/99	0.20	6.64	14.3	5.6	2.2	25.8	3.0-3.5	4.4
2se	0.13	2.65	2.3					
8/4/00	0.23	0.67	15.8	4.5	2.2	25.3	2.5-3.0	4.9
2se	0.09	0.38	6.0					
8/6/01	0.17	2.05	20.2	4.5	2.4	28.7	2.5-3.0	
2se	0.04	1.05	4.0					
Lake of the Isles								
9/14/95	1.45	5.21	1.8	57.4	0.5	20.3	0.5-1.0	0.5
2se	0.36	4.36	1.1					
8/30/96	0.28	9.30	10.0	6.9	1.1	24.6	1.5-2.0	2.0
2se	0.08	5.32	6.7					
8/13/97	0.71	8.48	16.2	26.2	1.4	22.5	1.0-1.5	3.7
2se	0.58	0.88	20.0					
8/31/98	0.25	29.33	23.9	54.3	0.3	24.3	0.5-1.0	3.3
2se	0.28	19.07	19.0					
8/16/99	0.15	0.54	24.2	83.7	0.5	22.5	0.5-1.0	3.0
2se	0.05	0.56	12.5					
6/28/00	0.72	0.57	41.1	8.8	2.3	22.9	1.5-2.0	
2se	0.87	0.23	13.3					
8/16/00	0.51	1.13	26.1	15.8	2.2	25.7	2.5-3.0	4.0
2se	0.39	1.09	12.8					
6/29/01	0.47	0.57	34.0	49.5	1.6	26.3	2.0-2.5	
2se	0.48	0.23	15.3					
9/7/01	0.51	1.13	26.0	42.8	0.8	23.5	1.0-1.5	
2se	0.39	1.09	12.8					

Plant coverage and occurrence (Table 14) showed trends similar to biomass. Milfoil, and to an extent other plant species, increased in Lake Calhoun in 2000 and 2001 although density and occurrence was still much less than in 1998. Very little weevil damage was noted. Cedar Lake remained dominated by milfoil, but density was lower in 2001 than in 1999 and 2000. Although 8 species were noted, only milfoil, coontail and *Nymphaea* were common. Milfoil continued to dominate Lake Harriet in 2000-2001, but not as extensively as in 1997-1999. The increasing biomass and coverage from 1998-2000 and the decrease in coverage in 2001 at Lake-of-the-Isles was apparent, as was the effect of poor water clarity. With good clarity in 2000 (Secchi disk > 2m) milfoil covered over 50% of the lake but in 2001, milfoil was estimated at 4% coverage and only was visually detected at 7% of stations; however, it was found at 25% of sites. Coontail was more abundant, probably because it was better able to tolerate the poor light conditions. As with Calhoun and Harriet, very little weevil damage was noted in any year.

Milfoil coverage continued to decrease at Gray's Bay, similar to the decline in biomass. The number of native plants species remained high and the plant community was mixed. It is not clear what is perpetuating the diverse community and less dominance by milfoil at Gray's Bay. Although weevils and other herbivores were present, they appear to be at very low density and the weevil damage rating in 2001 was only 0.1. Shady Island also retained a diverse community and, coincident with the decrease in biomass, milfoil coverage declined from 2000 (Table 14). More weevil damage was seen at Shady Island, but the damage rating was still quite low (0.2) and milfoil remains the co-dominant species. At Gray's Bay and Shady Island competition with native plants appears to be keeping the milfoil from dominating the system, yet the milfoil remains at much higher densities than at sites where declines clearly associated with herbivores have occurred.

Finally, it should be noted that we expected that alum treatments in the Minneapolis Chain-of-Lakes would eventually enhance native plant communities. Although we predicted that Eurasian watermilfoil would initially be enhanced by better water clarity, we expected than better water clarity would favor the native plants after several years, reducing the competitive advantage Eurasian watermilfoil appears to have in lower light environments. To date we have no indication that alum treatments have enhanced the native plant communities. Eurasian watermilfoil remains dominant in Cedar Lake, 5 years after treatment in 1996. The number of plant species remains low and the better clarity appears to have reduced seasonal fluctuations in milfoil biomass. Eurasian watermilfoil also remains dominant in Harriet and Calhoun, although the alum treatments are likely too recent to have resulted in a longer term shift in plant community composition.

Table 14. Estimates of plant coverage and occurrence for the whole-lake surveys (Calhoun, Cedar, Gray's, Harriet, Isles and Shady Island). Estimates of visual milfoil cover (% Vis MSP Cov), percent visual occurrence, occurrence on the drop hook and mean weevil damage rating (0-5) for the whole lake estimates were based on n = 66-82 stations at each lake. Jessen and Lound (1962) relative density ratings (0-5) were determined from a subset of 5-6 transects (n=24-29 stations). Relative density is the mean for all stations sampled. Species abbreviations are given in Appendix I.

Lake Calhoun		% Vis MSP Cov	% Occurrence (Visual)		% Occurrence (Drop Hook)		Density Rating	n = 27
Date	n	Mean ± 1SE	Spp.	% Occ. ± 1 SD	Spp.	% Occ. ± 1 SD	Spp.	Density ± 2SE
9/4/98	63	30.7 ± 4.4%	MSP	87.3 ± 4.2%	MSP	76.2 ± 5.4%	MSP	3.67 ± 0.49
			PEC	17.5 ± 4.8%	CRT	50.8 ± 6.3%	CRT	3.07 ± 0.53
Eurasian Watermilfoil			PRI	14.3 ± 4.4%	PEC	12.7 ± 4.2%	PCR	0.48 ± 0.38
Total Area: 22.3 ha.			CRT	11.1 ± 4.0%	PRI	3.2 ± 2.2%	PEC	0.48 ± 0.43
% of Litt. Zone: 44.8%			PCR	7.9 ± 3.1%	PZS	1.6 ± 1.6%	PRI	0.41 ± 0.36
% of Lake Area: 13.7%			NAJ	6.3 ± 3.1%			NAJ	0.33 ± 0.34
Survey Criteria: Visible milfoil			ELD	1.6 ± 1.6%			ELD	0.04 ± 0.07
Weevil Damage Rating: 0.698±0.133			HET	1.6 ± 1.6%			HET	0.04 ± 0.07

Lake Calhoun		% Vis MSP Cov	% Occurrence (Visual)		% Occurrence (Drop Hook)		Density Rating	n = 25
Date	n	Mean ± 1SE	Spp.	% Occ. ± 1 SD	Spp.	% Occ. ± 1 SD	Spp.	Density ± 2SE.
9/16/99	74	45.0± 4.5%	MSP	87.3 ± 3.9%	MSP	76.2 ± 5.0%	MSP	1.84 ± 0.75
			PEC	17.5 ± 4.4%	CRT	50.8 ± 5.8%	CRT	3.32 ± 0.47
Eurasian Watermilfoil			PRI	14.3 ± 4.1%	PEC	12.7 ± 3.9%	PRI	0.20 ± 0.23
Total area			CRT	11.1 ± 3.7%	PRI	3.2 ± 2.0%		
% of Litt. Zone:			PCR	7.9 ± 3.1%	PZS	1.6 ± 1.5%		
% Lake Area:			NAJ	6.3 ± 2.8%				
Surface area Criteria: Visible milfoil			ELD	1.6 ± 1.5%				
Weevil Damage Rating:			HET	1.6 ± 1.5%				

Lake Calhoun		% Vis MSP Cov	% Occurrence (Visual)		% Occurrence (Drop Hook		Density Rating	n = 26
Date	n	Mean ±1S.E.	Spp.	% Occ. ±1S.D.	Spp.	% Occ. ±1S.D.	Spp.	Density ±2S.E.
8/17/00	73	6.8±2.0%	MSP	26.0 ± 5.1%	MSP	24.7 ± 5.0%	MSP	1.62 ± 0.70
			PEC	1.4 ± 1.4%	CRT	11.0 ± 3.7%	PEC	0.04 ± 0.08
Eurasian Watermilfoil			PRI	2.7 ± 1.9%	NAJ	2.7 ± 1.9%	PZS	0.12 ± 0.17
Total area			NAJ	1.4 ± 1.4%	PRI	2.7 ± 1.9%	CRT	2.00 ± 0.63
% of Litt. Zone:			CHA	1.4 ± 1.4%	PZS	1.4 ± 1.4%	ELD	0.04 ± 0.08
% Lake Area:							PCR	0.38 ± 0.35
Surface area Criteria: Visible milfoil							NAJ	0.31 ± 0.29
Weevil Damage Rating:							PRI	0.12 ± 0.17
							HET	0.08 ± 0.15
							CHA	0.42 ± 0.32
							VAL	0.04 ± 0.08
							ZPA	0.15 ± 0.31

Lake Calhoun		% Vis MSP Cov	% Occurrence (Visual)		% Occurrence (Drop Hook		Density Rating	n = 26
Date	n	Mean ±1S.E.	Spp.	% Occ. ±1S.D.	Spp.	% Occ. ±1S.D.	Spp.	Density ±2S.E.
8/17/01	66	31.3 ± 4.9%	MSP	39.4 ± 6.0%	MSP	56.1 ± 6.1%	MSP	2.62 ± 0.62
			PEC	7.6 ± 3.3%	CRT	15.2 ± 4.4%	NAJ	0.54 ± 0.40
Eurasian Watermilfoil			CRT	3.0 ± 2.1%	PEC	7.6 ± 3.3%	CRT	0.46 ± 0.28
Total area			PCR	3.0 ± 2.1%	PRI	6.1 ± 2.9%	PRI	0.27 ± 0.38
% of Litt. Zone:			NAJ	1.5 ± 1.5%	NAJ	3.0 ± 2.1%	PCR	0.19 ± 0.19
% Lake Area:			PZS	1.5 ± 1.5%	PZS	3.0 ± 2.1%	PEC	0.15 ± 0.24
Surface area Criteria: Visible milfoil					PCR	1.5 ± 1.5%	PZS	0.15 ± 0.24
Weevil Damage Rating: 0.2					PFO	1.5 ± 1.5%	PPR	0.12 ± 0.23
							CHA	0.08 ± 0.11
							HET	0.04 ± 0.08
							PFO	0.04 ± 0.08

Table 14 Continued

Cedar Lake		% Vis MSP Cov	% Occurrence (Visual)	% Occurrence (Drop Hook)	Density Rating n = 26
Date	n	Mean ± 1S.E.	Spp.% Occ. ±1S.D.	Spp.% Occ. ±1S.D.	Spp.Density ± 2S.E.
9/27/99	75	50.1 ± 4.2%	MSP 78.7 ± 4.7%	MSP 90.7 ± 3.4%	MSP 3.96 ± 0.46
			NMP 13.3 ± 3.9%	CRT 25.3 ± 5.0%	CRT 1.50 ± 0.60
Eurasian Watermilfoil				NMP 6.7 ± 2.9%	NMP 0.12 ± 0.23
Total area					PRI 0.04 ± 0.08
% of Litt. Zone:					DRC 0.04 ± 0.08

Cedar Lake	n	Mean ± 1S.E.	Spp.% Occ. ±1S.D.	Spp.% Occ. ±1S.D.	Spp.Density ± 2S.E.
8/9/00	72	44.3 ±4.7%	MSP 68.1 ± 5.5%	MSP 87.5 ± 3.9%	MSP 3.58 ± 0.61
			CRT 9.7 ± 3.5%	CRT 23.6 ± 5.0%	CRT 1.29 ± 0.53
Eurasian Watermilfoil			NMP 15.3 ± 4.2%	NAJ 1.4 ± 1.4%	NMP 0.38 ± 0.38
Total area			PAM 1.4 ± 1.4%	NMP 6.9 ± 3.0%	NAJ 0.08 ± 0.17
% of Litt. Zone:			PEC 1.4 ± 1.4%	PAM 1.4 ± 1.4%	CHA 0.04 ± 0.08
% Lake Area:				PCR 1.4 ± 1.4%	
Surface area Criteria: Visible milfoil				CHA 1.4 ± 1.4%	
Weevil Damage Rating:					

Cedar Lake		% Vis MSP Cov	% Occurrence (Visual)	% Occurrence (Drop Hook)	Density Rating n = 24
Date	n	Mean 1S.E.	Spp.% Occ. 1S.D.	Spp.% Occ. 1S.D.	Spp.Density 2S.E.
8/21/01	75	36.3 ± 4.2%	MSP 66.7 ± 5.4%	MSP 81.3 ± 4.5%	MSP 2.83 ± 0.71
			NMP 16.0 ± 4.2%	CRT 34.7 ± 5.5%	CRT 0.71 ± 0.52
Eurasian Watermilfoil			CRT 9.3 ± 3.4%	NMP 5.3 ± 2.6%	NMP 0.08 ± 0.17
Total area			PEC 1.3 ± 1.3%	CHA 1.3 ± 1.3%	
% of Litt. Zone:			PRI 1.3 ± 1.3%	PEC 1.3 ± 1.3%	
% Lake Area:			PZS 1.3 ± 1.3%	PRI 1.3 ± 1.3%	
Surface area Criteria: Visible milfoil					
Weevil Damage Rating: 0.24					

Lake Harriet		% Vis MSP Cov	% Occurrence (Visual)	% Occurrence (Drop Hook)	Density Rating n = 29
Date	n	Mean ± 1 S.E.	Spp. % Occ. ± 1 S.D.	Spp. % Occ. ± 1 S.D.	Spp. Density ± 2S.E.
10/9/97	72	52.2 ± 3.8%	MSP 87.5 ± 3.9%	MSP 86.1 ± 4.1%	MSP 4.41 ± 0.36
			CRT 8.3 ± 3.3%	CRT 40.3 ± 5.8%	CRT 2.21 ± 0.49
Eurasian Watermilfoil:			HET 1.4 ± 1.4%	PRI 1.4 ± 1.4%	PRI 0.17 ± 0.14
Total Area: 28.6 ha.			PRI 1.4 ± 1.4%	PZS 1.4 ± 1.4%	ELD 0.03 ± 0.07
% of Litt. Zone: 83.2%					NAJ 0.03 ± 0.07
% of Lake Area: 21.1%					PEC 0.03 0.07
Survey Criteria: Visible milfoil					
Weevil Damage rating 0.507±0.072					

Lake Harriet		% Vis MSP Cov	% Occurrence (Visual)	% Occurrence (Drop Hook)	Density Rating n = 27
Date	n	Mean ± 1SE	Spp. % Occ. ± 1 SD	Spp. % Occ. ± 1 SD	Spp. Density ± 2SE
9/23/98	73	59.2 ± 4.2%	MSP 84.9 ± 4.2%	MSP 82. ± 4.5%	MSP 3.81 ± 0.68
			CRT 8.2 ± 3.2%	CRT 39.7 ± 5.7%	CRT 2.07 ± 0.55
Eurasian Watermilfoil			PRI 6.8 ± 3.0%	PRI 6.8 ± 3.0%	PRI 0.26 ± 0.31
Total Area: 23.1 ha.			NAJ 1.4 ± 1.4%	NAJ 5.7 ± 2.7%	PZS 0.19 ± 0.26
% of Litt. Zone: 67.2%			PZS 1.4 ± 1.4%	PEC 1.4 ± 1.4%	NAJ 0.15 ± 0.18
% of Lake Area: 17.1%				PZS 1.4 ± 1.4%	PEC 0.07 ± 0.10
Survey Criteria: Visible milfoil					HET 0.04 ± 0.07
Weevil Damage Rating: 0.493±0.088					

Lake Harriet		% Vis MSP Cov	% Occurrence (Visual)	% Occurrence (Drop Hook)	Density Rating n = 29
Date	n	Mean ±1S.E.	Spp. % Occ. ± 1S.D.	Spp. ± % Occ. ±S.D.	Spp. Density ±2S.E.
9/24/99	71	71.9 ±2.8%	MSP 79.2 ± 4.8%	MSP 93.1 ± 3.0%	MSP 3.86 ± 0.44
			CRT 11.1 ± 3.7%	CRT 59.7 ± 5.8%	PZS 0.03 ± 0.07
Eurasian Watermilfoil					CRT 3.14 ± 0.46
Total area					

Table 14 Continued

Lake Harriet		% Vis MSP Cov	% Occurrence (Visual)		% Occurrence (Drop Hook)		Density Rating	n = 25
Date	n	Mean ±1S.E.	Spp.	% Occ. ±1S.D.	Spp.	% Occ. ±1S.D.	Spp.	Density ±2S.E.
8/21/00	66	36.8 ±4.2%	MSP	71.2 ± 5.6%	MSP	74.2 ± 5.4%	MSP	3.56 ± 0.54
			CRT	24.2 ± 5.3%	CRT	62.1 ± 6.0%	PEC	0.12 ± 0.13
Eurasian Watermilfoil			NAJ	1.5 ± 1.5%	NAJ	1.5 ± 1.5%	PZS	0.08 ± 0.16
Total area			PZS	3.0 ± 2.1%	PZS	1.5 ± 1.5%	CRT	3.20 ± 0.60
% of Litt. Zone:			PEC	3.0 ± 2.1%			NAJ	0.12 ± 0.24
% Lake Area:							PRI	0.04 ± 0.08
Surface area Criteria: Visible milfoil							CHA	0.04 ± 0.08

Lake Harriet		% Vis MSP Cov	% Occurrence (Visual)		% Occurrence (Drop Hook)		Density Rating	n = 20
Date	n	Mean ± 1SE	Spp.	% Occ. ± 1 SD	Spp.	% Occ. ± 1 SD	Spp.	Density ± 2SE
8/14/01	71	46.4 ±4.7%	MSP	54.9 ± 5.9%	MSP	81.7 ± 4.6%	MSP	3.65 ± 0.55
			CRT	14.1 ± 4.1%	CRT	60.6 ± 5.8%	CRT	3.05 ± 0.59
Eurasian Watermilfoil			HET	1.4 ± 1.4%	PRI	1.4 ± 1.4%	HET	0.10 ± 0.14
Total area			PEC	1.4 ± 1.4%			NAJ	0.05 ± 0.10
% of Litt. Zone:							PRI	0.05 ± 0.10
% Lake Area:							PZS	0.05 ± 0.10
Weevil Damage Rating: 0.01								

Lake of the Isles		% Vis MSP Cov	% Occurrence (Visual)		% Occurrence (Drop Hook)		Density Rating	n = 25
Date	n	Mean ± 1 S.E.	Spp.	% Occ. ± 1 S.D.	Spp.	% Occ. ± 1 S.D.	Spp.	Density ± 2S.E.
8/13/97	72	15.4 ± 3.5%	MSP	31.9 ± 5.5%	MSP	59.7 ± 5.8%	CRT	2.48 ± 0.37
			CRT	26.4 ± 5.2%	CRT	62.5 ± 5.7%	MSP	1.84 ± 0.53
Eurasian Watermilfoil:			PZS	1.4 ± 1.4%	NAJ	2.8 ± 1.9%	PZS	0.04 ± 0.08
Total Area: 14.3 ha.					PZS	2.8 ± 1.9%		
% of Litt. Zone: 39.7%								
% of Lake Area: 32.4%								
Survey Criteria: Visible milfoil								

Lake of the Isles		% Vis MSP Cov	% Occurrence (Visual)		% Occurrence (Drop Hook)		Density Rating	n = 26
Date	n	Mean ± 1SE	Spp.	% Occ. ± 1 SD	Spp.	% Occ. ± 1 SD	Spp.	Density ± 2SE
8/31/98	73	8.5 ± 2.0%	MSP	28.8 ± 5.3%	MSP	56.2 ± 5.8%	CRT	2.85 ± 0.60
			CRT	15.1 ± 4.2%	CRT	39.7 ± 5.7%	MSP	2.81 ± 0.69
Eurasian Watermilfoil					CHC	2.7 ± 1.9%	NAJ	0.08 ± 0.15
Total Area: 36.0 ha.					NAJ	2.7 ± 1.9%	CHC	0.04 ± 0.08
% of Litt. Zone: 100.0%					PEC	1.4 ± 1.4%	PCR	0.04 ± 0.08
% of Lake Area: 49.6%							PEC	0.04 ± 0.08
Weevil Damage Rating: 1.411±0.320								

Lake of the Isles		% Vis MSP Cov	% Occurrence (Visual)		% Occurrence (Drop Hook)		Density Rating	n = 26
Date	n	Mean ±1S.E.	Spp.	% Occ. ±1S.D.	Spp.	% Occ. ±1S.D.	Spp.	Density ± 2S.E.
8/17/99	72	21.2 ± 2.8%	MSP	22.2 ± 4.9%	MSP	72.2 ± 5.3%	MSP	3.69 ± 0.57
			CRT	1.4 ± 1.4%	CRT	40.3 ± 5.8%	PEC	0.04 ± 0.08
Eurasian Watermilfoil							CRT	2.88 ± 0.52
Total area							NAJ	0.04 ± 0.08
% of Litt. Zone:							CHA	0.04 ± 0.08

Lake of the Isles		% Vis MSP Cov	% Occurrence (Visual)		% Occurrence (Drop Hook)		Density Rating	n = 26
Date	n	Mean ±1S.E.	Spp.	% Occ. ±1S.D.	Spp.	% Occ. ±1S.D.	Spp.	Density ± 2S.E.
8/14/00	82	50.7 ± 4.4%	MSP	82.2 ±14.2%	MSP	87.7 ±13.6%	MSP	3.73 ± 0.49
					CRT	24.7 ±14.8%	CRT	1.58 ± 0.58
Eurasian Watermilfoil							PCR	0.23 ± 0.26
Total area							NAJ	0.04 ± 0.08
% of Litt. Zone:							PRI	0.04 ± 0.08

Table 14 Continued

Lake of the Isles		% Vis MSP Cov	% Occurrence (Visual)			% Occurrence (Drop Hook)			Density Rating		n = 26
Date	n	Mean ±1S.E.	Spp.	% Occ.	±1S.D.	Spp.	% Occ.	±1S.D.	Spp.	Density ±	2S.E.
8/15/01	82	3.9% 1.4%	MSP	7.3	2.9%	MSP	25.6	4.8%	CRT	2.88	0.56
			CRT	7.3	2.9%	CRT	36.6	5.3%	MSP	1.65	0.68
Eurasian Watermilfoil						NAJ	1.2	1.2%	NAJ	0.08	0.15
Total area						PCR	1.2	1.2%	PCR	0.08	0.15
% of Litt. Zone:									PFO	0.04	0.08
% Lake Area:									PRI	0.04	0.08
Weevil Damage Rating: 0.15											

