1997 Project Abstract
For the Period Ending – June 30, 1999
This project was supported by Environmental and Natural Resources Trust Fund (ML 1997, [Chap. 216], Sec. [15], Subd. 15(b)).

TITLE: MINNESOTA RARE MUSSEL CONSERVATION
PROJECT MANAGER: MARK C. HOVE
ORGANIZATION: UNIVERSITY OF MINNESOTA
ADDRESS: 1980 Folwell Avenue, Saint Paul, Minnesota 55108
WEB SITE ADDRESS:
http://www.fw.umn.edu/Personnel/staff/Hove/Freshwater.Mollusk.Collection
(Includes survey results from this project.)
LEGAL CITATION: ML 1997, [Chap. 216], Sec. [15], Subd. 15(b)
APPROPRIATION AMOUNT: $91,000

Statement of Objectives

Freshwater mussels are one of the most threatened groups of animals in Minnesota. To assist natural resource managers with conservation of rare mussels we researched the following objectives:

(1) establish and monitor study refugia in the St. Croix River to protect freshwater mussels from invading zebra mussels,

(2) determine the suitable fish hosts for three threatened Minnesota mussels, and

(3) survey co-occurring mussel and fish resources in northern Minnesota's Little Fork and Big Fork rivers, providing crucial baseline data for water resource managers.

Overall Project Results

Objective 1: Native mussel refuge from zebra mussels

Resource managers have been requesting information on effective methods for relocating freshwater mussels to refugia from zebra mussel infestations, but this information is currently unavailable (Cope and Waller 1995). In 1997 an experimental refuge was established in the St. Croix River to examine the feasibility of relocation as a means of protecting populations of rare mussels from either zebra mussels or as a mitigation technique associated with human influences on river habitats. Analysis of the relocation and reference sites revealed the sites were similar. Based on the 0.25 m^2 quadrats taken, the average density of mussels was approximately 77 mussels/m^2 at each location. Similar numbers of mussels were studied at the two sites, 2344 from relocation site and 2118 from reference site. The main difference between locations is the greater percentage of mussels found dead at the reference site ranging from about 8-10% while at the relocation site the percentage of mussels found dead was only about 3-7%. The fact that there was

Result 3: Fish and mussel surveys in northern Minnesota

Natural resource managers need information on co-occurring mussels and their fish hosts in order to plan for both mixed use (tourism, forestry, agriculture) and conservation of healthy aquatic communities, including native mussels in their native habitats. A major gap in Minnesota's information on co-occurring mussel and fish species is the Rainy River drainage. The fish and mussel fauna of the Big Fork River and Little Fork River drainages in northern Minnesota was surveyed during late summer 1997 and 1998 respectively. Fish were collected using seines, backpack electroshockers, and boomshockers. Mussels were collected using quadrat analysis and timed searches. Ten mussel species and forty-two fish species were observed in the Big Fork River drainage, and eight mussel species and thirty-eight fish species were recorded from the Little Fork River watershed. Several new observations of mussels were made for the first time. Among these include the first recorded occurrence of the state-listed flutedshell from the Hudson Bay drainage. Strong linear relationships between mussel and fish abundance were rare. Strong relationships may be few because the relationships are more complicated. For example, the host requirements for these mussels may be far broader than we realize. In addition, fishes are more mobile than mussels and most species are shorter lived. It may be that the persistence of certain components of a fish community over decades is a more important independent variable. Future data analysis will incorporate cluster analysis and fish data from the 1970s and the 1980s.

Project Results Use and Dissemination

Project results were disseminated to natural resource managers, scientists, and the public via presentations, the Internet, and various publications with the goal of improving water resource management.

Date of Report: July 1, 1999

Date of Final Status Report: July 1, 1999
Date of Workprogram Approval: July 1, 1997
Project Completion Date: June 30, 1999

LCMR Final Work Program Update Report

I. Project Title: MINNESOTA RARE MUSSEL CONSERVATION

Project Manager: Mark C. Hove
Affiliation: University of Minnesota
Mailing Address: 1980 Folwell Avenue, Saint Paul, Minnesota 55108
Telephone Number: (612) 624-3019 E-mail: Mark.Hove@fw.umn.edu
Fax: (612) 625-5299

Web Page address:
http://www.fw.umn.edu/Personnel/staff/Hove/Freshwater.Mollusk.Collection
(Includes results from this project.)