Gray's Bay		% Vis MSP Cov	% Occurrence (Visual)			% Occurrence (Drop Hook)			Density Rating		n = 37
Date	n	Mean ± 1 S.E.	Spp.	% Occ. ± 1 S.D.		Spp.	% Occ. ± 1 S.D.		Spp.	Density ±	2S.E.
8/15/97	97	17.6 ± 2.7%	MSP	54.1 ± 5.1%		MSP	49.0 ± 5.1%		MSP	1.92 ±	0.45
			CHA	1.0 ± 1.0%		CRT	42.9 ± 5.0%		NAJ	1.76 ±	0.41
Eurasian Watermilfoil			PAM	1.0 ± 1.0%		NAJ	38.8 ± 4.9%		CRT	1.59 ±	0.39
Total Area: 58.4 ha.			VAL	1.0 ± 1.0%		PRI	38.8 ± 4.9%		PRI	1.41 ±	0.43
% of Litt. Zone: 113.7%						PZS	25.5 ± 4.4%		PZS	0.92 ±	0.37
% of Lake Area: 82.5%						PEC	12.2 ± 3.3%		CHA	0.76 ±	0.41
Survey Criteria: Visible milfoil						PAM	11.2 ± 3.2%		PAM	0.46 ±	0.25
Weevil Damage rating 0.000±0.000						ELD	5.1 ± 2.2%		PEC	0.43 ±	0.24
						PFO	5.1 ± 2.2%		PFO	0.24 ±	0.18
						CHA	4.1 ± 2.0%		VAL	0.24 ±	0.20
						VAL	3.1 ± 1.7%		ELD	0.08 ±	0.09
						PCR	2.0 ± 1.4%		MSI	0.05 ±	0.08
									PCR	0.05 ±	0.08

Gray's Bay	n	Mean ± 1SE	Spp.	% Occ. ± 1 SD	Spp.	% Occ. ± 1 SD	Spp.	Density ± 2SE
8/25/98	87	24.8 ± 3.3%	MSP	60.9 ± 5.2%	MSP	58.6 ± 5.3%	MSP	2.73 ± 0.60
			PRI	41.4 ± 5.3%	NAJ	55.1 ± 5.3%	NAJ	2.13 ± 0.63
Eurasian Watermilfoil			PAM	20.7 ± 4.3%	CRT	49.4 ± 5.4%	CRT	2.07 ± 0.57
Total Area: 14.2 ha.			VAL	19.5 ± 4.3%	PRI	37.9 ± 5.2%	PRI	1.97 ± 0.58
% of Litt. Zone: 27.6%			NAJ	18.4 ± 4.2%	PZS	16.1 ± 3.9%	PZS	1.03 ± 0.49
% of Lake Area: 20.0%			PEC	10.3 ± 3.3%	PAM	11.5 ± 3.4%	PAM	0.63 ± 0.44
Survey Criteria: Visible milfoil			PFO	5.7 ± 2.5%	VAL	11.5 ± 3.4%	VAL	0.63 ± 0.41
Weevil Damage Rating: 0.195±0.067			CRT	4.6 ± 2.2%	PFO	9.2 ± 3.1%	ELD	0.53 ± 0.30
			PNA	3.4 ± 2.0%	CHA	6.9 ± 2.7%	MSI	0.27 ± 0.32
			MGD	2.3 ± 1.6%	ELD	5.7 ± 2.5%	PEC	0.27 ± 0.16
			PZS	2.3 ± 1.6%	HET	5.7 ± 2.5%	PFO	0.27 ± 0.25
			HET	1.1 ± 1.1%	PEC	3.4 ±2.0%	CHA	0.23 ± 0.28
			NMP	1.1 ± 1.1%	ALG	1.1 ± 1.1%	HET	0.20 ± 0.15
			PCR	1.1 ± 1.1%	MGD	1.1 ± 1.1%	MGD	0.13 ± 0.19
			PNO	1.1 ± 1.1%	MSI	1.1 ± 1.1%	LTR	0.03 ± 0.07
			RAN	1.1 ± 1.1%	RAN	1.1 ± 1.1%	PCR	0.03 ± 0.07
							RAN	0.03 ± 0.07

Gray's Bay		% Vis MSP Cov	% Occurrence (Visual)			% Occurrence (Drop Hook)			Density Rating		n = 31
Date	n	Mean ± 1SE	Spp.	% Occ. ± 1 SD		Spp.	% Occ. ± 1 SD		Spp.	Density ±	2SE
8/11/99	87	44.8±3.5%	MSP	60.9	5.2%	MSP	58.6	5.3%	MSP	3.84	0.57
			PRI	41.4	5.3%	NAJ	55.2	5.4%	PEC	0.23	0.18
Eurasian Watermilfoil			PAM	20.7	4.3%	CRT	49.4	5.4%	PZS	0.97	0.52
Total area			VAL	19.5	4.3%	PRI	37.9	5.2%	CRT	1.77	0.50
% of Litt. Zone:			NAJ	18.4	4.2%	PZS	16.1	4.0%	ELD	0.74	0.44
% Lake Area:			PEC	10.3	3.3%	PAM	11.5	3.4%	NMP	0.03	0.06
Surface area Criteria: Visible milfoil			PFO	5.7	2.5%	VAL	11.5	3.4%	NUP	0.10	0.19
Weevil Damage Rating:			CRT	4.6	2.2%	PFO	9.2	3.1%	PAM	0.19	0.27
			PNA	3.4	2.0%	CHA	6.9	2.7%	NAJ	1.32	0.54
			MGD	2.3	1.6%	ELD	5.7	2.5%	PRI	1.65	0.55
			PZS	2.3	1.6%	HET	5.7	2.5%	HET	0.03	0.06
			HET	1.1	1.1%	PEC	3.4	2.0%	MGD	0.03	0.06
			NMP	1.1	1.1%	ALG	1.1	1.1%	CHA	0.68	0.47
			PCR	1.1	1.1%	MGD	1.1	1.1%	VAL	0.58	0.36
			PNO	1.1	1.1%	MSI	1.1	1.1%	PNA	0.10	0.11
			RAN	1.1	1.1%	RAN	1.1	1.1%	AMP	0.13	0.15

Table 14 Continued

Gray's Bay		% Vis MSP Cov	% Occurrence (Visual)		% Occurrence (Drop Hook)		Density Rating	n = 26
Date	n	Mean ± 1SE	Spp.	% Occ. ± 1 SD	Spp.	% Occ. ± 1 SD	Spp.	Density ± 2SE
7/25/00	77	29.2 ±3.7%	MSP	74.0 5.0%	MSP	63.6 5.5%	CRT	2.92 0.58
			PRO	18.2 4.4%	CRT	61.0 5.6%	MSP	2.69 0.52
Eurasian Watermilfoil			PAM	16.9 4.3%	NAJ	51.9 5.7%	NAJ	2.23 0.79
Total area			CRT	15.6 4.1%	PRI	40.3 5.6%	PRI	2.04 0.62
% of Litt. Zone:			PRI	14.3 4.0%	PZS	32.5 5.3%	ELD	1.85 0.57
% Lake Area:			NAJ	9.1 3.3%	ELD	26.0 5.0%	PZS	1.69 0.51
Surface area Criteria: Visible milfoil			PZS	9.1 3.3%	PRO	16.9 4.3%	PRO	0.92 0.52
Weevil Damage Rating:			ELD	7.8 3.1%	CHA	13.0 3.8%	VAL	0.81 0.44
			PEC	7.8 3.1%	PAM	11.7 3.7%	PAM	0.69 0.47
			VAL	6.5 2.8%	HET	10.4 3.5%	CHA	0.65 0.51
			CHA	3.9 2.2%	VAL	7.8 3.1%	MGD	0.46 0.40
			MGD	2.6 1.8%	MSI	2.6 1.8%	RAN	0.23 0.20
			RAN	2.6 1.8%	MGD	1.3 1.3%	HET	0.19 0.25
			HET	1.3 1.3%	PEC	1.3 1.3%	MSI	0.12 0.17
			MSI	1.3 1.3%	RAN	1.3 1.3%	PEC	0.08 0.11
			NMP	1.3 1.3%	UTV	1.3 1.3%	NMP	0.04 0.08
			PGR	1.3 1.3%			PGR	0.04 0.08
							UTV	0.04 0.08

Gray's Bay		% Vis MSP Cov	% Occurrence (Visual)		% Occurrence (Drop Hook)		Density Rating	n = 26
Date	n	Mean ± 1SE	Spp.	% Occ. ± 1 SD	Spp.	% Occ. ± 1 SD	Spp.	Density ± 2SE
8/3/01	79	9.4% 1.9%	MSP	46.8 5.6%	MSP	30.4 5.2%	CRT	2.54 0.55
			PZS	38.0 5.5%	NAJ	46.8 5.6%	MSP	2.31 0.63
Eurasian Watermilfoil			PEC	26.6 5.0%	PZS	43.0 5.6%	NAJ	2.12 0.56
Total area			PAM	13.9 3.9%	CRT	32.9 5.3%	PZS	1.92 0.54
% of Litt. Zone:			CRT	11.4 3.6%	PEC	15.2 4.0%	PRI	1.15 0.52
% Lake Area:			VAL	11.4 3.6%	VAL	13.9 3.9%	ELD	0.96 0.39
Surface area Criteria: Visible milfoil			PRI	10.1 3.4%	CHA	11.4 3.6%	PEC	0.88 0.50
Weevil Damage Rating: 0.11			NAJ	8.9 3.2%	PRI	10.1 3.4%	VAL	0.88 0.49
			NMP	8.9 3.2%	PRO	7.6 3.0%	CHA	0.81 0.52
			PRO	5.1 2.5%	ELD	6.3 2.7%	PAM	0.69 0.44
			NUP	2.5 1.8%	PAM	6.3 2.7%	PRO	0.38 0.32
			CHA	1.3 1.3%	NMP	2.5 1.8%	PCR	0.31 0.33
			PGR	1.3 1.3%	LTR	1.3 1.3%	NUP	0.15 0.31
					MGD	1.3 1.3%	MGD	0.12 0.17
					NUP	1.3 1.3%	NMP	0.08 0.11
					PCR	1.3 1.3%	MSI	0.04 0.08

Shady Island		% Vis MSP Cov	% Occurrence (Visual)		% Occurrence (Drop Hook)		Density Rating	n = 15
Date	n	Mean ± 1 S.E.	Spp.	% Occ. ± 1 S.D.	Spp.	% Occ. ± 1 S.D.	Spp.	Density ± 2S.E.
8/29/97	50	9.3 ± 2.9%	MSP	34.0 ± 6.7%	MSP	46.0 ± 7.0%	NAJ	2.13 ± 0.58
			NAJ	16.0 ± 5.2%	CRT	38.0 ± 6.9%	CRT	1.27 ± 0.66
Eurasian Watermilfoil:			VAL	10.0 ± 4.2%	NAJ	30.0 ± 6.5%	UTV	1.20 ± 0.68
Total Area:		8.6 ha.	UTV	6.0 ± 3.4%	CHA	22.0 ± 5.9%	PZS	1.13 ± 0.70
% ofLitt. Zone:		45.0%	PRI	4.0 ± 2.8%	PRI	22.0 ± 5.9%	VAL	1.13 ± 0.64
% ofLake Area:		45.0%	PZS	4.0 ± 2.8%	PZS	20.0 ± 5.7%	CHA	1.07 ± 0.60
Survey criteria: Visible milfoil					VAL	10.0 ± 4.2%	MSP	1.07 ± 0.63
Weevil Damage rating 0.000±0.000					ELD	8.0 ± 3.8%	PRI	0.93 ± 0.57
					UTV	6.0 ± 3.4%	ELD	0.53 ± 0.43
					PEC	4.0 ± 2.8%	PEC	0.40 ± 0.38
					PFO	4.0 ± 2.8%	PAM	0.27 ± 0.31
					ALG	2.0 ± 2.0%	PFO	0.27 ± 0.24
					BRA	2.0 ± 2.0%	MSI	0.07 ± 0.13
					PAM	2.0 ± 2.0%		

Table 14 Continued

Shady Island		% Vis MSP Cov	% Occurrence (Visual)		% Occurrence (Drop Hook)		Density Rating	n = 15
Date	n	Mean ± 1SE	Spp.	% Occ. ± 1 SD	Spp.	% Occ. ± 1 SD	Spp.	Density ± 2SE
8/27/98	64	26.3 ± 4.3%	MSP	67.2 ± 5.9%	MSP	59.4 ± 6.1%	MSP	3.38 ± 0.65
			VAL	21.9 ± 5.2%	NAJ	45.3 ± 6.2%	CRT	2.08 ± 0.67
Eurasian Watermilfoil			NAJ	17.2 ± 4.7%	CRT	40.6 ± 6.1%	NAJ	1.63 ± 0.67
Total Area: 17.0 ha.			PRI	14.1 ± 4.3%	PZS	26.6 ± 5.5%	CHA	1.13 ± 0.56
% of Litt. Zone: 89.5%			CRT	9.4 ± 3.6%	VAL	17.2 ± 4.7%	PRI	0.67 ± 0.48
% of Lake Area: 89.5%			PAM	9.4 ± 3.6%	CHA	15.6 ± 4.5%	VAL	0.63 ± 0.46
Survey Criteria: Visible milfoil			PZS	9.4 ± 3.6%	MGD	12.5 ± 4.1%	PZS	0.46 ± 0.34
Weevil Damage Rating: 1.250±0.194			CHA	7.8 ± 3.4%	PRI	12.5 ± 4.1%	ELD	0.29 ± 0.22
			MGD	7.8 ± 3.4%	HET	7.8 ± 3.4%	MGD	0.25 ± 0.28
			NMP	6.3 ± 3.0%	PAM	6.3 ± 3.0%	PAM	0.25 ± 0.22
			NUP	4.7 ± 2.6%	ELD	4.7 ± 2.6%	HET	0.21 ± 0.29
			PEC	4.7 ± 2.6%	NMP	3.1 ± 2.2%	NUP	0.17 ± 0.33
			PNA	4.7 ± 2.6%	NUP	3.1 ± 2.2%	PPR	0.08 ± 0.17
			ELD	3.1 ± 2.2%	PEC	3.1 ± 2.2%	UTV	0.08 ± 0.12
			HET	1.6 ± 1.6%	PNA	1.6 ± 1.6%	RAN	0.04 ± 0.08
			PCR	1.6 ± 1.6%	RAN	1.6 ± 1.6%		
			PNO	1.6 ± 1.6%				
			SCR	1.6 ± 1.6%				

Shady Island		% Vis MSP Cov	% Occurrence (Visual)			% Occurrence (Drop Hook)			Density Rating	n = 23	
Date	n	Mean ± 1SE	Spp.	% Occ. ± 1 SD		Spp.	% Occ. ± 1 SD		Spp.	Density ± 2SE	
8/6/99	70	19.9 ±2.8%	MSP	67.2	5.6%	MSP	59.4	5.9%	MSP	2.96	0.75
			VAL	21.9	4.9%	NAJ	45.3	5.9%	PZS	1.13	0.58
Eurasian Watermilfoil			NAJ	17.2	4.5%	CRT	40.6	5.9%	CRT	2.39	0.70
Total area			PRI	14.1	4.2%	PZS	26.6	5.3%	ELD	0.13	0.14
% of Litt. Zone:			CRT	9.4	3.5%	VAL	17.2	4.5%	NMP	0.22	0.35
% Lake Area:			PAM	9.4	3.5%	CHA	15.6	4.3%	NUP	0.17	0.35
Surface area Criteria: Visible milfoil			PZS	9.4	3.5%	MGD	12.5	4.0%	PCR	0.17	0.20
Weevil Damage Rating:			CHA	7.8	3.2%	PRI	12.5	4.0%	PAM	0.30	0.43
			MGD	7.8	3.2%	HET	7.8	3.2%	NAJ	1.30	0.72
			NMP	6.3	2.9%	PAM	6.3	2.9%	PRI	0.35	0.27
			NUP	4.7	2.5%	ELD	4.7	2.5%	HET	0.22	0.18
			PEC	4.7	2.5%	NMP	3.1	2.1%	MGD	0.17	0.20
			PNA	4.7	2.5%	NUP	3.1	2.1%	CHA	0.70	0.44
			ELD	3.1	2.1%	PEC	3.1	2.1%	PGR	0.04	0.09
			HET	1.6	1.5%	PNA	1.6	1.5%	VAL	0.48	0.40
			PCR	1.6	1.5%	RAN	1.6	1.5%	JUN	0.04	0.09
			PNO	1.6	1.5%				UTV	0.17	0.20
			SCR	1.6	1.5%				PNA	0.09	0.17

Shady Island		% Vis MSP Cov	% Occurrence (Visual)			% Occurrence (Drop Hook)			Density Rating	n = 25	
Date	n	Mean ± 1SE	Spp.	% Occ. ± 1 SD		Spp.	% Occ. ± 1 SD		Spp.	Density ± 2SE	
7/31/00	73	25.4 ± 3.8%	MSP	68.5	5.4%	MSP	65.8	5.6%	MSP	3.16	0.71
			NAJ	27.4	5.2%	CRT	56.2	5.8%	CRT	2.32	0.66
Eurasian Watermilfoil			CRT	26.0	5.1%	NAJ	34.2	5.6%	NAJ	1.72	0.75
Total area			MGD	17.8	4.5%	PZS	30.1	5.4%	CHA	1.52	0.61
% of Litt. Zone:			PZS	16.4	4.3%	CHA	12.3	3.8%	PZS	1.16	0.56
% Lake Area:			CHA	15.1	4.2%	ELD	9.6	3.4%	MGD	0.72	0.48
Surface area Criteria: Visible milfoil			PAM	13.7	4.0%	MGD	8.2	3.2%	PRI	0.56	0.38
Weevil Damage Rating:			NMP	11.0	3.7%	VAL	6.8	3.0%	VAL	0.56	0.46
			VAL	11.0	3.7%	PRO	5.5	2.7%	ELD	0.40	0.28
			NUP	9.6	3.4%	HET	4.1	2.3%	PRO	0.32	0.44
			PEC	9.6	3.4%	NUP	4.1	2.3%	PAM	0.28	0.29
			PRO	9.6	3.4%	PEC	4.1	2.3%	PGR	0.16	0.19
			PRI	8.2	3.2%	PRI	4.1	2.3%	PNA	0.16	0.32
			ELD	6.8	3.0%	PAM	2.7	1.9%	PNO	0.12	0.24
			PGR	2.7	1.9%	NMP	1.4	1.4%	NUP	0.08	0.16
			PNA	2.7	1.9%	PGR	1.4	1.4%	UTR	0.08	0.16
			HET	1.4	1.4%	PNA	1.4	1.4%	PEC	0.04	0.08
			PNO	1.4	1.4%	PPR	1.4	1.4%	NMP	0.04	0.08
						UTV	1.4	1.4%	PCR	0.04	0.08
									HET	0.04	0.08
									RAN	0.04	0.08

Table 14 Continued

Shady Island		% Vis MSP Cov	% Occurrence (Visual)		% Occurrence (Drop Hook)		Density Rating n = 27	
Date	n	Mean ± 1SE	Spp.	% Occ. ± 1 SD	Spp.	% Occ. ± 1 SD	Spp.	Density ± 2SE
8/9/01	75	19.1% 3.5%	MSP	56.0 ± 5.7%	MSP	49.3 ± 5.8%	CRT	2.52 ± 0.64
			PEC	21.3 ± 4.7%	CRT	49.3 ± 5.8%	MSP	2.19 ± 0.71
Eurasian Watermilfoil			NAJ	13.3 ± 3.9%	NAJ	33.3 ± 5.4%	NAJ	1.81 ± 0.68
Total area			NMP	13.3 ± 3.9%	PZS	25.3 ± 5.0%	PZS	1.07 ± 0.50
% of Litt. Zone:			PAM	10.7 ± 3.6%	VAL	16.0 ± 4.2%	CHA	0.74 ± 0.51
% Lake Area:			PRI	10.7 ± 3.6%	PEC	12.0 ± 3.8%	PRI	0.59 ± 0.44
Surface area Criteria: Visible milfoil			CRT	9.3 ± 3.4%	CHA	8.0 ± 3.1%	VAL	0.59 ± 0.40
Weevil Damage Rating:0.16			PZS	9.3 ± 3.4%	ELD	6.7 ± 2.9%	PEC	0.56 ± 0.40
			VAL	9.3 ± 3.4%	NMP	5.3 ± 2.6%	NMP	0.19 ± 0.26
			PNO	5.3 ± 2.6%	PAM	5.3 ± 2.6%	PAM	0.19 ± 0.19
			MGD	2.7 ± 1.9%	PRI	5.3 ± 2.6%	HET	0.11 ± 0.16
			PRO	2.7 ± 1.9%	PNO	2.7 ± 1.9%	MGD	0.11 ± 0.12
			SCR	2.7 ± 1.9%	HET	1.3 ± 1.3%	PNO	0.11 ± 0.16
					MGD	1.3 ± 1.3%	ELD	0.07 ± 0.10
					MGD	0.0 ± 1.3%	ELD	0.07 ± 0.10

Weevil Introduction/Manipulation:

Milfoil density at the 20 Cedar Lake plots in June 1999 (prior to weevil stocking) ranged from 3112± 909 g wet/m^2 to 3810± 664 g wet/m^2 (508 g dry/m^2) (Table 15); this was higher than these sites in 1998 and than our permanent transect sites in 1999. At the end of the experiment in late August, milfoil biomass declined to between 1512± 458 g wet/m^2 and 2551± 252 g wet/m^2. The mean number of species also declined.

Table 15. Wet and dry biomass (g/m^2 ± 1SE) of Eurasian watermilfoil (MSP) and non-milfoil plants, %Eurasian watermilfoil and mean number of species per sample for the 1999 cage experiment. The June sample was taken 3 weeks prior to stocking and the August sample was taken 8 weeks after initial stocking. Two samples per cage were taken in July and 3 samples per cage in August. N=5 replicate cages per treatment. Open cages allow fish entry, closed cages do not. A total of 150 adult weevils were stocked into each stocked cage.

Date	Cage Type	Stocked	MSP	NonMSP	%MSP	Mean No. spp.
6/3/99	Open	No	3810 ± 664	424 ± 195	89.9 ± 4.1%	2.30 ± 0.34
Dry			389 ±59	36 ± 17	91.3 ±4.2%	
6/3/99	Closed	No	3455 ±495	149 ± 76	95.5 ± 1.3%	2.00 ± 0.16
Dry			331 ± 37	8 ± 4	96.3 ± 0.9%	
6/3/99	Open	Yes	3112 ± 909	409 ± 187	81.8 ± 9.9%	2.50 ± 0.16
Dry			321 ± 88	36 ± 16	83.2 ± 9.6%	
6/3/99	Closed	Yes	3252 ± 430	350 ± 151	88.1 ± 7.0%	2.50 ± 0.22
Dry			346 ± 39	27 ± 10	90.1 ± 5.9%	
8/30/99	Open	No	2551 ± 252	363 ± 183	87.9 ± 5.8%	1.70 ± 0.20
Dry			175 ± 22	22 ± 12	89.3 ± 5.9%	
8/30/99	Closed	No	1512 ± 458	174 ± 173	92.5 ± 7.4%	1.30 ± 0.20
Dry			106 ± 33	13 ± 13	92.2 ± 7.8%	
8/30/99	Open	Yes	2241 ± 524	429 ± 311	82.8 ± 13.1%	1.80 ± 0.12
Dry			153 ± 45	25 ± 17	81.9 ± 13.8%	
8/30/99	Closed	Yes	2062 ± 250	319 ± 132	78.4 ± 10.0%	1.80 ± 0.20
Dry			140 ± 21	22 ± 9	78.6 ± 10.3%	

Weevil stocking appeared less successful than in 1998. Initially, higher densities of weevils were found in stocked vs non-stocked cages during visual surveys, but later in the summer higher

densities of weevils were found in closed compared to open cages (Table 16). Few significant differences in weevil density were found. By the last date there were significantly ($P > 0.1$) more total weevils, and more larvae and pupae per stem in the stocked cages but no effect of cage type.

There was no significant effect of cage or stocking on milfoil biomass (all $P > 0.1$); biomass generally decreased in all the cages after stocking. The failure to build substantially higher weevil densities in stocked cages and the relatively late stocking date may have prevented any effect on the watermilfoil. There was also no evidence of carryover effects from stocking in 1998. Fish invasion was a persistent problem and the experiment was conducted again in 2000.

Weevil stocking in 2000 was more successful than in 1999. For some unknown reason, adult and larval weevils also turned up in non-stocked cages (casual observation suggested no weevils prior to stocking). Some dispersal among cages may have occurred, particularly into the closed cages (see also the 1999 experiment), however, the presence of detectable weevils at our transect sites in 1999 and 2000 suggests that caging may have protected already occurring weevils. Throughout the experiment there were more weevils found in the stocked and closed cages than in not-stocked and open cages (Fig. 8, Table 17). A repeated measures ANOVA indicated a significant effect of cage type and stocking on larval density and cage type on adult density (all $P \leq 0.03$). At the end of the experiment, ANOVA indicated a significant cage effect ($p < 0.05$) for larvae (more larvae in closed than in open cages) and a significant ($p < 0.05$) cage and stocking effect for adults (more adults in closed cages and in stocked cages). No interactions were significant. These results suggest that fish (open cages) were reducing the establishment and abundance of weevils.

Table 16. Visual counts (mean number per 100 stems and 1 SE) of weevils in stocked and unstocked cages (open and closed) at Cedar Lake in 1999. There were 5 reps of each treatment combination.

Date	Cage type	Stocked	Eggs	Larvae	Pupae	Adults	Total
7/23/99	Open	No	0.3	6.4	1.7	0.1	8.5
		1 SE	0.3	3.6	1.4	0.1	3.2
	Closed	No	0	3.9	1.1	0.7	5.6
		1 SE	0.0	2.4	0.5	0.3	2.5
	Open	Yes	1.2	5.1	2.3	0.8	9.3
		1 SE	0.5	1.5	1.6	0.5	3.6
	Closed	Yes	0.7	20.1	5.1	1.3	27.2
		1 SE	0.3	11.9	2.9	0.6	11.1
8/5/99	Open	No	0.8	8.3	4.0	0.8	13.9
		1 SE	0.6	4.5	3.7	0.4	8.2
	Closed	No	0.5	4.1	1.6	4.1	10.4
		1 SE	0.3	1.0	0.7	2.9	3.9
	Open	Yes	0.4	2.3	0.3	0.4	3.3
		1 SE	0.4	1.5	0.2	0.3	2.2
	Closed	Yes	2.8	8.5	1.9	2.0	15.2
		1 SE	2.5	4.2	1.6	0.9	7.5
8/17/99	Open	No	0.4	1.2	0.4	0.8	2.8
		1 SE	0.2	0.6	0.3	0.5	0.5
	Closed	No	0.3	8.7	0.8	0.5	10.3
		1 SE	0.2	3.8	0.2	0.4	4.0
	Open	Yes	0.7	1.2	0.8	0.5	3.2
		1 SE	0.3	0.6	0.5	0.5	0.9
	Closed	Yes	0.9	8.8	2.1	1.5	13.3
		1 SE	0.5	5.1	0.7	0.9	5.3

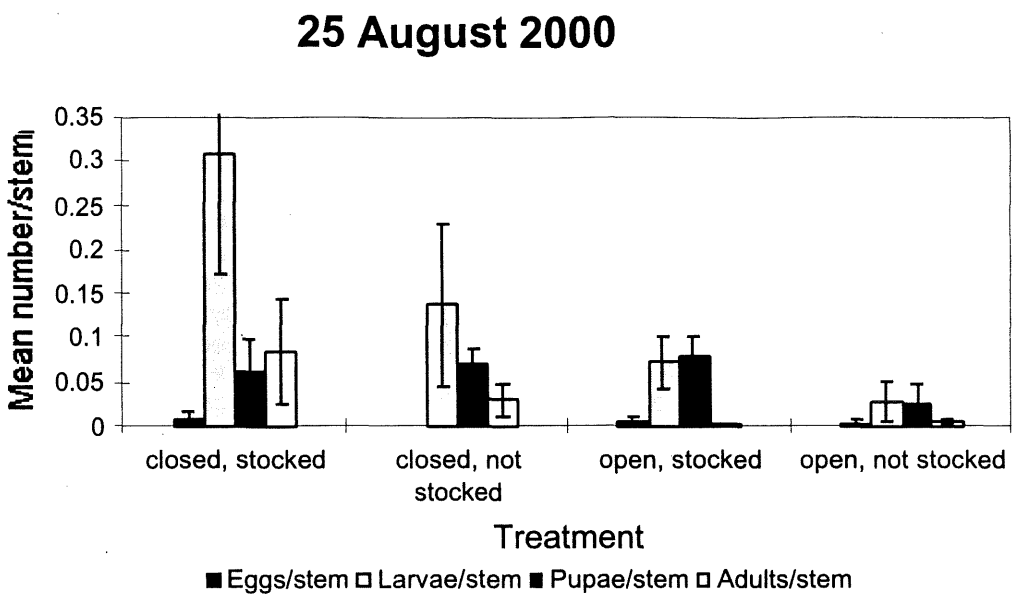

Fig. 8. Number of weevils per treatment (±2SE) at the end of the 2000 cage stocking experiment.

Table 17. Visual counts (mean number per 100 stems) of larvae, pupae and adult weevils in stocked and unstocked cages (open and closed) at Cedar Lake in 2000. There were 5 reps of each treatment combination. The first sample date was 1 week after stocking.

Date	Treatment	Larvae	Pupae	Adults
14-Jul-00	Closed Stocked	5.1	0.0	2.5
	Closed Not Stocked	2.3	0.1	2.9
	Open Stocked	3.2	0.0	1.4
	Open Not Stocked	0.0	0.0	1.2
26-Jul-00	Closed Stocked	18.6	1.5	2.6
	Closed Not Stocked	7.5	0.1	5.3
	Open Stocked	7.5	0.6	0.7
	Open Not Stocked	1.2	0.0	0.7
8-Aug-00	Closed Stocked	6.7	1.2	5.2
	Closed Not Stocked	7.6	1.0	1.7
	Open Stocked	8.7	0.5	4.3
	Open Not Stocked	3.9	0.3	0.3
25-Aug-00	Closed Stocked	30.8	6.2	8.5
	Closed Not Stocked	13.7	6.5	3.1
	Open Stocked	7.3	3.2	0.3
	Open Not Stocked	2.9	4.4	0.5

Table 18. Dry biomass ($g/m^2 \pm 1SE$) of Eurasian watermilfoil (MSP) and non-milfoil plants, % Eurasian watermilfoil and mean number of species per sample for the 2000 cage experiment. The June sample was taken 3 weeks prior to stocking and the August sample was taken 8 weeks after initial stocking. Two samples per cage were taken in June and also in August. N=5 replicate cages per treatment. Open cages allow fish entry, closed cages do not. A total of 150 adult weevils were stocked into each stocked cage.

Date	Cage Type	Stocked	MSP	NonMSP	%MSP	Mean No. spp.
6/14/00	closed	stocked	353.3	25.2	87.8%	2.7
			101.3	11.5	6.5%	0.2
6/14/00	closed	not	425.9	46.9	86.9%	2.3
			84.6	20.2	7.9%	0.3
6/14/00	open	stocked	147.4	13.5	83.4%	2.1
			52.8	6.0	8.6%	0.4
6/14/00	open	not	369.2	42.2	78.4%	2.0
			100.6	26.2	13.3%	0.4
8/31/00	closed	stocked	186.1	37.4	84.0%	1.9
			45.7	30.0	9.9%	0.3
8/31/00	closed	not	255.3	112.9	71.3%	2.0
			66.9	54.5	14.5%	0.0
8/31/00	open	stocked	151.2	14.1	91.7%	1.7
			35.3	10.4	5.9%	0.2
8/31/00	open	not	302.9	28.0	89.4%	1.7
			69.2	16.1	5.7%	0.2

Milfoil biomass was somewhat lower in 2000 than in 1999 and generally declined over the season (Table 18). There was a significant effect of cage ($p = 0.062$) on the difference in milfoil biomass from the beginning to end of the experiment. Stocking and the stocking by cage interaction were not significant. Milfoil biomass decreased more in closed vs open cages, suggesting that excluding fish predation (and the subsequent increase in weevil density noted above) resulted in a decrease in milfoil. In addition, there was a negative relation ($p = 0.1$) between change in milfoil biomass and final larval density (Fig. 9), further suggesting a decrease in milfoil density with more weevils.

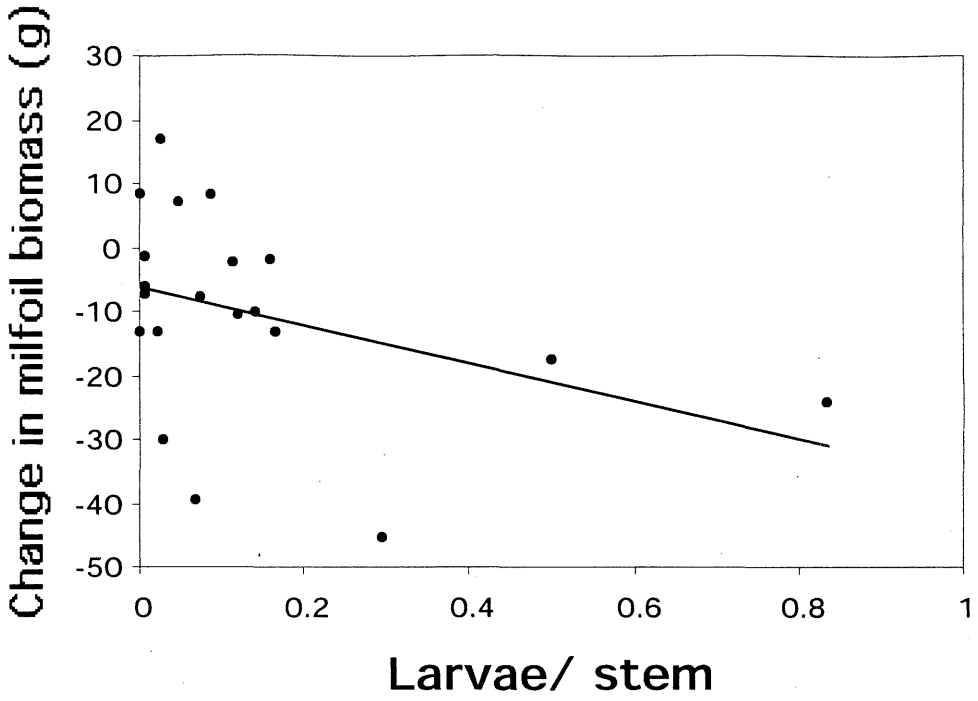

Figure 9. Change in milfoil dry biomass vs larval density at the end of the experiment.

The weevil introduction/manipulation experiments in 2001 allow us to compare effects of fish predation in a low-weevil, high-sunfish lake (Cedar) and a high-weevil, low-sunfish lake (Otter). We repeated our previous fish cage exclosure experiments in the NE bay of Cedar Lake, using the same cages (3mX3m) and treatments we used previously (four replicates of 4 treatments; stocked or not stocked, open or closed). The aim of this experiment was to determine the effect of sunfish (presence or exclusion) on augmented (stocked) weevil populations in a low weevil density lake. A more complete presentation and analysis is given by Ward (2002).

Stocking weevils increased larval, pupal, and adult density in 2001 (Fig. 10). Fish exclosure cages had increased larval and pupal density. Stocked weevil populations only established in exclosure cages, resulting in strong exclosure by stocking interaction effects on larval and pupal density ($p < 0.05$). There were significant time effects on adult density and time by treatment interactions on larvae ($p < 0.05$). On the final sampling date, nearly all weevils were found in a single stocked fish exclosure cage. While weevil density had increased initially in stocked exclosure cages, weevil density declined in these cages concurrent with fish invasions (Fig. 11). A model incorporating the number of fish observed and date as predictors explained 45% of the variability in weevil density in stocked exclosure cages ($p=0.01$). In 2001, 76% of the weevils from quantitative samples were recovered from a single stocked fish exclosure cage (Fig. 11); due to high variability and overall low density there were no significant treatment effects on weevil density from the plant samples although 4 times as many weevils (per area, per stem and per gram milfoil) were found in stocked fish exclosures than any other treatment. Similarly, there were no significant treatment effects on the plant community or milfoil density. The high variability of weevil density in stocked exclosure cages in 2001 (and thus lack of an effect on milfoil density) was largely explained by fish invasions (Fig. 11).

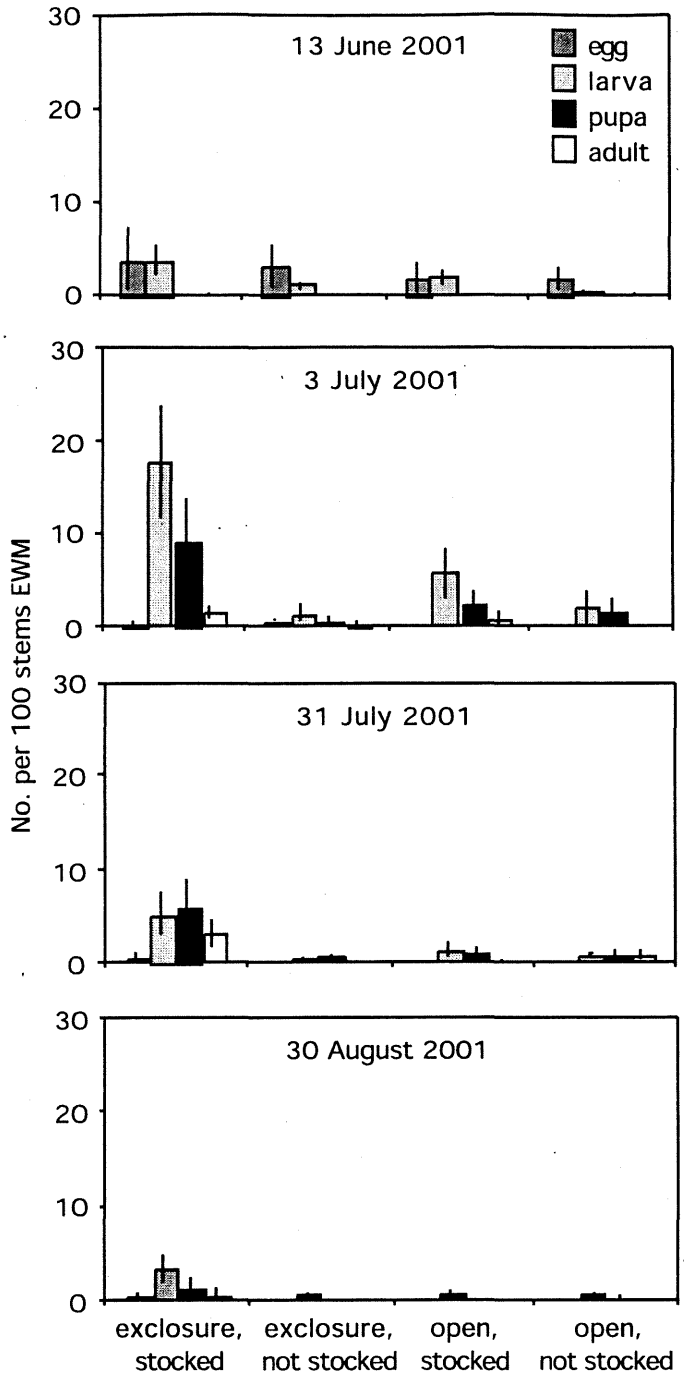

Fig. 10. Mean weevil density per 100 stems by treatment and life stage for four of the seven visual surveys in Cedar Lake cages, 2001. Error bars are ±1 SE. From Ward (2002).

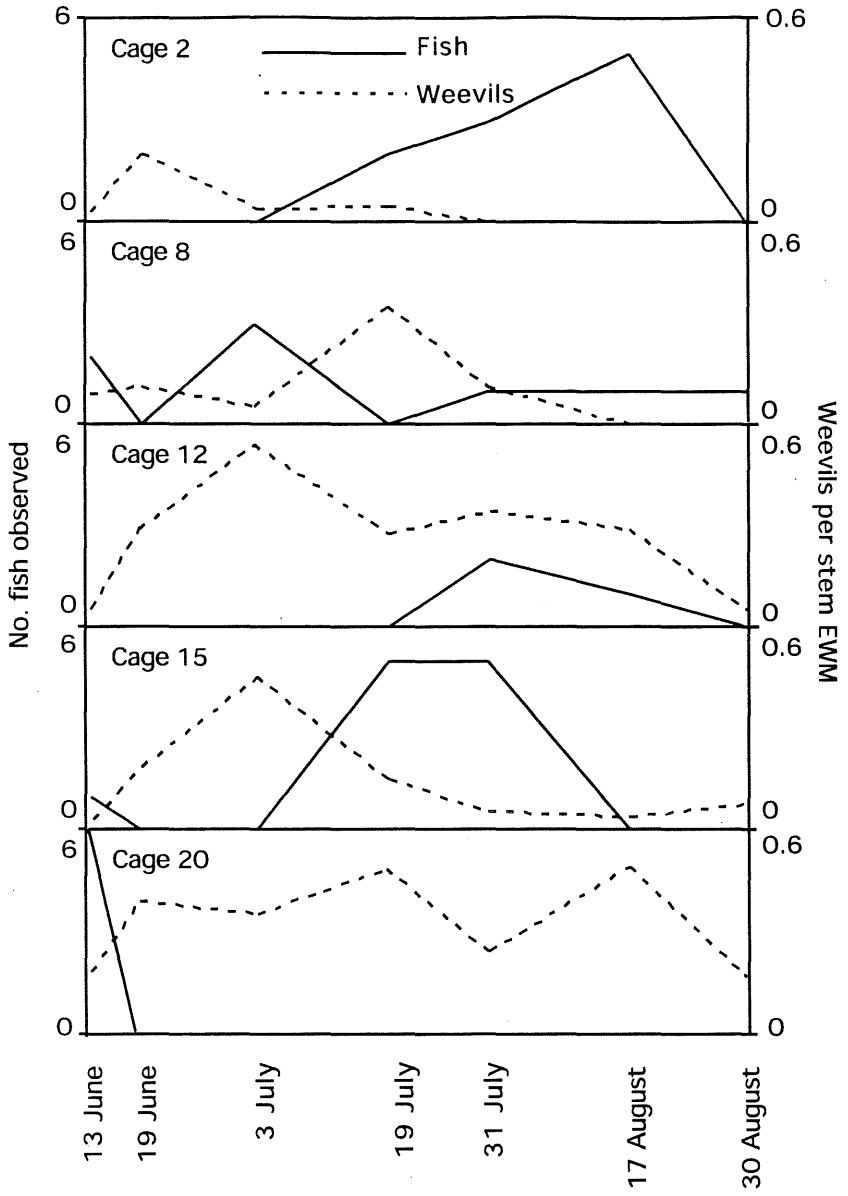

Fig. 11. The number of fish observed in Cedar cages during visual surveys (left axis) and weevil density per stem (right axis) for stocked fish exclosure cages in 2001. From Ward (2002).

At Otter Lake we added 5 sunfish (ca., 9-15 cm) to each of 4 enclosed (2mX2m) cages; 4 unstocked enclosed cages served as controls. An MN DNR fisheries survey during 2001 confirmed our visual observations of an extremely low density of sunfish in Otter Lake. The aim of this experiment was to determine the effect of increased fish density on an established weevil population. There was a strong effect of fish enclosure on milfoil weevil egg density in the visual surveys (Repeated measures ANOVA, p=0.006, Fig. 12) and possibly a treatment effect on larval density (p=0.15). Adult weevils may have been suppressed initially in cages with fish but there was no consistent effect of fish predation through the season (p=0.49; Fig.

12). Thus, presence of fish may have reduced weevil populations by suppressing oviposition rate.

Herbivorous invertebrate densities from the plant biomass samples were highly variable (Fig. 13); however, total EWM herbivore areal density (per m^2) was moderately suppressed in fish enclosures ($p=0.09$). Milfoil weevil mean adult and larval densities per gram dry EWM were lower in fish enclosures but there was no evidence of suppression ($p \geq 0.49$). Total lepidopteran density per m^2 was strongly suppressed in fish enclosures ($p=0.06$) due to a strong effect on *Parapoynx* spp. (Fig. 13). *Acentria ephemerella* was found at low density, similar in fish enclosures and fishless cages. Lepidopteran density per g total plants had a similar pattern to areal density but variability was higher.

Total invertebrate areal density did not differ between treatments, but the total number of invertebrates per g plant dry mass was moderately suppressed in fish enclosures ($p=0.1$). Most non-herbivore invertebrate taxa did not differ significantly between treatments, although the mean density of most was lower in fish enclosures.

Total plant dry weight did not differ significantly between treatments (Fig. 14). Eurasian watermilfoil, *Ceratophyllum demersum, Zosterella dubia, Potamogeton zosteriformis*, and *Stuckenia pectinatus* (formerly *Potamogeton pectinatus*) mean dry mass was higher in fish enclosures but only *Z. dubia* mass differed significantly between treatments ($p=0.05$, Fig. 14). No plant species were significantly depressed in fish enclosures. Overall, fish had the strongest effect on *Parapoynx* and suppression of *Parapoynx* apparently resulted in higher abundance of *Zosterella*. The weaker effects on the milfoil weevil are likely due to immigration of weevils from outside the cages and these small scale cage experiments may not reflect the impact that would be seen on a whole lake basis where immigration or emigration would not be as important. The lack of a treatment effect on Eurasian watermilfoil is not surprising as watermilfoil was at low density in both treatments and was already heavily damaged by milfoil weevils at the start of the experiment (personal observation), leaving little margin to detect effects. In fact, it was difficult to find enough locations with viable milfoil plants to place the cages.