Total Biennial Project Budget:

$ LCMR:	$91,000
- $ LCMR Amount Spent	$91,000
= $ LCMR Balance:	$0

A. Legal Citation: ML 97, [Chap. 216], Sec. [15], Subd. 15(b)

Project Number: K2
Subd. Fisheries MINNESOTA RARE MUSSEL CONSERVATION

Appropriation Language:

This appropriation is from the trust fund to the University of Minnesota to establish and monitor refugia in the St. Croix River to improve freshwater mussel conservation.

II. Project Summary and Results:

Native mussels are one of the most threatened groups of animals in Minnesota. To assist natural resource managers with conservation of rare species we researched the following objectives: 1) establish and monitor study refugia in the St. Croix River to protect freshwater mussels from invading zebra mussels, 2) determine the suitable fish hosts for three threatened Minnesota mussels, and 3) survey co-occurring mussel and fish resources in northern Minnesota's Little Fork and Big Fork rivers, providing crucial baseline data for water resource managers. Via coordination with the Macalester College, US Geological Survey (USGS), and National Park Service (NPS), we developed and tested a technique for establishing refugia as a means of conserving freshwater mussel biodiversity in the St. Croix River. This involved: relocating mussels; identifying possible refugia sites; and testing the effectiveness of these refugia. In summary it appears that there were few adverse impacts of relocation of mussels on either the mussels relocated or on the receiving mussel community. This study has all of the criteria suggested by Cope and Waller (1995) for an optimal relocation study. Dan Hornbach, Macalester College, and I will continue this monitoring effort for at least two more years.

We determined suitable host fish requirements of several rare mussels living in the St. Croix and Mississippi rivers, and the host fish requirements of a rare mussel found in the Zumbro, Cannon, and Root rivers. Laboratory studies revealed the mussel larvae of three rare, large-river mussels appear to have specific hosts in the catfish family. The stream-dwelling ellipse has more general host requirements. This study has identified previously unknown suitable hosts for four rare Minnesota mussels.

A survey of co-occurring mussels and fish was conducted at fifty sites in the Little Fork and Big Fork rivers of St. Louis, Koochiching, and Itasca counties. We generated species diversity lists, abundance lists, general habitat and water quality measurements for all sites. Voucher specimens were identified, preserved, and deposited at the Bell Museum of Natural History (state repository) and collection records were shared with the MN DNR's Natural Heritage and Nongame Reserch Program. A new mussel species to the Rainy River watershed was collected and voucher material was collected for two species that were previously known only from accounts in the literature. Statistical analysis of mussel catch rate and fish relative abundance revealed different mussel species appear to utilize different host fishes in the Big Fork and Little Fork river drainages.

Project results were disseminated to natural resource managers, scientists, and the public via presentations, the Internet, and various publications with the goal of improving water resource management.

fragile papershell
(*Leptodea fragilis*)

III. Project summary:

Objective 1: Native mussel refuge from zebra mussels

Resource managers have been requesting information on effective methods for relocating freshwater mussels to refugia from zebra mussel infestations, but this information is currently unavailable (Cope and Waller 1995). In 1997 an experimental refuge was established in the St. Croix River to examine the feasibility of relocation as a means of protecting populations of rare mussels from either zebra mussels or as a mitigation technique associated with human influences on river habitats. Analysis of the relocation and reference sites revealed the sites were similar. Based on the 0.25 m^2 quadrats taken, the average density of mussels was approximately 77 mussels/m^2 at each location. Similar numbers of mussels were studied at the two sites, 2344 from relocation site and 2118 from reference site. The main difference between locations is the greater percentage of mussels found dead at the reference site ranging from about 8-10% while at the relocation site the percentage of mussels found dead was only about 3-7%. The fact that there was no difference among treatments and that there were a lower number of mussels dying at the relocation site indicates that, at least for a one-year

period, relocation appears to have little negative impacts on mussel survivorship. Paired t-tests indicated there were significant increases in both shell length and wet weight between 1997 and 1998 at both the reference and relocation sites. These results indicate that the relocation of these species did not have a significant negative impact on their growth. In fact growth was greater at the relocation site. In summary it appears that there were few adverse impacts of relocation of mussels on either the mussels relocated or on the receiving mussel community. This study has all of the criteria suggested by Cope and Waller (1995) for an optimal relocation study. Dan Hornbach, Macalester College, and I will continue this monitoring effort for at least two more years.