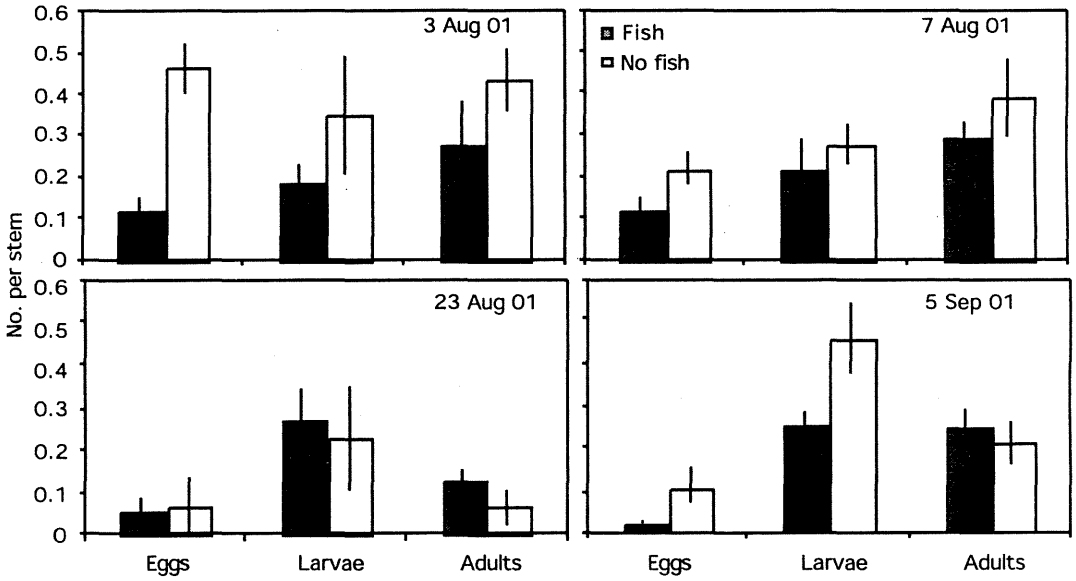

Fig. 12. Mean milfoil weevil density per stem by treatment and life stage, from visual surveys in Otter Lake cages. Error bars are ±1 SE. From Ward (2002).

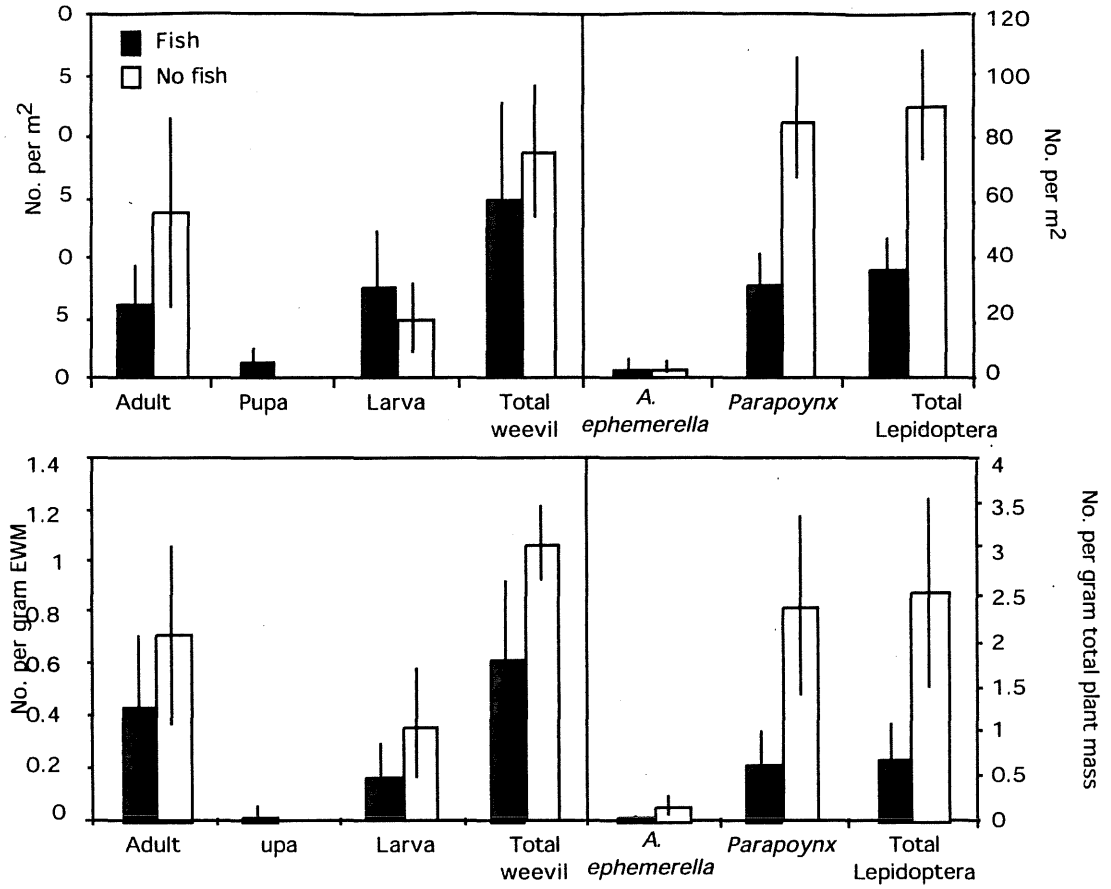

Fig. 13. Mean density of herbivorous invertebrates by treatment from final plant samples in Otter Lake cages. Top graphs are areal density, bottom are density per gram dry EWM mass for weevils (left axis) and per gram dry total plant mass for lepidoptera (right axis). Error bars are ±1 SE. From Ward (2002).

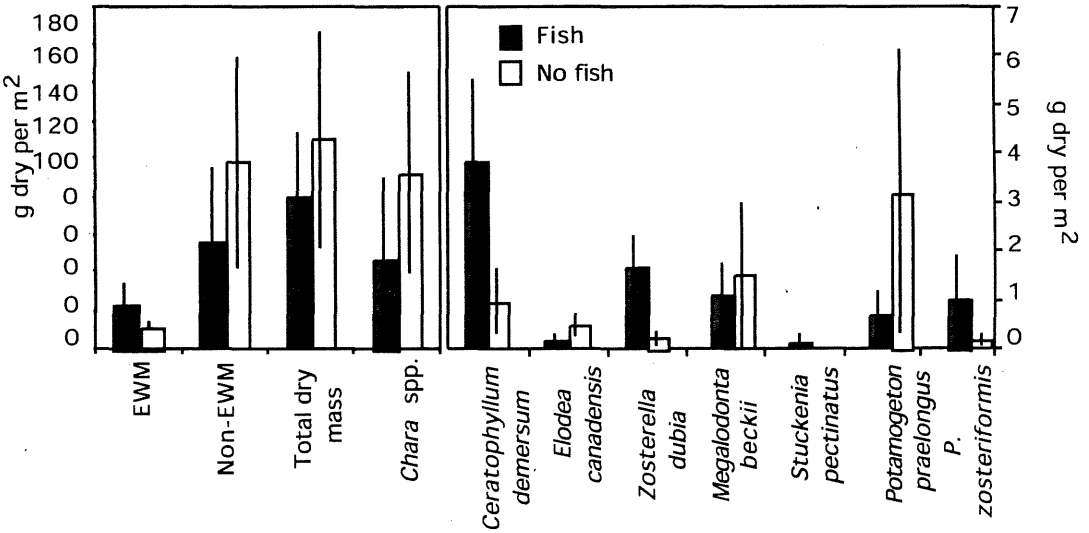

Figure 14. Mean final plant dry mass by treatment in Otter Lake cages. Note different scales on the left and right y-axes. Error bars are ±1 SE. From Ward (2002).

Weevil modelling:

Ward (2002) used milfoil weevil life history parameters derived from the literature and laboratory observations to construct a simple stage-structured matrix population model to project weevil populations (Fig. 15). Based on updated life history parameters (Table 19), we developed a refinement of the model we previously described in earlier reports. This model assumes unlimited space and resources so predicted densities should not be taken literally; projected populations represent the maximum potential growth of a population given stage-specific survival rates. The utility of the model is in identifying critical factors for milfoil weevil population growth.

The model is on a scale of heat accumulation in degree-days >10°C (hereafter DD), as milfoil weevil development rate is determined by temperature above a minimum threshold of 10°C (Mazzei et al. 1999). For convenience, survival and development rates were broken into 25 DD increments; 25 DD≈ 2 days at typical early season temperatures, but DD are not linearly related to time. Approximately 1500-1800 DD can accumulate in a typical Minnesota growing season. As weevils may take 6-10 days to develop eggs in the spring (Mazzei et al. 1999) and oviposition rates tend to tail off toward the end of the season, the model runs of the refined model were terminated at 1000 DD to focus on mid-season dynamics when parameters are more constant. The 1000 DD model runs approximate the time from early June to mid-August. The weevil population at the beginning of the simulations (15 larvae, 8, pupae, 2 adults) was based on the average larva and adult density of June samples from 4 MN lakes from 1994-2001 (data summarized in Newman et al. (2001a)); years when no weevils were found were excluded and pupae were under-represented in samples so initial pupal density was based on interpolation.

Relatively little information is available regarding the milfoil weevil's adult life span. Adult female milfoil weevils regularly survive and reproduce for >17 d in laboratory oviposition studies (Sheldon and Jones 2001, Solarz and Newman 2001). However, much longer reproductive adult life spans (>100 d) have been observed in the laboratory (Sheldon and O'Bryan 1996) and our fecundity experiments show no decline in fecundity over 40d (Newman 2002). We assumed a baseline adult mortality rate of 0.1 per 25 DD (average life span of 20 days at 22.5°C). Survival per DD of pre-reproductive adults was assumed to be equal to that of adults.

Runs of the initial model (Fig. 15) illustrate both the cyclical nature of the populations (increase in eggs, then larvae, then pupae, etc.) over the summer and also illustrate the potential importance of female egg laying longevity. Note that with shorter female longevity, peaks and valleys in abundance of each stage are apparent, whereas with longer female longevity, the stage structure begins to overlap and the generations become less distinct.

To determine the sensitivity of weevil populations to stage-specific survival rates with the improved model, we projected weevil populations with a range of juvenile (egg-adult) and adult survival rates (0%-50% additional mortality per 25 DD, Fig. 16). The distribution of juvenile survival rates across stages did not affect total population projections as long as total survival to adult remained constant. Sutter and Newman (1997) predict that, at high predation rates and low weevil density adult mortality due to fish predation could reach 51% per 25 DD (assuming 22.5°C). Therefore, adult survival rates used represent a reasonable range for milfoil weevil populations in the field.

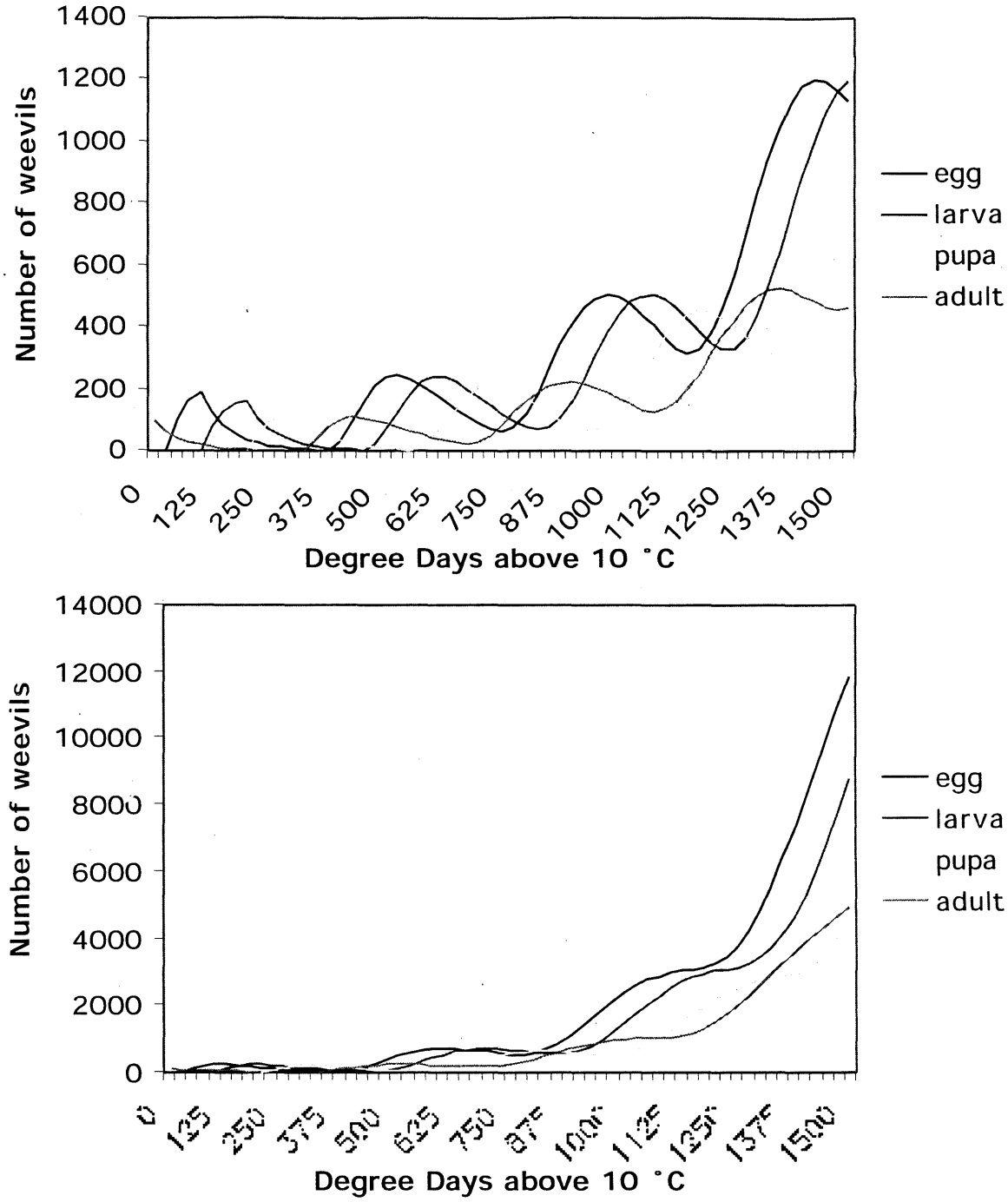

Figure 15. Predicted population density of egg, larvae, pupae and adults over a summer of 1500 degree days above 10 °C with the preliminary model. Top: Based on average adult longevity of 75 DD (about 5 days). Bottom: Based on average adult longevity of 150 DD (about 10 days). Initial density of adults was 100 and hatch and pupal survival were 0.8, larval survival was 0.7 and egg laying was estimated at 0.9 female eggs/female/25 DD. Development times for each stage were estimated from temperature-development relationships given by Mazzei et al. (1999).

Table 19. Weevil life history parameter estimates used for the improved weevil population model. Survival estimates are the mean of values in the sources. Development rates from Mazzei et al. (1999).

Stage	Degree Days >10°C required	Survival to next stage (range in sources)	Source
Egg	75	0.86 (0.81-0.89)	Sheldon and O'Bryan (1996), Newman et al. 1997, Mazzei et al. (1999), Sheldon and Jones (2001)
Larva	100	0.80 (0.71-0.9)	Newman et al. (1997), Mazzei et al. (1999)
Pupa	125	0.80 (0.75-0.83)	(Newman et al. (1997), Mazzei et al. (1999)
Adult (pre-reproductive)	50*	0.81	See text
Adult	-	0.9 per 25 DD	See text
Reproduction	-	6 eggs /female /25 DD (1.9-4.6 eggs /female /day)	Sheldon and O'Bryan (1996), Sheldon and Jones (2001), Marko, Krueger, and Newman, University of Minnesota, unpublished data

*Not estimated in Mazzei et al. (1999), may be up to 7 days (~90 DD) based on laboratory rearings (Marko, Krueger, and Newman, University of Minnesota, unpublished data).

In order to incorporate more biological reality and explore implications for EWM control, the weevil model was linked to a simple EWM growth model. The EWM model is a discrete logistic model; where Mt is EWM mass (g dry/ m2) at interval t, r is intrinsic growth rate, K is carrying capacity, p is a scalar term for herbivory, and Lt is larval density at interval t. Parameters were estimated from the literature and by fitting projections to the results of previous experiments (Table 20, see below).

Weevil density dependence was incorporated by setting a maximum larval density per stem above which recruitment to the larval stage is 0 and a maximum larval + pupal per stem density above which recruitment to the pupal stage is 0. This method of incorporating resource limitation simulates a limited number of suitable larval feeding and pupation sites that are utilized completely.

This model (model 2) was calibrated to the results of a tank experiment described in Newman et al. (1996). Parameters were estimated by projecting EWM and milfoil weevil populations for the same duration and initial conditions as the experiment (~425 DD, variable weevil stocking density) and altering density dependence and herbivory parameters to find one set of parameters that resulted in projections within 2 SE (or as close a possible) to all treatment levels of the experiment. Watermilfoil growth for the calibration runs was calculated from

growth observed in the control tanks (0 weevils stocked), based on change in stem length (r=0.06). Density dependence parameters may be underestimated, and herbivory parameters overestimated, due to limited EWM growth in the tanks. However, no other data were available with sufficient detail for similar calibrations. See Ward (2002) for details.

We projected EWM and milfoil weevil populations under different conditions to explore the potential impact of milfoil weevils on EWM growth. As milfoil weevil populations are sensitive to adult survival, populations were projected with a range of adult survival estimates to determine the implications for EWM control (Fig. 17). We also ran scenarios to address stocking situations (elevated initial weevil density, Fig. 18a) and high initial EWM mass (Fig. 18b).

Table 20. Parameters and baseline estimates used in model 2 projections.

Parameter	Estimate	Source
r- EWM growth rate	0.09	Auburn Lake, MN 1995*
K- EWM carrying capacity	1424 g dry/m2	Auburn Lake, MN 1996*
p- herbivory constant	0.12	Fit to Newman et al. 1996 results, see Figure 2
Max. larva density for larval recruitment	1/stem†	Fit to Newman et al. 1996 results, see Figure 2
Max. larva+pupa density for pupal recruitment	1.5/stem†	Fit to Newman et al. 1996 results, see Figure 2
Initial EWM mass	190 g dry/m2	Mean of early season samples

*Calculated from unpublished data summarized in Newman et al. (2001a). K is maximum density in a single sample, r maximum growth rate based on whole-lake means, initial density is mean of June samples; from samples from 4 MN lakes from 1994-2001. Growth rate was converted to DD scale assuming a temperature of 21.5°C.

†Grams EWM converted to stem counts assuming 1.16 g dry/stem. Estimates based on regression of stem counts and dry weights for data from 4 MN lakes sampled from 1994-2001. Data summarized in Newman et al. (2001a).

Milfoil weevil populations have the potential for dramatic increase within a growing season (Fig. 16). However, these projections are based on laboratory survival rates and the assumption of unlimited resources. Baseline runs probably represent the maximum potential increase of weevil populations under ideal conditions.

The rate of weevil population growth is more sensitive to adult mortality than juvenile mortality (Fig. 16). With 50% additional juvenile mortality per 25 DD weevil populations still have a 25-fold increase through the summer, whereas populations with 50% additional adult mortality per 25 DD decline thorough the season. Even at 30% additional adult mortality per 25 DD, which could be associated with a moderate fish predation rate (Sutter and Newman 1997), weevil populations fail to increase dramatically; additional juvenile mortality rates of ~90% are required to similarly limit weevil populations. Even given the assumption of unlimited resources, observed fish predation rates or similar sources of adult mortality could limit weevil populations.

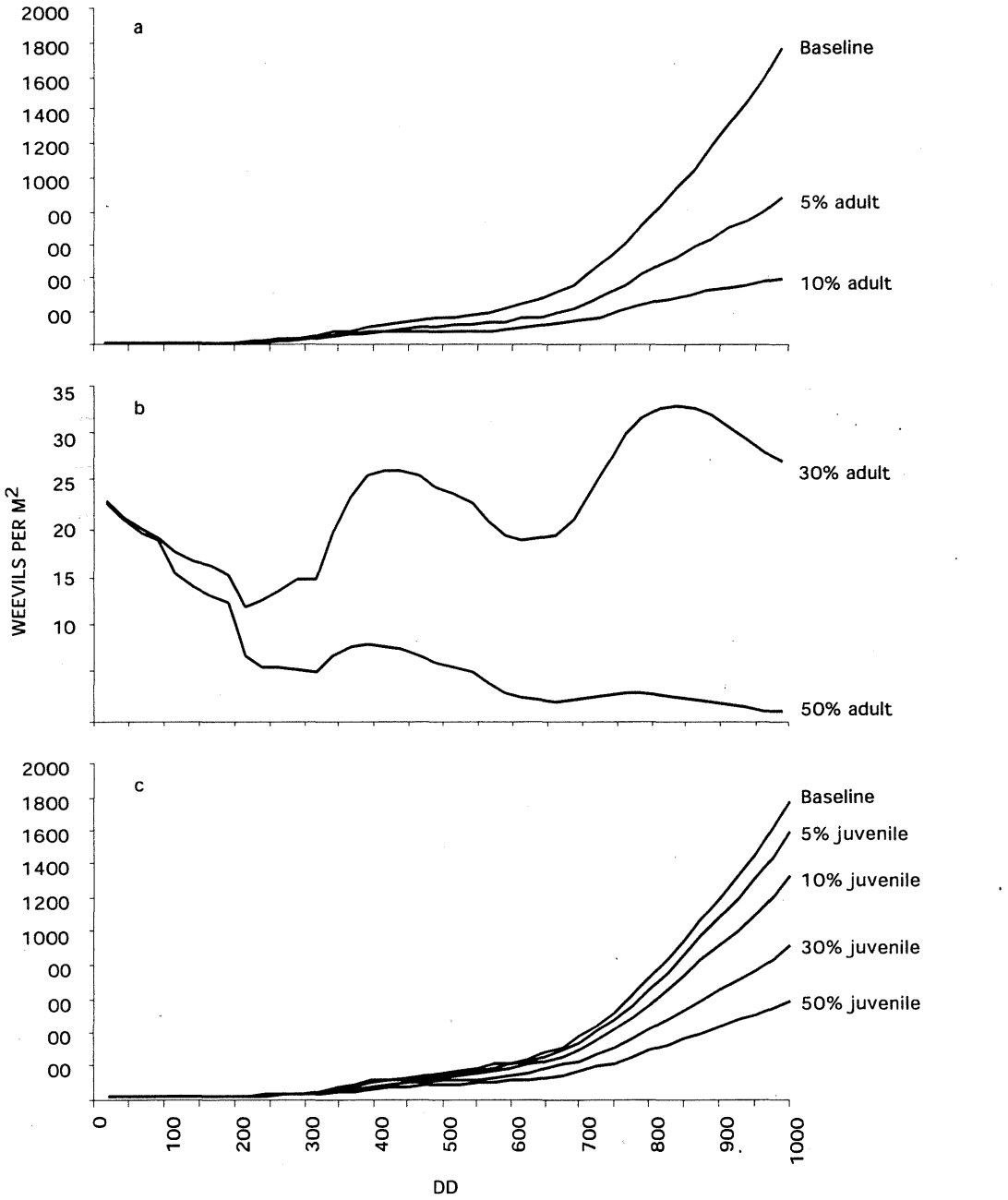

Fig. 16. Model 1 projections with different levels of additional adult (a and b) and juvenile (c) mortality as indicated on the graphs. Additional mortality rates indicated are per 25 DD (~2 days) and are additive above baseline rates. Note the different y-axis scale on graph b. Milfoil weevil density graphed is larvae + pupae + adults.

Model 2 predicts that, under conditions of low adult mortality, milfoil weevils can suppress EWM dramatically within a growing season (Fig. 17a). However, as adult mortality increases EWM control is less successful (Fig. 17b-d); even at low levels of adult mortality EWM mass increases through most of the season (Fig. 17b). Although larval weevils cause the majority of damage to EWM plants (Newman et al. 1996), according to these models adult

survival is the key factor in determining EWM suppression. This makes sense because female weevils lay only several eggs per day, and thus female longevity is key to final population size.

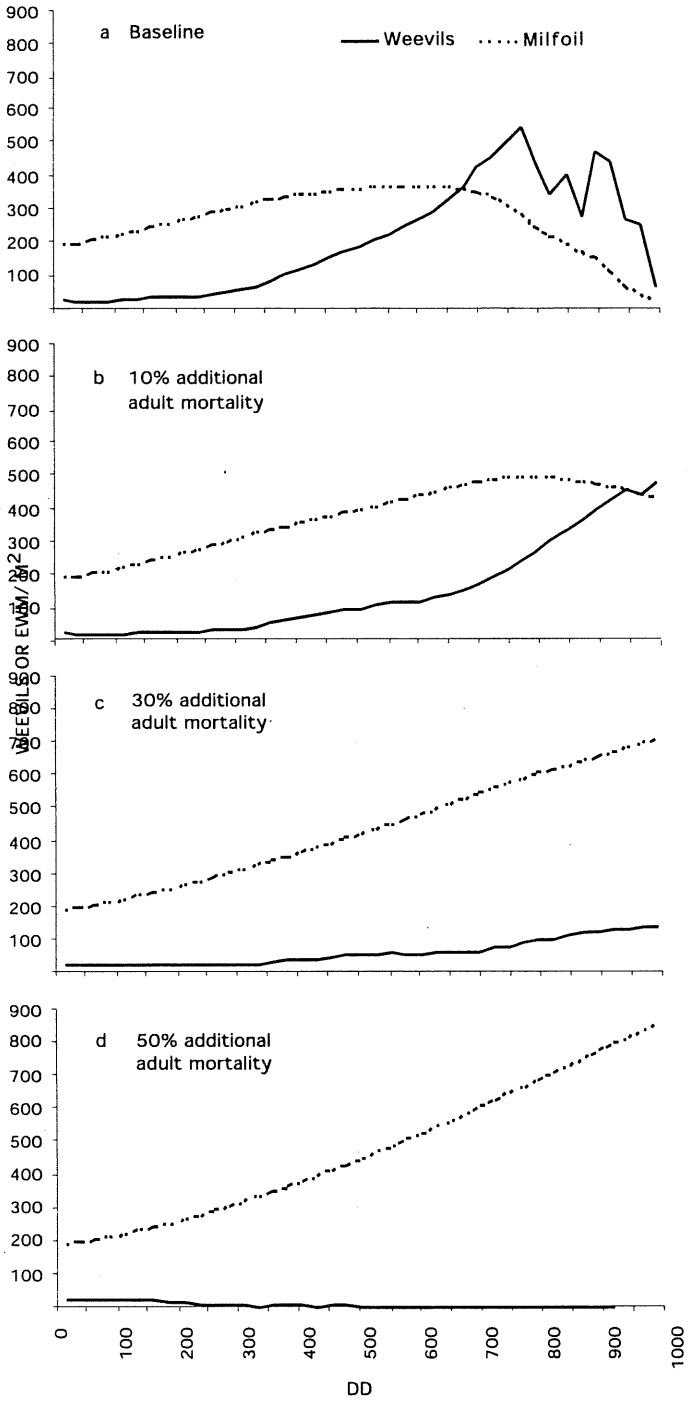

Fig. 17. Model 2 projections with different levels of adult mortality. The projection in a uses baseline parameters from Tables 16 and 17; projections in b-d include 10-50% additional adult mortality. The y-axis is larva + pupa + adult weevil density per m^2 and EWM dry mass per m^2.

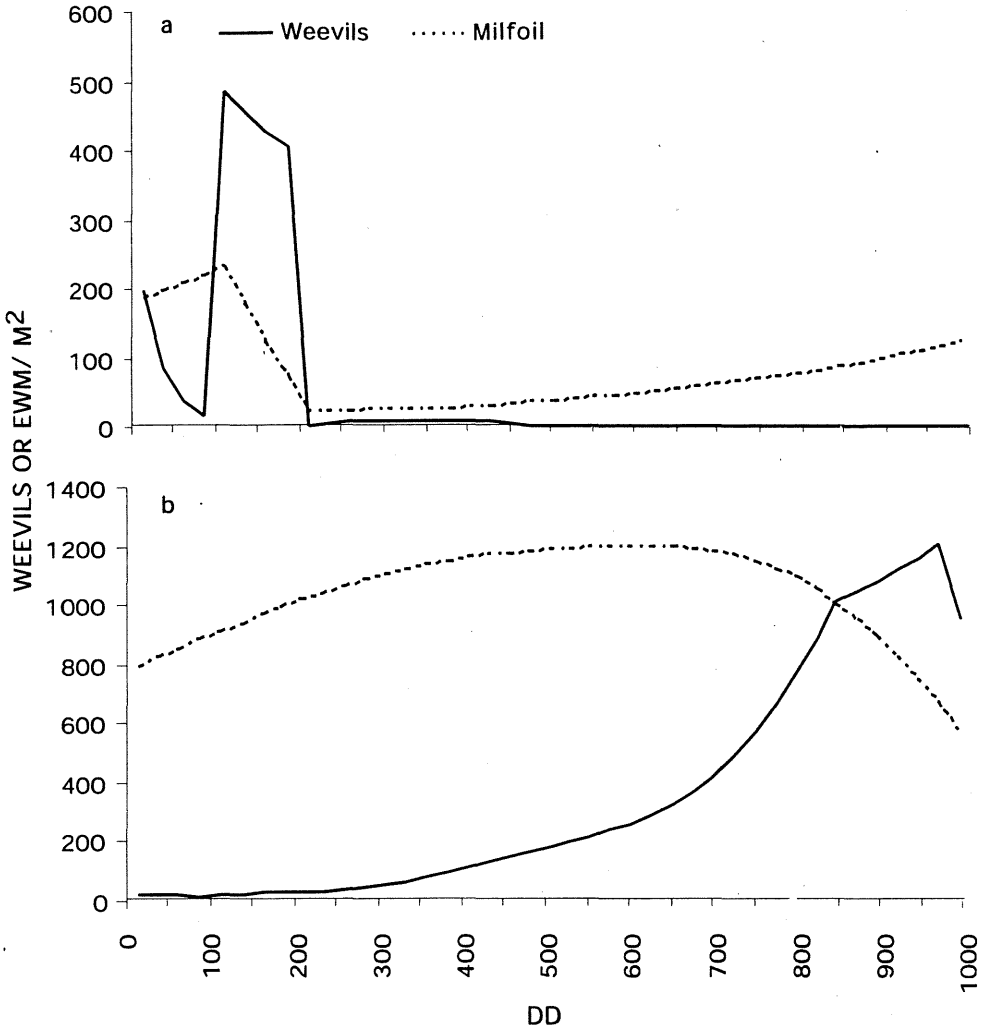

Fig. 18. Model projections with baseline parameters from Tables 16 and 17 except 18a has 50% additional adult mortality and high initial weevil density (200 adults/ m2, simulates weevil stocking) and 18b has high initial EWM density, and no fish predation. The y-axis is larva + pupa + adult weevil density per m^2 and EWM dry mass per m^2.

According to Model 2, stocking adult milfoil weevils at high density will result in suppression of EWM; however, weevil populations are not sustained if adult mortality is high (Fig. 18a). Sheldon and Creed (1995), Creed and Sheldon (1995) observed suppression of EWM with high milfoil weevil stocking densities in enclosures. However, open stocking trials in Minnesota lakes did not increase weevil density or impact milfoil weevils (Newman et al. 1998), possibly due to fish predation and adult weevil emigration. Stocking weevils in littoral fish exclosures did result in increased weevil density, but populations did not increase as predicted with low adult mortality, possibly due to adult emigration (Ward 2002). Loss of adult weevils must be minimized if milfoil weevil stocking is to be successful.

Model 2 also predicts that, at high initial EWM density, milfoil weevils would have to reach very high density to suppress EWM (Fig. 18b). Although the weevil densities predicted

at high initial EWM density are probably unreasonably high (Fig. 18b), the projection does raise the important point that the milfoil weevil density necessary to suppress EWM is a function of EWM density. Increasing EWM growth rate or carrying capacity would have similar effects. Therefore, recommendations of milfoil weevil densities required for EWM control should take into account EWM density and growth rate.

In the simple EWM growth model utilized in Model 2, milfoil weevils are the only factor limiting EWM growth. Therefore, in model projections with low weevil density, EWM increases to carrying capacity and reaches densities seldom observed in the field (e.g. Fig. 17d). However, numerous factors can alter local EWM growth dynamics (depth, water clarity, nutrient availability, etc.). Furthermore, the density dependence and herbivory parameters are preliminary estimates (see discussion above). Model 2 projections should be considered general patterns rather than quantitative predictions. An improved EWM growth model and quantitative information on milfoil weevil damage rates will be required to make quantitative predictions.

Model 2 relies on the assumption that EWM growth and additional adult mortality sources depend on temperature similarly to weevil development rate above 10°C. Best and Boyd's (1999) simulation model of EWM growth is based on temperature accumulation >3°C but the relationship between temperature and EWM growth varies in phases through the season where we assumed a linear relationship. Sunfish maximum foraging rate is approximately linearly related to temperature between 10°C and 30°C but foraging rate does not decline linearly below 10°C (Kitchell et al. 1974). Neither EWM growth nor fish foraging rate has a minimum threshold of 10°C whereas milfoil weevils do not reproduce or develop below 10°C. Furthermore, adult milfoil weevils take 6-10 days after feeding in the spring to begin reproduction. The model projections were based on initial populations after weevil reproduction had begun. However, given that fish foraging and EWM growth are not limited below 10°C, the spring cool-water period before reproduction begins and when development is slow is likely a critical period for milfoil weevil populations. Use of milfoil weevils to control EWM may be best suited for locations where water temperature warms rapidly to >10°C in spring.

The potential for longer-term (across year) effects of weevil herbivory on EWM growth is not addressed in the models. Relatively low densities of milfoil weevils may result in decreased over-winter survival of EWM even if within-season control is not apparent (Newman and Biesboer 2000). Across-year effects may be an important mechanism in observed EWM declines; Model 2 suggests that if initial EWM density the following year were lower, milfoil weevil control would be more effective.

There are many other interactions that are not addressed in Model 2 that may have implications for EWM control. Sunfish foraging is less efficient at high macrophyte density; therefore, dense EWM may be both a refuge and a resource for milfoil weevils. Across years, there are potential feedbacks between EWM growth and sunfish population structure due to the effect of macrophytes on sunfish foraging and growth, and the refuge dense EWM offers sunfish from its predators (Olson et al. 1998).

These models offer guidance for the use of milfoil weevils in managing EWM. Potential sources of adult mortality should be evaluated when considering expensive milfoil weevil population augmentations. Lakes with high sunfish density may not be suited for biological control of EWM using milfoil weevils if rapid, within-year control is the goal. However, there are many potential improvements to these models that will require integration of an improved EWM growth model and more detailed information on weevil herbivory.

Plant community manipulation:

Resampling of the plots that were manipulated in 1998 was conducted in early and late summer 1999. In Lake Auburn, Eurasian watermilfoil composed a lower percentage of the plant community in all plots in 1999 (Table 20) compared to 1998, when Eurasian watermilfoil composed from 22 to 49% of plant biomass. Coontail was the dominant plant is all the treatments.

Somewhat surprisingly, Eurasian watermilfoil biomass was higher in the remove MSP plots than in control plots, however, 2-way ANOVAs revealed no significant differences in plant biomass among dates or treatments. The mean number of species per sample did decrease between July and August 1999 ($p<0.08$). One way analyses for each date also revealed no significant differences due to treatment.

Table 20. Mean biomass ± 1SE (g wet/m^2) of all plants (Total), Eurasian watermilfoil (MSP), all other plants (NAT) and the most common plants (coontail (CRT), and sago pondweed (PEC)) by treatment for the plant community manipulation at Lake Auburn 1999. The percent of total plant biomass composed by MSP and CRT along with the mean number of species per sample (Spec) are also given. Treatments were: No removal (Control), Remove all plants (Remall), remove Eurasian watermilfoil (RemMSP) and remove all plants except MSP (Remnat). Plant manipulations occurred in August 1998. n = 4 plots per treatment.

Date	Treatment	Total	MSP	CRT	PEC	NAT	%MSP	%CRT	Spec
7/1/99	Control	1844	124	1573	93	1666	6.0%	84.2%	2.4
		333	83	367	93	300	3.7%	8.9%	0.4
7/1/99	REMALL	1060	186	795	0	795	10.8%	82.0%	2.4
		262	178	266	0	266	10.1%	14.9%	0.5
7/1/99	REMMSP	1972	521	1145	0	1151	19.0%	67.2%	2.5
		872	464	441	0	437	10.5%	10.6%	0.2
7/1/99	REMNAT	2676	1085	1475	17	1496	38.9%	56.6%	2.9
		966	484	514	17	507	13.6%	13.6%	0.6
8/18/99	Control	1851	620	1212	1	1231	28.4%	69.7%	2.0
		532	333	242	1	245	13.6%	12.8%	0.4
8/18/99	REMALL	1308	663	644	0	644	35.2%	64.8%	1.8
		435	589	273	0	273	22.2%	22.2%	0.3
8/18/99	REMMSP	1669	676	981	0	987	39.2%	59.9%	2.3
		863	394	495	0	494	10.5%	10.5%	0.1
8/18/99	REMNAT	1621	337	1264	12	1284	17.0%	76.6%	2.3
		431	130	311	12	305	5.3%	1.5%	0.1

Otter Lake had a more diverse plant community (Table 21) and was not dominated by either Eurasian watermilfoil or coontail. Two-way ANOVAs indicated a significant treatment effect on coontail ($p<0.02$). One way ANOVAs indicated this effect was apparent in August when coontail was significantly more abundant in the remove milfoil plots than the control and remove all plots. No other treatment effects were significant. Although both total plant biomass and biomass of native species was highest in the remove MSP plots during both seasons, high variability amongst plots resulted in a significant effect only for coontail. In fact, milfoil biomass was also highest in these plots suggesting that removal of milfoil in the previous year enhanced growth of all plants.

Table 21. Mean biomass ± 1SE (g wet/m^2) of all plants (Total), Eurasian watermilfoil (MSP), all other plants (NAT) and the most common plants (coontail (CRT), *Elodea* (ELD), *Zosterella dubia* (*Heteranthera* (HET)), flatstem pondweed (PZS), sago pondweed (PEC), *Potamogeton richardsonii* and *P. praelongus* combined (PRI*) and *P. robinsii* (PRO)) by treatment for the plant community manipulation at Otter Lake, 1999. The percent of total plant biomass composed by MSP along with the mean number of species per sample (Spec) are also given. Treatments were: No removal (Control), Remove all plants (Remall), remove Eurasian watermilfoil (RemMSP) and remove all plants except MSP (Remnat). Plant manipulations occurred in August 1998. n = 4 plots per treatment.

Treat	Total	MSP	CRT	ELD	PZS	HET	PEC	PRI*	PRO	NAT	%Spic	Spec.
6/18/99												
Control	1632	449	42	715	4	412	0	0	0	1173	38.6%	3.5
	509	366	23	478	4	345	0	0	0	547	20.0%	0.6
Remall	2043	820	38	963	0	77	3	31	0	1222	34.0%	4.5
	928	450	18	602	0	39	3	31	0	597	14.8%	0.7
RemMSP	3416	1292	102	701	17	1155	0	130	0	2109	38.2%	4.9
	1254	633	51	509	17	626	0	130	0	838	13.9%	0.7
Remnat	1794	161	34	1262	0	239	0	0	0	1626	8.0%	3.5
	723	88	12	707	0	165	0	0	0	638	1.7%	0.2
8/20/99												
Control	1896	867	46	512	242	0	13	0	196	1029	32.4%	3.6
	871	397	36	311	177	0	13	0	103	475	11.3%	0.8
Remall	1691	305	26	220	1040	0	17	50	12	1386	21.5%	4.7
	894	273	14	89	995	0	17	50	12	991	18.7%	0.8
RemMSP	2824	1096	480	315	612	0	4	38	261	1728	27.3%	4.5
	1026	484	268	129	91	0	4	38	261	551	6.1%	0.5
Remnat	1702	244	68	398	552	0	168	2	131	1458	18.9%	4.5
	610	99	36	201	377	0	168	2	131	513	2.8%	0.5

In 2001, new plots were established earlier in the growing season (early June). At Lake Auburn, the community was dominated by coontail and Eurasian watermilfoil (Table 22). The plant removals were successful at manipulating the plant community; total plant biomass was reduced in the removal all treatment and milfoil biomass was reduced in the remove milfoil treatment. Due to high variability among the plots, no significant date or treatment effects were found for plant biomass with a 2-way ANOVA (treatment by date). There was, however, a significant treatment effect on the percent Eurasian watermilfoil ($p = 0.1$) and percent coontail ($p=0.05$); coontail composed a greater proportion of the community in the remove all and remove MSP treatments and MSP composed a lower percentage in these treatments relative to control and remove native treatments. These results suggest that coontail was able to quickly colonize and take advantage of removal of MSP and that proportional representation of MSP was reduced through the summer in the plots from which it was removed. An analysis of the changes in responses over the season (difference of pre vs post manipulation samples) also showed no significant effects with plant biomass but confirmed the effect on the contribution of coontail ($p=0.06$); the proportion of coontail increased after removal in the remove all treatment and decreased in the control treatment.

The difference analysis also showed a significant treatment effect on number of species ($p= 0.06$); the number of species increased in the control and declined in the remove all treatment. Analysis (one way ANOVA on treatment) of the September samples supported the previous analyses. Due to high variability among plots no significant differences in total, MSP, or non-MSP biomass were found but significant effects (both $p<0.05$) on the proportion of MSP and CRT were noted. The proportion of MSP was lower and the proportion of CRT higher in the remove all and remove MSP treatments than in the control and remove natives treatments. In the lower diversity and poorer water clarity system of Lake Auburn, Eurasian watermilfoil retained dominance in the control or when natives were removed, but coontail was able to become dominant where Eurasian watermilfoil was removed, even in the remove all treatment.

Table 22. Mean biomass ± 1SE (g wet/m^2) of all plants (Total), Eurasian watermilfoil (MSP), all other plants (NAT) and the most common plants (coontail (CRT), flatstem pondweed (PZS), sago pondweed (PEC) and *Nymphaea* (NMP)) by treatment for the plant community manipulation at Lake Auburn 2001. The percent of total plant biomass composed by MSP and CRT along with the mean number of stems (per m^2) and number of species per sample (Spec) are also given. Treatments were: No removal (Contr), Remove all plants (Remall), remove Eurasian watermilfoil (RemMSP) and remove all plants except MSP (Remnat). Plant manipulations occurred just after the initial sampling in June. n = 5 plots per treatment.

Treat	Total	MSP	CRT	PZS	PEC	NMP	NAT	%MSP	%CRT	Stems	Spec.
6/13/01											
Contr	2120	990	1017	0	0	113	1129	45.4%	51.3%	113	1.8
	271	309	370	0	0	113	441	12.9%	10.9%	39	0.2
Remall	3063	1186	1616	5	0	256	1877	39.6%	54.7%	84	2.3
	546	465	534	5	0	208	731	13.2%	10.9%	24	0.1
RemMSP	2398	956	1439	3	0	0	1442	39.3%	60.6%	140	2.0
	219	193	366	3	0	0	369	11.5%	11.4%	51	0.2
Remnat	2907	1335	1393	0	0	179	1572	41.8%	51.4%	127	2.2
	399	400	458	0	0	75	427	11.3%	13.0%	34	0.2
9/21/01											
Contr	3052	2254	712	0	64	22	798	63.8%	30.8%	268	2.6
	1420	1392	301	0	50	22	291	12.7%	13.2%	131	0.2
Remall	1273	116	1153	4	0	0	1158	15.3%	84.5%	47	1.8
	214	81	274	4	0	0	276	12.7%	12.6%	47	0.2
RemMSP	1897	394	1479	19	5	0	1503	18.5%	78.8%	60	2.2
	360	235	341	19	5	0	329	11.5%	10.9%	60	0.5
Remnat	3404	2277	816	0	0	311	1128	56.8%	37.3%	146	2.0
	1052	1094	296	0	0	311	449	18.1%	18.8%	60	0.3

Otter Lake had a much more diverse plant community (Table 23) with 3 to 6 species per sample commonly collected. Date was a more significant factor in Otter Lake; total plant biomass declined significantly from June to September ($p < 0.001$) and this was primarily due to a

significant decline in Eurasian watermilfoil from over 500 g wet/m^2 to less than 20 g wet/m^2. There was no significant effect of session or treatment on non-Eurasian watermilfoil biomass or biomass of other species. The decline in milfoil was likely due to weevil damage (see above). The percent contribution of Eurasian decreased and the percent coontail increased from June to September and the mean number of species also decreased over time (all $p < 0.05$) but no significant treatment effects were found for these variables. Analysis of the change in plant biomass (difference between June and September) revealed significant effects of treatment on native plant biomass, but not other response variables. Native biomass increased in the remove milfoil plots and decreased in the remove all plots, suggesting that despite the low density of Eurasian watermilfoil it was competing with the native plants. Had milfoil not been suppressed in all the plots by weevil activity, the effects of competition may have been more evident.

Table 23. Mean biomass ± 1SE (g wet/m^2) of all plants (Total), Eurasian watermilfoil (MSP), all other plants (NAT) and the most common plants (coontail (CRT), *Elodea* (ELD), *Najas* (NAJ), flatstem pondweed (PZS), sago pondweed (PEC), Richardson's pondweed (PRI) and *Chara* (CHA)) by treatment for the plant community manipulation at Otter Lake 2001. The percent of total plant biomass composed by MSP along with the mean number of species per sample (Spec) are also given. Treatments were: No removal (Contr), Remove all plants (Remall), remove Eurasian watermilfoil (RemMSP) and remove all plants except MSP (Remnat). Plant manipulations occurred just after the initial sampling in June. n = 5 plots per treatment.