Result 2 - Rare mussel life history studies

Natural resource managers frequently need to know the host requirements of mussel larvae (glochidia). Persistence of mussel populations in rivers depends on co-occurrence of their fish hosts. We conducted host suitability tests on the three rare mussels Minnesota mussels: spectaclecase, pistolgrip, and ellipse. Through coordination with other agencies we expanded the host suitability study to include an additional three species, including the federally endangered winged mapleleaf.

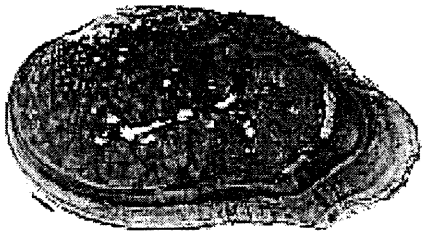
Suitable host fishes were identified for several mussels. Three-fold shell growth was observed on pistolgrip juveniles collected from yellow and brown bullheads. Several darter species, brook stickleback, and two sculpins serve as hosts for ellipse glochidia. Although twenty-five fish species and mudpuppy were exposed to spectaclecase glochidia, none of the species tested facilitated glochidial metamorphosis. Snuffbox glochidia were exposed to four fish species and blackside darters and logperch served as hosts. Four of seven catfish species were found to be suitable hosts for purple wartyback glochidia. Although 81 trials were conducted on 53 fish species and mudpuppies none of them facilitated glochidia metamorphosis. However, growth of winged mapleleaf glochidia was observed among those attached to black and brown bullheads, and flathead catfish, which suggests they may serve as hosts. Host suitability trials using winged mapleleaf glochidia will continue in fall 1999. In this study we identified previously unknown suitable hosts for four rare Minnesota mussels.

Juvenile mussels were collected from fish naturally infested with glochidia and molecular markers for identifying these mussel larvae were developed. Fish naturally infested with mussel larvae were collected from the St. Croix River. Thousands of juvenile mussels were collected from several fish species; primarily drum. We optimized methods in White *et al.* (1994) for our laboratory and were able to reproduce results published in White (1994). Markers were developed for the five mussel species of interest. Future studies should verify the consistency of these markers for mussels in other river systems, and reliable methods need to be developed to extract and amplify DNA from mussel larvae.

Result 3: Fish and mussel surveys in northern Minnesota

Natural resource managers need information on co-occurring mussels and their fish hosts in order to plan for both mixed use (tourism, forestry, agriculture) and conservation of healthy aquatic communities, including native mussels in their native habitats. A major gap in Minnesota's information on co-occurring mussel and fish species is the Rainy River drainage. The fish and mussel fauna of the Big Fork River and Little Fork River drainages in northern Minnesota was surveyed during late summer 1997 and 1998 respectively. Fish were collected using seines, backpack electroshockers, and boomshockers. Mussels were collected using quadrat analysis and timed searches. Ten mussel species and forty-two fish species were observed in the Big Fork River drainage, and eight mussel species and thirty-eight fish species were recorded from the Little Fork River watershed. Several new observations of mussels were made for the first time. Among these include the first recorded occurrence of the state-listed flutedshell from the Hudson Bay drainage. Strong linear relationships between mussel

and fish abundance were rare. Strong relationships may be few because the relationships are more complicated. For example, the host requirements for these mussels may be far broader than we realize. In addition, fishes are more mobile than mussels and most species are shorter lived. It may be that the persistence of certain components of a fish community over decades is a more important independent variable. Future data analysis will incorporate cluster analysis and fish data from the 1970s and the 1980s.



pistolgrip
(*Tritogonia verrucosa*)

IV. Outline of Project Results:

Result 1: Native mussel refuge from zebra mussels

LCMR Budget:	$21,795	Balance:	$0
Match:	$0	Match Balance:	$0

- Criteria for selecting suitable freshwater mussel relocation sites.
- Guidelines for conducting freshwater mussel relocation projects.
- Determination of the suitability of potential refugia for native freshwater mussels in the St. Croix River.

Project Activities	Completion Date
1) Evaluate first refuge sites, improve design if necessary	August 1997
2) Construct refuges in upper & lower St. Croix River	August 1997
3) Collect & measure mussels, and move them to refuges	September 1997
4) Measure mussel growth, survival, population density and diversity at refuges	September 1998
5) Analyze data and develop general recommendations for future relocation efforts	April 1999
6) Prepare final report; give public presentations on findings	June 1999
After grant period	
1) Combine findings with those of NPS & NBS (their study ends Sept. '99) in scientific publications	1999/2000
2) Pursue additional funding to continue monitoring the refuges beyond grant period	

Result 2: Rare mussel life history studies

LCMR Budget:	$39,523	Balance:	$0
Match:	$0	Match Balance:	$0

- Determine host fish requirements of two rare mussels (spectaclecase and pistolgrip) living in the St. Croix and Mississippi rivers, and one rare mussel (ellipse) found in the Zumbro, Cannon, and Root rivers.