Treat	Total	MSP	CRT	ELD	PZS	NAJ	PEC	PRI	CHA	NAT	%Spic	Spec.
6/7/01												
Contr	1572	526	238	367	206	32	0	71	131	532	37.9%	5.8
	269	232	154	167	96	21	0	20	81	242	16.6%	0.1
Remall	1233	418	84	134	150	421	0	22	2	816	40.7%	4.7
	385	134	29	96	80	325	0	14	2	373	14.1%	0.6
RemMSP	1286	444	149	344	301	40	0	0	8	404	40.5%	4.8
	289	200	64	237	99	37	0	0	7	199	15.3%	0.3
Remnat	1392	788	140	165	214	10	0	0	72	604	65.7%	5.1
	225	164	91	131	130	5	0	0	70	330	12.5%	0.1
9/20/01												
Contr	716	26	155	207	33	120	7	14	92	690	2.2%	3.9
	208	26	68	133	16	59	7	12	92	204	2.2%	0.5
Remall	279	4	90	91	9	35	0	7	0	275	1.8%	3.1
	107	4	47	55	8	27	0	7	0	108	1.8%	0.4
RemMSP	758	1	171	176	48	216	67	64	0	757	0.1%	3.7
	321	1	73	69	23	114	67	59	0	320	0.1%	0.9
Remnat	545	5	44	136	52	179	29	19	40	540	1.0%	3.8
	198	5	14	77	45	81	24	9	40	198	1.0%	0.5

Influence of milfoil genotype and rearing sediment on weevil performance:

Analysis of the sediment showed a significant effect (two-way ANOVA, $\alpha = .05$) of sediment source on bulk density, percent water, and organic content and a significant effect of plant source on NH4+ concentration. Cedar sediment had lower bulk density and higher organics than Otter sediment and Cedar plants appeared to result in lower ammonium concentrations (Table 24). Despite the big differences in sediment character, no significant effects were found for hatch, larval, pupal or egg to adult development for either sediment or plant source (Fig. 19). There were also no significant differences in stem diameter at pupation. Factors such as bulk density and organic content can affect plant growth, however, we purposely grew the plants to similar size to eliminate plant size effects. The lack of a significant plant or sediment source effect on weevil development in 1999 suggests that plant size or stem diameter (not measured in 1998) may be more important that other measures of plant quality. In fact, no differences in plant nitrogen content were noted among the treatments.

Table 24. Sediment ammonium (mg/L), bulk density (g dm/ ml) and organic matter for sediment used in the plant and sediment source rearing experiment.

Sediment/Plant	NH4+	% water	bulk density	% organic
OtterSed/CedarPlant	0.72	46.13%	0.805	1.08%
OtterSed/OtterPlant	4.17	45.15%	0.823	0.92%
CedarSed/CedarPlant	1.26	75.38%	0.299	1.23%
CedarSed/OtterPlant	3.53	75.52%	0.281	1.26%

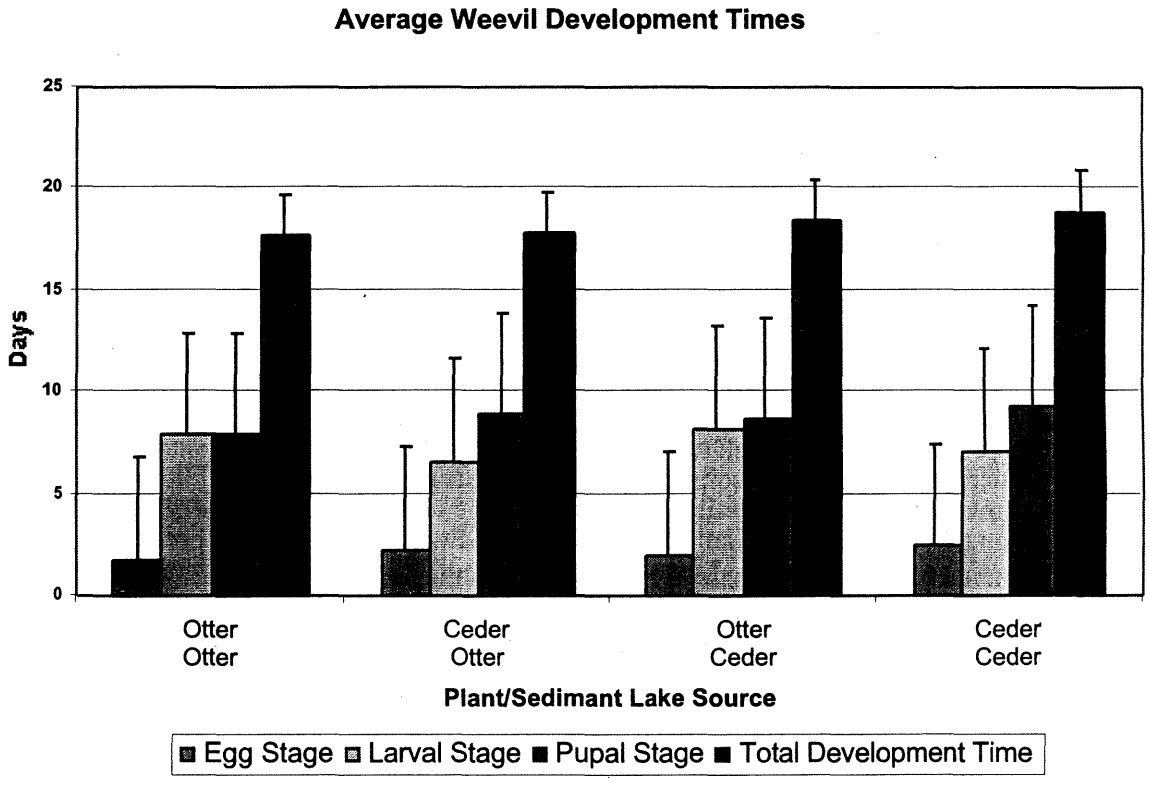

Figure 19. Influence of sediment and plant source (Cedar or Otter) on milfoil weevil development times. There was no effect of sediment or plant source on development time, survival or adult eclosion mass.

Summary

We have now documented two declines clearly attributable to weevil stem mining (Cenaiko and Otter). We also have evidence that weevil damage, at least in the shallower sites, at Lake Auburn and Smith's Bay have reduced milfoil abundance, but the persistence of these declines depends in part on the persistence of weevil populations. In Lake Auburn, weevils disappeared in July 1998 and were not present in 1999. Although the milfoil continued to decline during this time, perhaps due to poor water clarity or competition with other plants, it increased in 2000 and 2001 when weevils returned but at very low densities (< 6/m^2). It appears that there have been two declines in Lake Auburn, the first between 1993 and 1994 (see our earlier reports) and the second in 1996-1997. However, high weevil populations did not persist after each decline and milfoil subsequently rebounded. The decline of weevils populations was almost certainly not due to lack of milfoil because much higher densities of weevils have been maintained in Cenaiko and Otter Lakes with much less milfoil persisting after a decline.

The response of Lake Auburn remains puzzling. The early season decline of milfoil in 1998 was associated with relatively low weevil densities but much apparent damage (personal observation). The cause of weevil population crash is unknown, but the poor light conditions probably prevented regrowth of milfoil and other plants. The weevils returned in 2000 and although they did not reach high densities the population increased and persisted through the summer. It appeared that weevil populations might recover in 2001, but densities declined below detection in August and September. Due to poor visibility it is difficult to tell if sunfish populations are high, however surveys conducted by Pothoven (1996) in Cedar and Auburn suggest similar high densities of sunfish in both lakes during 1993-1995, with sunfish increasing from 1993 to 1995. DNR Fisheries surveys reported 62 bluegill per trapnet in Auburn in 1995; this density increased to 110 per trapnet in 2000. In some ways, the recent milfoil decline is similar to that observed in 1993; weevil populations declined in 1995 and milfoil increased to record levels. It remains to be seen weevil populations will recover and if milfoil will remain suppressed, at least below the high densities of the mid 1990s. If not, we suspect milfoil will continue its increase in 2002.

The decline at Cenaiko Lake has persisted through 2001; milfoil remained at < 75g/m^2 during 2000 and 2001 and did not exceed 8% of total plant biomass. It is not certain what permits development of high weevil populations in Cenaiko Lake, however, low predation by sunfish appears to be an important factor. All life stages persist throughout the summer and adult densities in September 2001 were as high as seen all summer, although densities in 2001 were lower than previous years. It is unclear if this lower density was due to high water levels or an increasing sunfish population. Observations in 2002 should clarify this, however, we are concerned that sunfish populations may be increasing and causing the decrease in weevil densities compared to previous years. It does appear that the response of the native plant community in Cenaiko is also important in suppressing Eurasian watermilfoil, however the apparent decrease in diversity in 2001, perhaps associated with high water levels, is also cause for concern. Coontail appears to becoming increasingly dominant component of the plant community and it may be less able to suppress growth of Eurasian watermilfoil.

The longer and much less dramatic suppression of Eurasian watermilfoil continued at Smith's Bay. The June 2001 milfoil biomass (31 g dry/m^2) was the lowest we have seen there since sampling began in the early 1990's. Milfoil did increase through the summer to moderate levels and weevil densities failed to increase to previous levels. At the shallowest sites milfoil remains suppressed and native plants dominate. Northern watermilfoil has returned to the shallowest stations and it also supports weevils. At deeper sites, with little evidence of weevil damage, Eurasian watermilfoil remains quite dense, but well beneath the surface. Weevils do not appear to be a factor regulating milfoil biomass beyond 200-300m from short (or water deeper than 2.5m). We do not have good estimates of sunfish densities at Smith's Bay.

Milfoil increased greatly at Otter Lake in the spring of 2000, to a biomass similar to historic highs, but weevil populations increased and the milfoil declined and remained below 90 g dry/m^2

in 2000. The decline continued in 2001 with high weevil densities. By August 2001, milfoil was < $2g/m^2$ and 7% of total plant biomass. Furthermore, as noted from our removal plots, the decline was not localized but occurred throughout the lake. This decline indicates that weevils can suppress the plant at Otter Lake. Climatic factors may have been generally favorable to weevils in 2000-2001 because these are the highest density of weevils we have observed at Otter. A more likely explanation is low sunfish densities. DNR Fisheries surveys in Otter in 1997 indicated a low density of bluegills (2.1 per trapnet) and even lower densities were found in 2001 (<1/trapnet) following a suspected winter kill. Unfortunately, the Fisheries surveys are not frequent enough to determine if fish populations were higher in the mid 1990s when milfoil was dense. It is clear that the weevil population did not rapidly increase until spring 2000 when milfoil became abundant, although it should be noted that although weevil density per m^2 was low prior to this, weevil abundance per stem in 1997 and 1998 was much higher than in previous years at Otter Lake.

In Cedar Lake, fair water clarity and the very low weevil densities have resulted in a continued high density Eurasian watermilfoil that persists through the summer. Although we found higher weevil densities in 2000 and 2001 than in previous years, weevil and other herbivore densities at Cedar remain the lowest among our regularly sampled lakes and populations decline rather than increase over the summer. DNR fisheries surveys have consistently indicated a high density of bluegills at Cedar Lake (60-90 per trapnet) and the other lakes in the Minneapolis Chain-of-Lakes.

Milfoil and plant densities varied with time and among our survey sites but we have little evidence that weevils or other herbivores were instrumental in these changes. At Lake-of-the-Isles, clarity is clearly an important factor. The alum treatment appears to be less important at improving water clarity that the early development of an extensive plant bed which may further promote summer-long clarity. At Gray's Bay and Shady Island, competition with native plants species may be important. There was no clear effect of alum treatments on native plants in the city lakes and alum treatments in Cedar may have reduced previously seen late summer decreases in milfoil due to poor water clarity. Weevil and herbivore densities remained low or below detection at all of the survey sites.

Sediment may affect plant growth and quality but when plants were reared to the same size we found no effect of plant genotype (Otter vs Cedar) or rearing sediment on plant nutrient content or weevil performance. Further investigation of plant quality effects may be worth pursuing but in this case fecundity or oviposition rate should also be studied. Overall, we have no evidence that differences in plants or rearing sediments among lakes explain differences in weevil populations.

The results of our plant community manipulations show some subtle effects but few major effects within a growing season. Removal of milfoil seems to promote growth of all plants including milfoil and coontail is clearly an important player in plant community dynamics. Field observations do suggest that competition and maintenance of native plants (other than coontail) is important for sustained biological control and we will continue to monitor and manipulate more sites. In addition we are examining the potential importance of exchangable nitrogen for plant community dynamics and this may be an important factor for nuisance levels of milfoil.

Our fish predation experiments indicate that predation by sunfish can be important at limiting both weevil and other herbivore populations. Immigration and emigration may have masked larger changes in our relatively small scale cage experiments. However, both the fish experiments and our models stress the potential importance of fish predation (or other mortality sources) on adult longevity and subsequent population size. Larger scale observations may help to define limiting levels of sunfish density.

Finally, our work and that of others is starting to demonstrate that weevil populations do best in large expanses of milfoil or at shallower sites rather than on steep edges of the bed. Tamayo et al. (2000) found that milfoil beds with weevils were shallower than beds without weevils. Jester et al. (2000) found that milfoil weevil abundance was negatively associated with depth. This effect was not due to a greater distance from shore preventing weevil access to plants because they also found that weevil abundance was positively correlated with distance from shore to the middle and deep edges of the plant bed, but was not related to distance to the shallow edge of the bed. Thus weevil populations may be higher in large shallow expanses of milfoil rather than steep shoreline

with plants below the surface (Jester et al. 2000). Lillie (2000) found the highest densities of weevils and greatest damage in the shallow and middle portions of beds and much lower densities at the deep edges. Johnson et al. (2000) found weevil densities negatively correlated with lake depth and size and suggested that the milfoil weevil is more suited to smaller and shallower lakes rather than large deep lakes. Our work also shows higher density (and more control) at shallower sites, but this does not appear to be related to distance from shore. Deeper plants may provide less refuge for the weevil than plants that approach the surface, both from access to fish predation and to wave action. Deeper plants may also be less accessible to adults that would need to dive to reach the plants.

A key to success in Cenaiko, Otter and Smith's Bay appears to be the summer-long persistence or increase in weevil density, particularly adults, which in the past, has not been maintained at the other lakes. Our modelling results also suggest adult longevity is key to both population density and to potential suppression of Eurasian watermilfoil. Before herbivorous insects can be used as biological control agents factors limiting their populations must be identified. Our work is making progress at identifying the most likely limiting factors.

Previous research in Auburn and Smith's Bay suggests that overwinter mortality is not limiting and that in-lake factors are more important (Newman et al. 2001). Although overwinter habitat may be limiting (Jester et al. 2000, Tamayo et al. 2000), it does not appear limiting at most of our intensive study sites. It could be an important limiting factor in some of the Minneapolis Chain-of-Lakes (e.g., Calhoun and Harriet) but extensive wooded and undeveloped areas along Cedar Lake and Lake-of-the-Isles suggest that overwinter habitat is not limiting in these lakes either. Furthermore, parasites and parasitoids do not appear important, at least at Lake Auburn and Smith's Bay (Newman et al. 2001) and our plant quality experiments suggest that plant source and rearing sediment may not be important limiting factors. It does appear that stem size and perhaps plant growth could be limiting but these are more likely artifacts of small experimental plants rather than the larger (e.g. >0.5m long) plants typically found in the field. We did not evaluate fecundity of adult weevils reared on different Eurasian watermilfoil populations grown on different sediments and this factor might be worth exploring. Results comparing Eurasian and northern watermilfoil (Newman 2002) suggested that realized fecundity is much higher on Eurasian than northern watermilfoil so there is a potential for differences in weevil population development between Eurasian watermilfoil populations. Fish may also indirectly influence weevil populations by reducing oviposition rate; Ward (2002) speculated that apparent reduced oviposition in the presence of sunfish could be a factor resulting in lower populations in the presence of sunfish.

Factors limiting larval and pupal life stages could also be important and little work has been done on predators of juvenile stages (Creed 2000). However, our modelling results and the life history of the weevil suggests that juvenile mortality would need to be extremely high to be as influential as adult mortality. In addition we have no evidence that invertebrate predators are affecting weevil populations and Ward (2002) actually found a significant positive correlation of weevil larvae with zygopteran nymphs.

The fish exclusion/enclosure results in Cedar and Otter Lakes further suggest that fish may be limiting weevil populations and our population models underscore the importance of female reproductive longevity on summer-long population density. Small increases in adult mortality, which would be explained by relatively small increases in fish predation, can have a disproportionate effect on population size. This is because females lay only a few eggs per day. If predation by sunfish is further shown to be an important limiting factor, and levels of sunfish that permit development of adequate weevil populations can be determined, it may be feasible to explore fisheries enhancements to the sunfish population and size structure through enhancement of predator populations or fishing regulations. It would be particularly fortuitous if enhancing sport fishing populations would aid in the biological control of Eurasian watermilfoil. We will collate additional fisheries information to determine if there is any relationship between sunfish density and weevil densities. Unfortunately, the typical 5 or more years between fisheries surveys may not capture important changes in fish populations. For example, sunfish density in Cenaiko declined from 95 per trapnet in 1992 to 5 per trapnet in 1998. This decline is consistent with the high weevil density found in 1996, but does not allow us to draw direct relationships between

sunfish and weevil density.

It is possible that other herbivores in addition to the milfoil weevil are affecting milfoil populations. Johnson et al. (1998, 2000) have shown milfoil declines in New York associated with high densities of *Acentria*. They suggest that in many lakes *Acentria* may be more important than the milfoil weevil and they also suggested competition between *Acentria* and *Euhrychiopsis*. *Acentria* and *Parapoynx* have been at low densities in all of our lakes with the exception of Cenaiko Lake and, in 1996-1997, Otter Lake. The high densities in Otter Lake (20-100 per m^2) were noted the summer following the decline of milfoil when milfoil densities ranged from not detectable to <25 g wet/m^2. Most caterpillars were associated with plants other than Eurasian watermilfoil. Furthermore, although caterpillar were found in 2000 and 2001, the densities were low, particularly in mid summer when the greatest declines occurred. Thus, the caterpillars may be assisting with milfoil suppression following a decline but we have little evidence that they are initiating declines. Furthermore, if fish predation is limiting weevil densities it likely would limit caterpillar densities (see also Ward 2002). We do not have high caterpillar densities in our lakes that have few weevils and high sunfish densities. More analysis of these interactions is required and a comparison of weevil and fish densities among lakes is planned. .

Two conditions are needed for successful biological control of weeds: adequate agent densities and a negative response of the target to the control agent (Newman et al. 1998). At sites with persistent control of milfoil, the native plant community has expanded. It is also clear that at many of our sites weevil populations have not built to adequate densities, although weevil densities in 2000 appeared higher in all lakes, and these populations appear to have at least contained milfoil growth in all except Cedar and Auburn during 2000. Cenaiko Lake and now Otter Lake provide clear examples of the potential for high weevil populations and subsequent effects on milfoil in one or two years. Given the potential for population increase in the summer, and the lack of a strong correlation between in-lake and onshore densities, it does not appear that overwinter populations are the main limiting factor (Newman et al. 2001) at least at Lake Auburn and Smith's Bay where detectible populations have been found in early summer each year. Fish exclusion experiments suggest that fish predation may be an important factor.

Although our experiments manipulating plant community structure are still inconclusive, field observations suggest that positive native plant response is variable and may also be affecting sustained control. In lakes with many rooted plant species, milfoil may not be abe to expand or recover as quickly after it is damaged by herbivores. We will continue experiments and observations on this topic.

It is clear that we do not yet have adequate information to reliably predict if and when insects will cause declines in milfoil populations or if the declines will persist (Creed 2000). It is also clear that milfoil suppression can be obtained given adequate densities of weevils throughout the summer, and perhaps positive plant community response. On-going focused research should shed additional light on the factors that regulate weevil populations and their effects on plant communities. Once these factors have clearly been identified, management strategies, such as piscivore enhancement or water clarity improvements can be tested to determine their feasibility for enhancing the biological control of Eurasian watermilfoil.

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Appendix I. Abbreviations and dry mass (g/m^2) of plants collected from 1994 through 2001.

Key to plant abbreviations used in this report.

CHA	*Chara* spp. (muskgrass)
CRT	*Ceratophyllum demersum* (coontail)
ELD	*Elodea canadensis* (Canada waterweed)
HET	*Heteranthera dubia* (mud plantain) = *Zosterella dubia*
LMR	*Lemna minor* (lesser duckweed)
LTR	*Lemna trisulca* (star duckweed)
MGD	*Megalodonta beckii* (water marigold)
MSI	*Myriophyllum sibiricum* (northern watermilfoil)
MSP	*Myriophyllum spicatum* (Eurasian watermilfoil)
NAJ	*Najas* spp.
NMP	*Nymphaea* spp.
NUP	*Nuphar* spp.
PAM	*Potamogeton amplifolius* (largeleaf pondweed)
PBE	*Potamogeton berchtoldi* (Berchtolds' pondweed)
PCR	*Potamogeton crispus* (curled pondweed)
PDI	*Potamogeton diversifolius*
PEC	*Potamogeton pectinatus* (sage pondweed)
PFO	*Potamogeton foliosus* (leafy pondweed)
PGR	*Potamogeton gramineus* (variable pondweed)
PIL	*Potamogeton illinoensis* (Illinois pondweed)
PNA	*Potamogeton natans*(floating leaf pondweed)
PNO	*Potamogeton nodosus* (river pondweed)
PRI	*Potamogeton richardsonii* (claspingleaf pondweed)
PRO	*Potamogeton robbinsii* (Robins' pondweed)
PSP	*Potamogeton spirillus* (snailedseed pondweed)
PZS	*Potamogeton zosteriformis* (flatstem pondweed)
RAN	*Ranunculus* spp. (white water buttercup)
SPO	*Spirodela polyrhiza* (greater duckweed)
VAL	*Vallisneria americana* (wild celery)
UTV	*Utricularia vulgaris* (bladderwort)

Biological Control of Purple Loosestrife

Final Report

by

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I. Abstract.

To facilitate biological control purple loosestrife (*Lythrum salicaria*) we undertook a mass rearing program of the root weevil, *Hylobius transversovittatus*. Other insects have been successfully reared and released in the purple loosestrife biological control program include, two species of leaf feeding beetles, *Galerucella calmariensis* and *G. pusilla*. The root weevil proved to be far more challenging to rear and although several hundred adults were successfully reared and used in collaborative research or released into wetlands in Minnesota, 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. *Hylobius* weevils were as active in plants with a history of *Galerucella* defoliation as without. Initial studies with a newly isolated fungal isolate resulted in documented reductions in growth of purple loosestrife plants. However, later experiments conducted with the isolate did not produce consistent results. This may have been caused by changes in the fungal isolate while in culture which led to a reduction in pathogenicity. 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. The majority of these research results will be published in appropriate scientific journals.

II. Background.

Purple loosestrife is a perennial emergent wetland plant introduced into North America from Europe. In this proposal, the common name, purple loosestrife, will refer to weedy, naturalized populations of the taxa, *Lythrum salicaria* and *Lythrum virgatum*. Purple loosestrife occurs north of the 35th parallel in the contiguous United States and Canada (Stuckey, 1980; Anderson and Ascher, 1993). Purple loosestrife is extremely abundant in the glaciated area of eastern North America, and with the exception of the Colorado, Arkansas and the Rio Grande rivers, has colonized all major watersheds of the arid west (Thompson et al., 1987). It is found in all Canadian provinces with the exception of the Yukon and North-West Territories (Mal et al., 1992). Purple loosestrife is a popular garden plant and often escapes into nearby wetlands and establishes along the edges of rivers and ponds, in roadside ditches, in low, wet meadows and marshes and other disturbed sites (Stuckey, 1980). Due to the growing concern of its spread, purple loosestrife has been declared a primary noxious weed in Minnesota, and similar or less restrictive legislation has been enacted in other states (Anderson and Ascher, 1993; Rendall, 1989).

Exotic European populations of *Lythrum*, cultivars of *Lythrum* grown in North America, as well as native *Lythrum* spp. may all contribute traits to weedy populations of purple loosestrife through introgression or gene flow among species. Morphological and isozyme analysis of Minnesota populations of purple loosestrife and native *L. alatum* provide evidence of introgression between the two species (Anderson and Ascher 1993, 1994a,b; Strefeler et al., 1996a,b).

Weediness of purple loosestrife has been confirmed by the fact that it has displaced valuable wetland plant species as an extremely successful colonizer of disturbed wetland ecosystems in North America. It is also adaptable to a wide range of soil nutrient levels (Shamsi and Whitehead 1973b, 1977) and soil types (Thompson et al., 1987). Purple loosestrife has the ability to make morphological adjustments as a result of changes to its immediate environment. For example, under flooded conditions, submerged stems develop aerenchyma tissue, and under decreased light levels, leaf size increases (Shamsi and Whitehead, 1974).

Purple loosestrife is highly competitive (Rawinski and Malecki, 1984) and in a controlled experiment, purple loosestrife had a higher relative competitive ability as compared to wetland species such as *Cyperus*, *Juncus* and *Eleocharis* (Johansson and Keddy, 1991). In a related study, purple loosestrife had a higher competitive ability, as a function of biomass, than 44 common wetland species including broad-leaved cattail (*Typha latifolia*) (Gaudet and Keddy, 1988), a species often associated with purple loosestrife (Thompson et al., 1987). In field experiments, stem densities of purple loosestrife increased while stem densities of cattail (*Typha* spp.) decreased as a result of establishment of purple loosestrife seedlings on moist exposed areas of a marsh (Rawinski and Malecki, 1984). Lythrum seedlings were shown to be very competitive, but inundative seedings of Italian ryegrass (*Lolium perenne* var. aristatum) did reduce its presence (Welling and Becker, 1993). Other stressors, such as biological control insects, are purported to do the same in an established, perenniated system.

Purple loosestrife is a herbaceous perennial and forms a woody crown which sends up new shoots every year, however, lateral spread of the crown is limited and averages one-half meter (Shamsi and Whitehead 1973a; Thompson et al., 1987). Seed dispersal is the major source of spread of this weed. Purple loosestrife plants are prolific in their seed production, averaging 2.7 million seeds per plant (Thompson et al., 1987) which results in the formation of an extensive seedbank (Welling and Becker, 1990). Once present in the seedbank, seedlings of purple loosestrife recruit more successfully than native species (Welling and Becker, 1993). In North America, plants form dense monospecific stands and crowd out native wetland species (Mal et al., 1992; McKeon, 1959) which result in a decline in species diversity and extinction of rare species (Moore and Keddy, 1989). However, in the presence of native predators and diseases in Europe, the pattern of community dynamics is different. With natural predators or diseases present, purple loosestrife plants may form dense monospecific stands in areas of disturbance but within a few years become mix-species stands (Shamsi and Whitehead, 1973a).

In Minnesota, the purple loosestrife biological control program has focused on rearing and releasing the leaf beetles, *Galerucella calmariensis* and *G. pusilla*. Currently, there are over 500 sites throughout the state where *Galerucella* spp. have been released. Reproducing populations of leaf beetles are present in a majority of these sites. Although some sites have a substantial area where defoliation is severe and native plant vegetation competing with purple loosestrife, other sites are less affected. In Europe, a suite of insects is commonly associated with declines in purple loosestrife. A total of five insect species have been approved by the USDA for release in the United States for purple loosestrife biological control. The remaining three species of insects are in the family Curculionidae (weevils). Two weevils feed directly on the seed capsules and the third feeds as a larva on the root crowns. This root feeding weevil, *Hylobius transversovittatus* is the focus of this study. To date, this weevil has been released in several

Minnesota wetlands by carefully placing eggs in or near the root crown. Unfortunately, no adults have been observed in any of the release sites, although a few larvae have been recovered from infested root crowns. It appears that releasing eggs results in high larval mortality and consequently, low adult survival. Even if a few larvae do survive, their population density is too low to result in establishment of *Hylobius*.

As reported by Katovich et al. (1999) purple loosestrife plants accumulate storage carbohydrates, such as starch, in late summer and fall, require minimal amounts of carbohydrates for respiration and maintenance growth during the winter, and use stored carbohydrate reserves the following spring to provide energy for the growth of developing shoots.

It was hypothesized that anything which disrupts the production and storage of carbohydrates in the roots and crowns of purple loosestrife could have an impact on the survivability of this species from one year to the next. Root and crown boring weevils, such as *Hylobius* are such disruptions which could significantly lower the production, translocation and storage of sugars or starch in the roots and crowns.

Reduction in levels of root and crown carbohydrates are an indication of plant stress (Wargo et al., 1972). Carbohydrate levels in roots and crowns of purple loosestrife were not consistently reduced by *Galerucella* feeding, even after two years of nearly complete leaf defoliation by the beetles (Katovich et al., 1999). The combination of *Hylobius* and *Galerucella* feeding on different plant parts may provide enough stress to cause plant mortality. The impact of previous *Galerucella* leaf defoliation on *Hylobius* performance in crowns of purple loosestrife is also not known.

Habitat has aided or impeded the success of introduced biological control agents. Remote sensing may enhance the study of the interaction of wetland type and the success or failure of biological control agent introductions. Remote sensing technology has been used successfully to track the spread of invasive rangeland weed populations such as yellow starthistle (*Centaurea solstitialis*) and St. Johnswort (*Hypericum perforatum*) (Lass et al., 1996) . Purple loosestrife stands have also been successfully detected using standard aerial color photography (Frazier and Moore, 1993; Balogh and Bookhout, 1989). During peak flowering, it is possible to discriminate flowering purple loosestrife plants from other background wetland vegetation. Global positioning systems (GPS) can be used to record site and boundary data directly from the field and when linked to geographic information systems (GIS), data can be combined and generated into maps (Lass and Callihan, 1993). Integrating remote sensing, GPS and GIS technologies could enable one to determine the impact of *Galerucella* spp. on purple loosestrife stands. This is of particular value since purple loosestrife stands occur in remote and inaccessible areas which make accurate assessment on the ground expensive, labor intensive, and at times potentially hazardous.

III. Methodology

Result I. Development of an Artificial Rearing Protocol for *Hylobius.*

An analysis of weed biological control programs in north temperate climates demonstrates that the two most important insect families involved in successful biological control program are Curculionidae and Chrysomelidae (Andow, Ragsdale and Nyvall, 1997). Although *Galerucella* spp. have been established throughout Minnesota, and some purple loosestrife stands have been substantially reduced by the leaf feeding beetles alone, it may take the root feeding weevil to obtain complete biological control throughout the weed's range. For that reason we focused on establishing this insect in Minnesota. We proposed to continue the developement of artificial rearing methods for *Hylobius,* to evaluate the impact of *Hylobius* on rootstock survival, to determine if there was an interaction between *Galerucella* spp. and *Hylobius*, and to release lab reared *Hylobius* into cages erected in Minnesota wetlands.

Diet preparation. In December 1998, approximately 25 purple loosestrife root crowns were collected and washed to remove excess dirt and most of the epidermal layer, leaving only exposed root tissue. Dead root parts were clipped away and fibrous roots were easily removed following a short drying time. Clean, clipped roots were stored in water until they were chipped. Clean root crowns were then mulched using a chipper/shredder. The mulch was stored in a Ziplock® freezer bag at –70° C.

After 24 h at –70° C the mulch was pulverized and passed through a series of wire mesh screens and the material that was 1 mm or less in size was collected. The root mulch was ground to a fine powder using a 1 L commercial Waring® blender. Approximately 240 ml of root crown mulch was placed into the blender along with an equal amount of chipped dry ice. The dry ice was used to dissipate any heat generated in the process of grinding the mulch into a fine powder. The finished product or "root crown powder" was kept in a cooler on dry ice so it remained frozen during processing. The "root crown powder" was stored at –70° C until needed. The root crown powder is an essential ingredient in the *Hylobious* diet developed by Bernd Blossey at Cornell University. An artificial diet was prepared, dispensed into 30 ml plastic insect rearing cups (Bioserve®) and stored in plastic bags at 4°C until needed.

Hylobious rearing protocol. On December 10, 1998 we received 23 *Hylobious* adults and 30 eggs from Cornell University to begin our colony. The adults (13 females and 10 males) were placed in metal screened cages inside a plant growth chamber and allowed *ad lib.* access to cut stems of purple loosestrife. Cut stems were inserted into florist foam and adult weevils readily oviposited in the stem and foam material. The plant growth chamber was set at 17°C with a 16:8 h light:dark cycle. The environmental chamber contained a Plexiglass® panel that reduced the amount of harmful ultraviolet light being emitted from the light source since ultraviolet light is considered detrimental to *Hybolious* adult and egg development (Blossey, pers. comm.). *Hylobious transversovittatus* eggs that were shipped to us from New York had been placed on a water agar plate. We placed the agar plant into a darkened incubator set at room temperature. Within a few days egg hatch began and we transferred first instar larvae into diet cups by placing them in a depression in the surface of the diet made by a sterile pin. We used a camel's hair brush to transfer the larvae that had been sterilized by emersing it in 70% ethanol for several

minutes. Once the larvae were in the diet cups, lids were sealed with parafilm and diet cups were placed in a plastic container in which the bottom was lined with a with a damp cloth to elevate the relative humidity. *Hylobius* larvae were incubated at 27°C that was maintained without light.

***Hylobious* egg collection**. Screen cages housing the ovipositing adults were cleaned periodically of any beetle excrement. Purple loosestrife bouquets (stems and florist foam) were replaced weekly and eggs were harvested each week. Because *Hylobius* adults are nocturnal it was observed that adults lay more eggs when disturbed less, thus time between cage cleanings and replacement of purple loosestrife bouquets was increased to 2 weeks. Eggs of *Hylobius* were collected from the stems and florist foam by first removing them from the screen cage and storing the foam and stems overnight at room temperature. Doing so helped the egg's chorion laid the previous night to sclerotize before we handled the eggs. Eggs were removed from the purple loosestrife stems by splitting the stems and gently lifting eggs out with a paintbrush. Eggs were removed from the florist foam by gently scratching the surface at an angle with the paintbrush thus exposing the eggs. Eggs were then placed on a water agar plate. The edges of the water agar plates were sealed with parafilm®, covered with a paper towel and stored in an incubator prior to egg hatch. *Hylobious* eggs were incubated at room temperature for 7-14 d with no light source. Newly hatched larvae were transferred to diet cups as described previously and each cup was dated.

Revised rearing protocol.

Diet cups were assessed every 2-4 wk for contamination and larval development. As larvae in the diet grew, as evidenced by the tunneling in the diet, Parafilm® and plastic bags were removed to allow for air exchange for the remainder of larval development. Less than 2% of the diet cups were thrown away due to contamination indicating our rearing procedures and diet development was as sterile as possible. After 12 wk (June 1999) over 70% of the diet cups were discarded because there was no evidence of further development of *Hylobious* larvae. Because of high mortality and slow larval development, we began a systematic evaluation of the artificial diet. Many of the large larvae had molted into a larval-pupal intermediate indicating a key nutrient was missing in the diet. A careful review of diet preparation resulted in the following modifications to our diet preparations.

Variations of diet ingredients and protocol. After an unsuccessful attempt at reproducing adult *Hylobius* in artificial diet prepared according to the Cornell University protocol we began a systematic variation in the diet suggested by various people who rear insects on artificial diet. We varied procedures for preparing the diet, diet ingredients and sources of essential ingredients.

In March 1999, we obtained purple loosestrife root crown powder prepared by B. Blossey at Cornell University and 50 diet cups were prepared with the only change in procedure being replacing the University of Minnesota source of root crown powder used in previous experiments. In May, an additional 50 diet cups were made by reducing the temperature of the autoclaved ingredients to 60°C before adding the remaining vitamins. This change in procedure was warranted after reviewing diet preparation steps looking for areas where diet ingredients could be degraded, as preliminary results indicated the diet lacked some key essential ingredient. Such a change would result in a diet that retained essential nutrients and other heat labile

ingredients necessary for larval development and adult emergence. The third variable we tested was relative humidity. The comparison was with a saturated environment (ca. 95% RH) vs. a relative humidity approaching 35%.

A more thorough evaluation of diet ingredients was undertaken and we varied key ingredients of the original protocol and tested for differences among diets as measured by adult emergence (Guthrie et al. 1985). We chose to vary these six ingredients based on conversations with scientists at other laboratories across the country who have extensive experience rearing a wide variety of insects on artificial diets. Each diet variation was made with root crown powder prepared as prepared in December 1999 and designated the University of Minnesota source. Each diet treatment was autoclaved and then cooled for 20 min at 60°C prior to adding any vitamins or other materials that cannot be autoclaved. We used 30 diet cups of each variation to evaluate the change in diet ingredients (Table 1). Wheat Germ is known to provide essential fatty acids and is a component of many insect diets, cholesterol or the backbone sterol must be provided to insects as they cannot synthesize this ingredient *de novo* and sterol hormones such as ecdysone appeared to be lacking in previous diets. Ascorbic acid was increased to prevent bacterial contamination and water volume was increased to keep the diet in a higher moisture state than had been used previously.

Table 1. Variations the *Hylobius* artificial diet.

Diet Additives (Color Code)	**Wheat Germ (g)**	**Cholesterol (g)**	**Ascorbic Acid (g)**	**Water Volume (ml)**	**pH**
All additional ingredients added (yellow)	41.4	2.4	9.6	600	4.0
Wheat Germ (orange)	20.7	0.0	0.0	300	4.5
Cholesterol (white)	0.0	1.2	0.0	300	4.5
Ascorbic Acid (red)	0.0	0.0	3.3	300	4.0
Standard Diet? (blue)	41.7	2.4	9.6	600	4.0
2X Wheat Germ (green)	82.8	2.4	9.6	600	4.5

All diet cups were covered with Parafilm® and placed in an autoclave bag containing moist paper towels. Larvae were added each day to an equal number of experimental diets until all diet cups were used (replication over time). The diet cups were then placed in the environmental chamber at 27°C and a pan of water was placed in the bottom of chamber to maintain a high relative humidity.

Comparison of NY and MN rearing protocol. In mid-December of 1999, A. Milles visited Cornell University in Ithaca, NY to observe rearing protocols used in this laboratory and to set up an experiment comparing diet prepared in Minnesota and diet prepared in New York. The objectives were to test for differences in diet preparation techniques and rearing conditions between MN and NY and to observe any differences in environmental factors, diet preparation, or other factors that might be contributing to the low *Hylobious* production observed in Minnesota.

In addition to the study described above, we also tested three sources of purple loosestrife root crown powder. Root crowns from two Minnesota sites (a wetland within the Minneapolis-St. Paul metro area where the insecticide altosid is commonly used in wetlands for mosquito control and a wetland located in Sherburne county, Minnesota outside the metro area where no mosquito control is practiced. The third source was from New York. Diet cups were prepared according to the protocol developed at Cornell University. Each treatment contained 120 replicates with a total of 360 diet cups. To limit variables due to multiple handling, the laboratory technician at Cornell made all of the diet cups, and A. Milles placed a newly hatched larva from Cornell's *Hylobius* colony onto each diet cup. Half of the larvae in their respective diet cups remained at Cornell and the other half returned with A. Milles to the University of Minnesota where an environmental chamber was set to the identical parameters as the chamber at Cornell. *Hylobius* larvae were observed one week after placing them in the environmental chamber to observe for evidence of larval tunneling and to ensure that the Minnesota cohort survived transport from New York. Following this initial inspection, diet cups were kept in an environmental chamber consistent with rearing conditions observed at Cornell (25°C, 16:8, light:dark, 33% RH) and were undisturbed for five weeks. Beginning six weeks from the date larvae were placed in diet cups, weevil development was assessed weekly until adult eclosion. Twenty-nine of the 180 diet cups being incubated in Minnesota were discarded following the first week due to high levels of fungal contamination during diet preparation at Cornell. Those that remained in the study did not develop any further fungal contamination.

Rearing location. An additional experiment was conducted to compare environmental conditions involved during the long larval incubation period. The objective of the experiment was to determine if conditions in Hodson Hall were for some reason not conducive to larval development. It was observed that insect control procedures were being practiced in the hallway and in adjacent labs and there was a slight possibility that cockroach controls practiced in the building could be interfering with *Hylobius* growth and development. By taking a set of diet cups out of the building and rearing them away from any possible contamination we could determine if Hodson Hall was the source of our rearing problem.

Diet cups were prepared according to the Cornell protocol using two sources of root crown powder (NY and MN metro). Eggs and subsequent first instar larvae were from the established *Hylobius* colony located at the University of Minnesota. Approximately 45 diet cups of each root crown source were made for each experiment. As *Hylobius* larvae became available, they were placed on the previously prepared diet. Diet cups and larvae at the off-campus location were kept in a dark closet with an average temperature of 68°F and 15-20%

relative humidity. Diet cups located in the laboratory were kept at the same environmental chamber used in previous attempts to rear the insects with conditions set as described earlier.

Result 2. Criteria for establishing *H. tranversovittatus* in *Galerucella* spp. stressed and non-stressed purple loosestrfie plants.

Since *Galerucella* spp. are well established in many Minnesota wetlands, it will be important to ascertain how *Hylobius* perform on purple loosestrife plants in the presence of Galerucella and on plants previously stressed by *Galerucella* leaf defoliation. *Hylobius* is potentially the most important of the biological agents introduced to the United States to date for the control of purple loosestrife. It is also one of the more difficult insect biological control agents to study because of its reproductive cycle. Once inoculated with eggs, it may require one to two years for adult weevils to emerge. Since *Hylobius* has such a long generation time, it will be important to know whether the establishment of *Hylobius* on purple loosestrife crowns is impeded by the presence of *Galerucella.* If *Hylobius* does not establish successfully in wetlands previously infested with *Galerucella,* natural resource managers will need to consider releasing *Hylobius* into wetlands where *Galerucella* has not yet become established.

Previous work (Katovich et al., 1999) had shown that root reserves, particularly starch, were reduced as intensity and duration of *Galerucella* defoliation continued. We hypothesized that *Hylobius* activity would be lower on root crowns which have experienced one or more years of *Galerucella* defoliation. Root and crown starch levels were monitored and used as physiological indicators of plant stress to better understand the impact of *Hylobius* alone and in combination with *Galerucella.*

This experiment was initiated in the spring of 1999 and repeated in the spring of 2000. In early May, purple loosestrife root crowns were harvested from Circle Lake (Rice County) where *Galerucella* populations have caused a minimum of four years of severe leaf defoliation. Purple loosestrife plants with no previous *Galerucella* feeding stress were collected in an area of Circle Lake where the insects had not yet spread as indicated by the presence of inflorescences remaining from the previous year.

These purple loosestrife crowns were planted in a standard potting mix into pots and placed into wading pools. Treatments were as follows: *Hylobius* only, *Hylobius* in combination with *Galerucella* and control plants. Each set of treatments was randomly assigned to plants with and without previous *Galerucella* feeding. All treatments were repeated on the same plants the following year so that each plant received two seasons of treatment. In early May 1999, 10 adult lab reared *Hylobius* were placed on plants in screened cages. When there was evidence of ample egg laying, adult *Hylobius* were removed. Prior to the second year of the study, in 2000, extra treated crowns from the previous year were dissected and checked for the presence of *Hylobius* larvae. Larvae were present so the addition of adult *Hylobius* was not required for the second season. When the study was repeated in 2000, 8 adult *Hylobius* were added to each plant in May and June. Again, adult *Hylobius* were removed when there was evidence of ample egg laying. *Hylobius* adults were not released on plants in the spring of 2001, the second year of the 2000 study, as numerous larvae were found when purple loosestrife crowns were dissected.

Plants with *Galerucella* defoliation treatments were caged after adult feeding and egg laying. Plant assigned to control and *Hylobius* treatments were caged at the same time.

For all years of the study, feral adult *Galerucella* were the source of insects for *Galerucella* treatments. In the summers of 2000 and 2001, control plants were treated with soil applications of a systemic insecticide to prevent unwanted *Galerucella* spp or *Hylobius* feeding. Screened cages were removed from all plants after the first generation of *Galerucella* spp. had emerged to enable seed set. In late October of 2000 and 2001, the experiments were harvested and purple loosestrife roots and crowns were sampled as described in Katovich et al. (1999). Root and crown samples were freeze-dried and analyzed with Near Infrared Reflectance Spectrometry (NIRS) for determination of starch levels (Katovich, 1999).

Result 3. Effect of wetland type on successful establishment of purple loosestrife biocontrol agents.

The objective of this study was to 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 used the National Wetlands Inventory System based on the wetland classification system of Cowardin et al. (1979). This study explored the correlation between success of *Galerucella* establishment and wetland type. The success of *Galerucella* establishment has been monitored in up to 120 releases sites to date by DNR personnel (Luke Skinner, personal communication). The success of *Galerucella* spp. establishment and defoliation was layered the digitized National Wetland Inventory data for Minnesota with GIS to determine wetland type. It was then determined whether there were relationships between wetland type and success of Galerucella populations.

A second study explored the possibility of using aerial photography of purple loosestrife to delineate the impact and spread of Galerucella in selected release sites. Remote sensing using color aerial photography (Lass and Callihan, 1993; Balogh and Bookhout, 1989) has been successfully used to delineate purple loosestrife stands. Areas of Galerucella feeding can easily be mapped because flowers are not produced on purple loosestrife plants with high levels of Galerucella defoliation. Balough and Bookhout used aerial slides taken yearly by the USDA Farm Services Administration, on a county by county basis, to record the presence of purple loosestrife in Ohio. The use of remote sensing could allow fast and efficient characterization of the impact of *Galerucella* in wetland habitats which often are inaccessible, or require considerable time and labor to access on the ground.

For this study, we explored the possibility of determining the spread of *Galerucella* by examining existing yearly aerial photos taken by USDA Farm Services Administration at Circle Lake in Rice County. At this site, adult and larval *Galerucella* feeding resulted in a tremendous reduction in number of purple loosestrife inflorescences (Katovich et al., 2001). We examined aerial photographs taken by USDA- FSA at this location because aerial photographs had been taken annually from1992, prior to release of *Galerucella* beetles to 1998, after suppression of flowering. Due to the expense of aerial photography, we wanted to explore whether existing aerial photos could be used for our study.

Result 4. 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. Previous work by Welling and Becker (1990) delineated the importance of purple loosestrife seedbanks in recruitment and establishment in wetlands. A biological control agent, such as *N. marmoratus,* could reduce the numbers of seed in the seedbank by reducing seed production.

In early July of each year, 12 purple loosestrife inflorescences were tagged at early flowering when adult *N. marmoratus* were feeding on developing inflorescences. Only plants with adult *N. marmoratus* present were tagged. From 1997 through 2000, plants were tagged in a wetland at Larpenteur and Century Ave. in St. Paul. Plants were also tagged at a wetland at Afton, MN from 1998 through 2000. Additional plants not damaged by *N. marmoratus* (with all seed capsules present) were tagged later in the season at both sites after seed capsules had formed to serve as control plants. In late September of each year, all tagged shoots were harvested at each site. The number of seed capsules were counted on each inflorescence.