Project Activities	Completion Date
1) Collect fish for first year host studies	September 1997
2) Spectaclecase and pistolgrip host studies and collection of naturally infested fish	August 1997
3) Collect additional fish for ellipse host studies	October 1997
4) Ellipse host studies	March 1998
5) Development & testing of spectaclecase and pistolgrip genetic markers	March 1998
6) Collect fish for second year host studies	September 1998
7) Spectaclecase and pistolgrip host studies and collection of naturally infested fish	August 1998
8) Collect additional fish for ellipse host studies	October 1998
9) Ellipse host studies	March 1999
10) Continued development & testing of spectaclecase and pistolgrip genetic markers	May 1999
11) Application of genetic markers to identify juvenile mussels collected from naturally infested fish	May 1999
12) Submit results for publication and post on Internet	June 1999

Result 3: Fish and mussel surveys in northern Minnesota

LCMR Budget:	$29,682	Balance:	$0
Match:	$0	Match Balance:	$0

- Generate species diversity lists, abundance lists, general habitat and water quality measurements for fifty sites in the Little Fork and Big Fork rivers of St. Louis, Koochiching, and Itasca counties. (Survey data will be compatible with Minnesota's Natural Heritage Database.)
- Voucher specimens were identified, preserved, and deposited at the Bell Museum of Natural History (state repository).

Project Activities	Completion Date
1) Fish and mussel survey of the Little Fork River	October 1997
2) Catalogue material from Little Fork River into the Bell Museum	June 1998
3) Fish and mussel survey of the Big Fork River	October 1998
4) Catalogue material from Big Fork River into the Bell Museum and update specimen database	June 1999
5) Submit survey results for publication and presentations, and submit results for use by MN DNR County Biological Survey and National Forest Service	June 1999

V. Dissemination

Project results were disseminated several ways. Results were shared informally and through copies of workprograms with the following organizations: host fish and survey data with local MN and WI DNR fisheries offices, survey data with the MN DNR Natural Heritage and Nongame Research Program, Minnesota County Biological Survey Program, National Forest Service, National Park Service, U.S. Fish and Wildlife Service (USFWS), and Bell Museum of Natural History. Voucher specimens were deposited at the Bell Museum of Natural History. Collection information was added to the Bell Museum database to facilitate review and dissemination. Results and recommendations were also presented in the following written and oral media:

Selected Papers, Presentations, and Reports

(1) Kurth, J. E. and M. C. Hove. 1997. Host fish suitability studies and host attracting behaviors of *Tritogonia verrucosa*, the pistolgrip. Triannual Unionid Report. Report No. 12, p. 10.

(2) Lee, C. and M. C. Hove. 1997. Spectaclecase (*Cumberlandia monodonta*) host(s) still elusive. Triannual Unionid Report. Report No. 12, p. 9.

(3) Hove, M. C. 1997. Mississippi River Research Consortium abstracts. Triannual Unionid Report. Report No. 13, p. 22-29.

(4) Hove, M., S. Strong, A. Jacobson, J. Schussler, and V. Kurth. 1997. Northern Minnesota river holds three state-listed mussels. Triannual Unionid Report. Report No. 13, p. 22.

(5) Hove, M. C. and J. E. Kurth. 1997. *Cyclonaias tuberculata* glochidia transform on catfish barbels. Triannual Unionid Report. Report No. 13, p. 21.

(6) Hove, M. C. 1998. Life history of the federally endangered winged mapleleaf, *Quadrula fragosa*. Population and habitat viability analysis of the winged mapleleaf workshop. St. Cloud, Minnesota.

(7) Hove, M. C. 1998. Mussel power: advances in native mussel biology and management. 31th Annual Meeting of the Minnesota Chapter of the American Fisheries Society, February 24-26, 1998, Camp Ripley, Minnesota.

(8) Hove, M., M. Nelson, S. Weller, R. Buech, and R. Bright. 1998. Organizing Minnesota's freshwater mollusks into a GIS-compatible database. 31th Annual Meeting of the Minnesota Chapter of the American Fisheries Society, February 24-26, 1998, Camp Ripley, Minnesota.