Result 5. Development of a plant pathogen of purple loosestrife

In the spring of 1996, it was observed that greenhouse grown plants of the purple loosestrife cultivar 'Morden Gleam' were wilting and dying from what appeared to be a plant disease. The Morden Gleam cultivar is a hybrid created by crossing two species of *Lythrum*; *Lythrum alatum* and *Lythrum virgatum*, different species from the weedy purple loosestrife (*Lythrum salicaria*). An unidentified fungus was isolated from diseased plant tissue. The isolated fungus was cultured and then sprayed onto weedy purple loosestrife plants.

Preliminary experiments had shown relative ease of gaining infection with the fungus on weedy purple loosestrife plants and they developed similar symptoms as plants from the Morden Gleam cultivar. Initial studies indicated complete necrosis of above ground shoots and death of weedy purple loosestrife plants. The fungus caused the formation of a debilitating stem canker at the leaf axil of an individual infected leaf. Additional experiments with the fungus also resulted in disease symptoms on weedy purple loosestrife and indicated that additional research was warranted. Exploratory work was conducted with the fungus as a biocontrol agent.

Studies were designed to determine the effect of the fungus on plant growth the year of inoculation, as well as in succeeding years.

<u>Seedling growth stage study</u>. A study was designed to determine the effect of fungus inoculation on purple loosestrife seedlings at different stages of growth, specifically at one and two months after emergence when plants were at the vegetative and early flower stage of growth respectively. Plants were grown in the greenhouse in the spring of 1999. When plants were one or two months old, they were sprayed with the fungus in a liquid formulation at a spore population of 2×10^6 spores/ml with 1% (v/v) methylated soybean oil. Plants were sprayed with a hand held sprayer then placed into a dew chamber for 24 hours. Plants were then placed outside in plastic wading pools. Shoots and regrowth were harvested and dry weight obtained.

The experiment was replicated 4 times.

Winter survival study. A study was initiated to determine the effect of overwintering on purple loosestrife plants sprayed with fungus. In the spring, purple loosestrife crowns were dug from a wetland, planted into pots and placed into children's wading pools for continual sub-irrigation. In early September, plants were sprayed with a plant mister with the fungus at a spore population of 4 X 10^6 spores/ml in combination with water and 3% methylated soy bean oil (v/v). After spraying, plants were placed into a dew chamber for 24 hours, then placed outside into plastic wading pools. Control plants were not sprayed with fungus. Plants were mulched and overwintered to determine whether the fungus re-infected plants year following inoculation. Purple loosestrife shoots were harvested in late June of 2000, shoot regrowth was harvested in August 2000 and shoot dry weights were obtained for both harvesting dates. The experiment was replicated 7 times.

Field study. A field study, was initiated to determine the efficacy of the fungus in a wetland environment. In late September 1999, the fungus was sprayed with a hand held sprayer at a spore population of 4 X 10^6 spores/ml in combination with water and 1% methylated soybean oil (v/v) at the base of 10 purple loosestrife plants growing in a wetland. Ten additional plants growing in the same wetland were selected as controls and were not sprayed. In the summer of 2000, treated and untreated plants were evaluated for shoot height, shoot number, as well as presence of visual disease symptoms.

Greenhouse Study. A greenhouse study was initiated to determine whether creating entry wounds on purple loosestrife plants would increase activity of the fungus. The tops of greenhouse grown plants in the flowering stage were removed to create entry wounds for the fungus prior to spraying. Plants were sprayed with the original isolate of the fungus at a rate of 7 X 10^5 spores/ml with 1% (v/v) methylated soybean oil. Sprayed plants were placed in the dew chamber for 24 hours. Control plants were not sprayed. One month after spraying, shoots were removed. After 3 months, regrowth dry weights were obtained. The experiment was replicated 12 times.

Result 6. Seedling Survival Study

The objective of this experiment was to determine spring survival of overwintered purple loosestrife seedlings planted at weekly intervals in late summer in Minnesota. Results of this study would enable wetland managers to understand how purple loosestrife seedlings present one season contribute to stands of purple loosestrife the succeeding season. Results of this study have been submitted to *Weed Science* for publication (draft of paper is attached).

IV. Results and Products.

This project investigated the impact of insect biocontrol agents on purple loosestrife, to improve the effectiveness and expedite the realization of benefits from the release of these agents in reducing purple loosestrife populations in Minnesota. Work was done with *Galerucella calmariensis* and *G. pusilla, Hylobius* and *Nanophyes marmoratus*, biocontrol agents introduced

into North America from Europe. Rearing of *Hylobius* on an artificial diet was refined and *Hylobius* was released into Minnesota wetlands. Root feeding by *Hylobius* and subsequent effects on purple loosestrife populations through degradation of crown root carbohydrate storage structures was explored. The presence of *Galerucella* spp. on the performance of *Hylobius* was also be investigated. With the foundation laid with carbohydrate work on this project, we can better determine the expected outcome of successful *Hylobius* establishment in Minnesota wetlands. Other aspects of this research examined the impact of *N. marmoratus* on seed production and the pathogenicity of a previously unidentified fungus on shoots and crowns of purple loosestrife. Use of remote sensing as a tool to determine the suppression of flowering caused by *Galerucella* spp. on purple loosestrife was also examined.

Result I. Development of Artificial Rearing Protocol for *Hylobius*.

Diet preparation. None of the variations in the standard diet improved adult production. Neither the source of the root crown powder (NY or MN) nor cooling diet prior to adding the vitamin mixture following autoclaving improved the number of adult *Hylobius* reared. Only 2 deformed adults were produced by the termination of the experiment on 8 October, 2000.

Specifically, percent larval survival was very low when the standard diet was amended with a combination of wheat germ, ascorbic acid, and cholesterol or in a diet in which wheat germ was added at 2X. Initial larval survival was good in the standard diet amended with cholesterol, ascorbic acid or 1X wheat germ, but relatively few adults emerged (Table 2). The final results of this study were as follows: 1 adult and 1 half larva/half pupa were found in diet containing just wheat germ (orange label); 1 desiccated adult was found in diet containing just cholesterol (white label); and 2 adults in diet containing additional ascorbic acid (red label). The final results showed no advantage to any specific diet ingredient(s), however, it did suggest that the environmental chamber may be a factor contributing to improper insect development.

Table 2. Percent survival of *Hylobius* larvae to a late instar larvae using various amendments to the standard Cornell diet, August 9, 1999.

Diet Amendment	All Additives	1X Wheat Germ	Cholesterol	Ascorbic Acid	All Additives	2X Wheat Germ, 1X other additives
% Larval Survival	3	82	96	97	45	0

Comparison of NY and MN rearing protocol. In a separate study we compared diets prepared at Cornell University with a diet prepared at the University of Minnesota using the same constituents and using a common source of larvae (eggs hatched at the University of Minnesota). No adults were produced in either diet. There was evidence of development of late instar larvae, but no pupae or adults were found at the termination of the experiment.

Rearing of *Hylobius* at the U of MN improved after A. Milles returned from Cornell University where she observed their protocols and participated in diet preparation. Production of

Hylobius adults was similar among the three diets and two locations the insects were reared at (Tables 3 and 4). The only modification done in the rearing protocol involved maintaining relative humidity at 35% in Minnesota (similar to the level at Cornell) and not surface sterilizing the paintbrush after each larval transfer (from water agar to diet). In our previous attempts relative humidity was maintained in excess of 75% and may have contributed to poor larval survival. However, elevated RH cannot explain the appearance of larval-pupal intermediates. Prior to lowering the percent humidity, we observed water pooling in tunnels produced by neonate larvae resulting in apparent drowning of the small larvae soon after they were placed on the diet surface. After lowering the relative humidity, larval survival increased.

Comparison of the three sources of purple loosestrife root crown powder did not seem to make a difference in the number of adults produced (Table 4). Similarly, mortality between those insects reared in New York and those reared in environmental chambers in Minnesota was not statistically different (χ^2=3.18, 1 df, n.s P=0.05). Overall, there were no differences in the percent adult production between the MN/NY diets or the percentage typically reared at Cornell University (40-60%) (Table 4).

Table 3. Comparison of environmental chambers between Minnesota and New York used to rear *Hylobius*. Conditions were: 25°C, 35% RH, 16:8 (light:dark).

Treatment	Proportion of larvae that eclosed as adults	Proportion alive larvae and adults at the termination of the experiment	Proportion of dead larvae at the termination of the experiment
MN Growth Chamber	40	62	36
NY Growth Chamber	51	75	25

Table 4. Comparison of three sources of purple loosestrife root crown powder with respect to the proportion of larvae that eclosed as adults.

Root crown Source	Proportion of live adults at the end of the experiment
MN (Metro)	31%
MN Sherburne County	35%
NY	34%

Rearing location. To further isolate the possible reason for low adult production larvae were reared in a location off campus in a heated but uncontrolled environment. The experiment produced adults at both locations with the cooler temperatures in the home closet likely resulting in fewer adults produced (Table 5). It appears that either the subtle changes in diet preparation observed at Cornell improved the quality of the diet and that where insects were reared did not substantially affect mortality (Table 6). There was no significant difference in mortality using two different sources of purple loosestrife root powder in these experiments further corroborating the results of the experiment conducted in tandem between Cornell University and the University of Minnesota.

Table 5. Effect of rearing location on successful eclosion of *Hylobius*.

	Percent larvae alive and live adults produced in different rearing location	
	Hodson Hall growth chamber	Home (closet)
Adults produced	70%	36%
Alive (larvae and adults)	86%	81%
Dead	14%	19%

Table 6. Percent live adults and larvae found in the root crown sources used for both the off-campus and laboratory experiment.

Root Crown Source	Percent of larvae resulting in live adults	
	Lab (MN growth chamber)	Home (closet)
MN Metro	56%	52%
NY	44%	48%

In summary, rearing of *Hylobius* is time consuming and fraught with problems some of which has not been completely identified. The life cycle of this insect is long and as such this represents unique problems with mass rearing. It appears the insects are sensitive to moisture with relative humidity requirements above 35% for maximum survival but humidity in excess of 90% can slow development and cause substantial mortality. Moreover, because the artificial diet is not completely defined, i.e, the diet requires a substantial amount of pulverized purple loosestrife root crown tissue for the insects to successfully emerge as an adult. Our experiments concluded that the three sources of root crown powder did not differ substantially in quality, but that this key ingredient was essential yet difficult to prepare in large quantities needed for mass rearing. It is unclear what component of the root crown powder is needed but likely it is more a phagostimulant effect than an essential nutrient. The rearing procedures that we used in Minnesota did not differ substantially than those used at Cornell University but it would require substantial investment in incubator space to mass rear sufficient quantities of *Hylobius transversovittatus* for distribution statewide. Given the long time needed for adult eclosion (>12 weeks), the large amount of incubator space needed, the need to keep adults that emerge in winter in reproductive diapause, sensitivity of larvae to disturbance during larval development and the need to maintain relative humidity above 35% but below 75% makes this insect a poor candidate for a mass rearing program.

Addenda:

1999 *Hylobius* Production. A total of 950 eggs were collected from the purple loosestrife bouquets and florist foam used in the rearing cage containing *Hylobius transversovittatus* adults New York. A total of 840 (88%) of the eggs that were collected hatched on water agar plates and only 275 larvae (29%) developed into later instars. The 275 large larvae remained on diet cups in the environmental chamber, and only one completed its life cycle on May 3, 1999 after fifteen weeks of incubation. The newly emerged adult was placed in the rearing cage with the others for egg laying.

2000 *Hylobius* Production.

Diet Variations. A total of 153 adults were successfully produced from the diet experiments described in the body of the final report. These adults were fed on mature purple loosestrife plants for 4 weeks before they were recollected and used for collaborative experimental field plots. It is difficult to explain exactly what conditions allowed for the successful rearing following the visit to Cornell University. There was no apparent difference among the three sources of root crown powder nor any interference with larval maturation depending upon where insects were reared. The most significant effect was the change in relative humidity with a reduction from 75% to 35%. Regardless, artificial rearing did not result in a high level of production and with the time of development exceeding 3 months mass rearing of this insect is at best limited.

Mass Rearing. Additional mass rearing began April 2000, to produce adults for release in future field trials. Root crowns from a MN metro site were chipped and ground to powder for diet ingredients, and diet cups were made according to protocol. A total of 457 adults were produced, of which only 40 were used to supplement experimental plots in which competition between *Galerucella calmariensis* and *Hylobius transversovittatus* was being monitored (see report by Drs. Katovich and Becker). We were able to release 263 adults at a wetland site in New Brighton throughout the month of August. In October, it was considered too late in the season for any newly emerged adults to be released in the field and successfully overwinter, so we decided to keep the remaining 133 adults in the lab and prepare them to overwinter in the refrigerator. The adults then could be released in the spring or be used in subsequent experiments. The 133 adults were kept in the rearing cage in the environmental chamber with a bouquet of loosestrife stems for food, a temperature of 18°C and a 12:12 L:D (light:dark). Beginning Oct. 5, daylight was reduced by 2h every week until it was only 6h, and then they were completely shut off. The temperature was reduced after 3 weeks to 12°C and reduced by 2°C every week thereafter until 8°C was reached. On November 28, 2000 the rearing cage was cleaned and the *Hylobius* were placed in a plastic container filled with moist florist foam and a mesh lid. They were returned to the environmental chamber with 8°C and no daylight. There apparently was insufficient moisture in the containers and no adults survived when they were checked for morbidity in February 2001.

Result 2. Criteria for establishing *H. tranversovittatus* in *Galerucella* spp. stressed and non-stressed purple loosestrfie plants.

In general, starch levels of purple loosestrife crowns were higher in control plants compared with plants with *Hylobius* or *Hylobius* plus *Galerucella* feeding (Table 7 and Table 8). Starch levels of roots from Trial 2 proved to be the exception to this, where no differences were found among control plants and insect treatments. Previous experiments (Katovich et al., 1998) have shown that purple loosestrife crowns are more responsive to stress than roots. No differences were found between levels of starch in roots or crowns between the *Hylobius* only or *Hylobius* plus *Galerucella* treatment. These results demonstrate that *Hylobius* larvae alone are able with stress crowns of purple loosestrife after two years of feeding. *Galerucella* spp. feeding did not reduce *Hylobius* larvae activity, as measured by root and crown starch levels. *Hylobius* weevils

were as active in plants with a history of *Galerucella* defoliation as without. This was demonstrated by the fact that there was no significant effect in starch levels between plants with or without a history of previous *Galerucella* spp. feeding.

Table 7. Effect of *Hylobius* plus *Galerucella* spp. feeding on starch levels in roots and crowns of purple loosestrife. 1999-2000.

Treatment	Starch		Shoot dry weight
	(μg starch/mg dry weight)		(g)
	Root	Crown	
Control	320	247	95
Hylobius	285	192	72
Hylobius + *Galerucella* spp.	282	176	15
LSD (0.05)	32	45	26

Table 8. Effect of *Hylobius* plus *Galerucella* spp. feeding on starch levels in roots and crowns of purple loosestrife. 2000-2001.

Treatment	Starch		Shoot dry weight
	(μg starch/mg dry weight)		(g)
	Root	Crown	
Control	428	334	78
Hylobius	373	233	72
Hylobius + *Galerucella* spp.	408	227	21
LSD (0.05)	NS	78	41

Result 3. Effect of wetland type on successful establishment of purple loosestrife biocontrol agents.

Release sites were rated for successful *Galerucella* establishment by Luke Skinner and co-workers at the Department of Natural Resources. Wetland type was determined for each release site and correlated with the successful establishment of *Galerucella* beetles. There were no correlations found among wetland type and success of *Galerucella* establishment. It is thought that this was due to the relatively few sites where beetles had been present for a long enough period to be considered successfully established.

Because of the expense involved in aerial photography, slides routinely taken by the Farm Services Agency of the Circle Lake area from 1992 to 1998 were examined for the presence of purple loosestrife. When aerial slides were viewed for presence of purple loosestrife, it was found that there was not enough color resolution in photographs be able to determine flowering of purple loosestrife plants. *Galerucella* beetle feeding suppresses flowering and it is necessary to photograph purple loosestrife at flowering to determine successful spread of *Galerucella* beetles by aerial photographic means.

Result 4. Impact of previously released *Nanophyes marmoratus* on purple loosestrife seed production.

This study was conducted at the St. Paul and Afton sites during the summers of 1997-2000 and 1998-2000, respectively. At the St. Paul site, there were reductions in number of seed capsules from plants with known activity during all years with the exception of the summer of 2000, however, reductions were not always significant at the 5% level. In the summer of 2000, extensive, *Galerucella* feeding was observed at this site and it was difficult to find control plants which did not have *Galerucella* feeding injury. Feeding by *Galerucella* beetles on shoot tips of purple loosestrife plants may have reduced number of capsules on tagged control plants at the St. Paul site and confounded the results (Table 9). At the Afton site, number of seed capsules was consistently reduced on plants with *N. marmoratus* activity for all three years when compared with control plants (Table 10). Results of this study indicate that *N. marmoratus* is established at both sites and is reducing purple loosestrife seed production at the Afton site.

Table 9. Effect of *Nanophyes marmoratis* on number of seed capsules and inflorescence length in purple loosestrife. 1997-2000. Century and Larpenteur, St. Paul, MN

Treatment	Number of seed capsules				Inflorescence length (cm)			
	1997	1998	1999	2000	1997	1998	1999	2000
Control	101	244	220	124	20	41	28	19
Nanophyes	35	197	141	146	11	38	24	25
LSD (0.05)	58	NS	52	NS	NS	NS	NS	4

Table 10. Effect of *Nanophyes marmoratus* on number of seed capsules and inflorescence length in purple loosestrife. 1998-2000. Afton, MN.

Treatment	Number of seed capsules			Inflorescence length (cm)		
	1998	1999	2000	1998	1999	2000
Control	214	210	133	36	30	21
Nanophyes	141	155	83	46	27	19
LSD (0.05)	51	51	44	9	NS	NS

Result 5. Development of plant pathogen of purple loosestrife.

A. History of development of a plant pathogen on purple loosestrife. As mentioned in the Materials and Methods section, during the spring of 1996 it was observed that greenhouse grown plants of the purple loosestrife cultivar 'Morden Gleam' were dying from what appeared to be a plant disease. The symptomology was described as plant wilting followed by crown death. An unidentified fungus was isolated from diseased plant tissue. The isolated fungus was cultured and then sprayed onto weedy purple loosestrife plants. Preliminary experiments showed relative ease of gaining infection with the fungus on weedy purple loosestrife plants and they developed similar symptoms as plants from the 'Morden Gleam' cultivar. In initial studies, complete necrosis of above ground shoots and death of crown tissue was documented on weedy purple loosestrife plants. Exploratory work was conducted with the fungus as a biocontrol agent.

In 1999, Encore Technologies obtained a licencing agreement from the University of Minnesota for development of the fungus as a bioherbicide. An isolate of the fungus was deposited with the ATCC and the University of Minnesota pursued a patent of the deposited culture. The patent of the ATCC culture was obtained in July 2001 (U. S. Patent Number 6,268,203).

During this period, another isolate of the fungus was sent to Amy Y. Rossman, a mycologist with the Systematic Botany and Mycology Laboratory, USDA Agricultural Research Service, Beltsville, MD. The fungus was identified as a combination of two fungi, *Coniella fragariae* and a previously unidentified fungus that was subsequently named *Harknessia lythri* (Farr and Rossman, 2001) (reprint attached). During the last two years, greenhouse experiments with the individual fungi were conducted by John Gronwald, of the USDA-ARS with field trials to be conducted by Elizabeth Katovich. Initial greenhouse studies have showed inconsistent results with the individual fungi.

B. Experimental results.

<u>Seedling growth stage study.</u> This study was initiated to determine the effect of a fungus application on purple loosestrife seedlings at different stages of growth. When plants in the

vegetative stage of growth were sprayed with the fungus, plants wilted and died approximately one month after treatment. After shoots were cut at soil level, no regrowth occurred on plants treated with the fungus, indicating death of crown tissue (Table). Control plants had greater shoot and regrowth dry weights. Symptoms of the fungus were positively identified on treated plants. When the fungus was sprayed on plants at the early flower bud stage, no disease symptoms were present and there were no differences in shoot or regrowth dry weights (Table 11). Although excellent results were obtained with the fungus sprayed on vegetative plants, repeating the experiment with older plants failed to produce diseased plants. Activity of the fungus was inconsistent from experiment to experiment.

Table 11. Effect of fungus applications at different stages of growth of purple loosestrife seedlings.

Treatment	Shoot dry weight (g)	Regrowth dry weight (g)
Vegetative stage		
fungus	1.0	0
fungus + soybean oil (1% v/v)	1.0	0
control	5.0	4.0
LSD (0.05)	2.0	3.0
Early flower bud		
fungus	15	6
fungus + soybean oil (1% v/v)	16	5
control	15	9
LSD (0.05)	NS	NS

Winter survival study. This study was initiated to determine the effect of a late season application of fungus on survival and growth of purple loosestrife plants the following summer. No visual injury symptoms were evident on plants after inoculation in September of 1999 or in the spring or summer of 2000. There were also no differences in June shoot or August regrowth dry weights between treated and untreated plants the summer following treatment.

Table 12. Effect of late season fungus treatments on survival of purple loosestrife plants. Shoot dry weights and regrowth dry weights the summer following fungus application. 1999-2000.

Treatment	Shoot dry weight (g)	Regrowth dry weight (g)
fungus + soybean oil (3% v/v)	33	24
control	41	25
LSD (0.05)	NS	NS

Field study. This study was initiated to determine the effect of late season application of the fungus on the survival and growth of established purple loosestrife plants in the field. In the summer following inoculation, there were no disease symptoms present on plants treated the previous autumn (Table 13). In addition, no differences were found in shoot number or height.

Table 13. Effect of a late season fungus treatment on purple loosestrife shoot height and number the summer after application. Field study, 1999-2000.

Treatment	Shoot height (cm)	Shoot number
fungus + soybean oil (1% v/v)	198	8
control	173	8
LSD (0.05)	20	8

Greenhouse study. This study was initiated on greenhouse grown purple loosestrife seedlings to determine whether creation of entry wounds would increase activity of the fungus. There were no consistent symptoms of the fungus present on sprayed plants. There were also no differences in regrowth dry weights between purple loosestrife plants treated with fungus and control plants (Table 14).

Table 14. Effect of entry wounds on activity of fungus. Greenhouse study, 2000.

Treatment	Regrowth dry weight (g)
fungus + soybean oil (1% v/v)	14
control	18
LSD (0.05)	NS

Initial studies conducted prior to the 1999-2001 LCMR funding cycle with the original fungal

isolate resulted in documented reductions in growth of purple loosestrife plants. However, later experiments conducted did not produce consistent results. This may have been caused by changes in the fungi while in culture which led to a reduction in pathogenicity. At this time, research on the fungi has been terminated due to lack of success.

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