(9) Hove, M. C., K. R. Hillegass, J. E. Kurth, V. E. Pepi, C. J. Lee, P. A. Mahoney, A. R. Kapuscinski, and M. Bomier. 1998. Considerations for conducting host suitability studies. Freshwater mussel symposium: conservation, captive care, and propagation. March 6-8, 1998, Columbus, Ohio.

(10) Hove, M. C., K. R. Hillegass, J. E. Kurth, V. E. Pepi, C. J. Lee, P. A. Mahoney, A. R. Kapuscinski, and M. Bomier. *In press*. Considerations for conducting host suitability studies. Proceedings of the Freshwater mussel symposium: conservation, captive care, and propagation. Columbus, Ohio.

(11) Hove, M. C. and J. E. Kurth. 1998. Darters, sculpins, and sticklebacks serve as suitable hosts for *Venustaconcha ellipsiformis* glochidia. Triannual Unionid Report 14: 8.

(12) Kurth, J. E., M. C. Hove, and A. R. Kapuscinski. 1998. Determination of suitable fish hosts of two rare freshwater mussels. Pages 1392-1394 *In* Yearout, R. D. ed. Proceedings of the National Conference on Undergraduate Research. Volume IV. University of North Carolina, Asheville, North Carolina.

(13) Sieracki, J. L., M. C. Hove, M. M. Tenpas, and P. W. Sorenson. 1998. A preliminary report of an investigation of whether brooding pistolgrip and purple wartyback release chemical attractants. Triannual Unionid Report. Report No. 15, p. 15.

(14) Hove, M. C., J. E. Kurth, and A. R. Kapuscinski. 1998. Brown bullhead suitable host for *Tritogonia verrucosa*; *Cumberlandia monodonta* host(s) remain elusive. Triannual Unionid Report. Report No. 15, p. 13.

(15) Hove, M. C. 1998. Freshwater mussels of Minnesota and research at the University of Minnesota, Bell Museum of Natural History and Department of Fisheries and Wildlife. Science workshop for St. Paul elementary school teachers.

(16) Hove, M. C., J. E. Kurth, D. J. Heath, R. L. Benjamin, M. B. Endris, R. L. Kenyon, A. R. Kapuscinski, K. R. Hillegass, T. W. Anderson, V. E. Pepi, and C. J. Lee. 1998. Hosts and host attracting behaviors of five upper Mississippi River mussels. Page 159 *In* R. Bieler and P. M. Mikkelsen, eds. Abstracts: World Congress of Malacology, Washington, D. C.

(17) Hove, M. C., J. E. Kurth, D. J. Heath, R. L. Benjamin, M. B. Endris, R. L. Kenyon, A. R. Kapuscinski, K. R. Hillegass, T. W. Anderson, V. E. Pepi, and C. J. Lee. 1998. Hosts and host attracting behaviors of five upper Mississippi River mussels. St. Croix River Research Rendezvous. October 20, 1998, Marine on St. Croix, Minnesota.

(18) Cunningham, L. A., D. J. Hornbach, and M. Hove. 1998. Effects of relocation on freshwater mussels. St. Croix River Research Rendezvous. October 20, 1998, Marine on St. Croix, Minnesota.

(19) Hove, M., J. Gustafson, J. Sieracki, J. Kurth, P. Mahoney, and M. Tenpas. 1998. Special concern mussels found in northern Minnesota watershed. Triannual Unionid Report 16: 32.

(20) Heath, D., M. Hove, R. Benjamin, M. Endris, R. Kenyon, and J. Kurth. 1998. *Quadrula fragosa* exhibit unusual reproductive behaviors. Triannual Unionid Report 16: 33.

(21) Hove, M. C., J. E. Kurth, D. J. Heath, R. L. Benjamin, M. B. Endris, R. L. Kenyon, A. R. Kapuscinski, K. R. Hillegass, T. W. Anderson, V. E. Pepi, and C. J. Lee. 1999. Hosts and host attracting behaviors of five upper Mississippi River mussels. 32nd Annual Meeting of the Minnesota Chapter of the American Fisheries Society, January 5-7, 1999, La Crosse, Wisconsin. (*Awarded Best Poster*)

Interviews and Invited Lectures

(1) Mussel power. Fall 1997. The Spectrum 3(2): 5 (A University of Minnesota, College of Natural Resources outreach publication.)

(2) Fish attracting behavior of rare midwest mussels and unionid research at the University of Minnesota. October 1997. Tour and presentation requested by the 1997 midwest federal and state threatened and endangered species coordinators. Presentation and tour provided at the University of Minnesota.

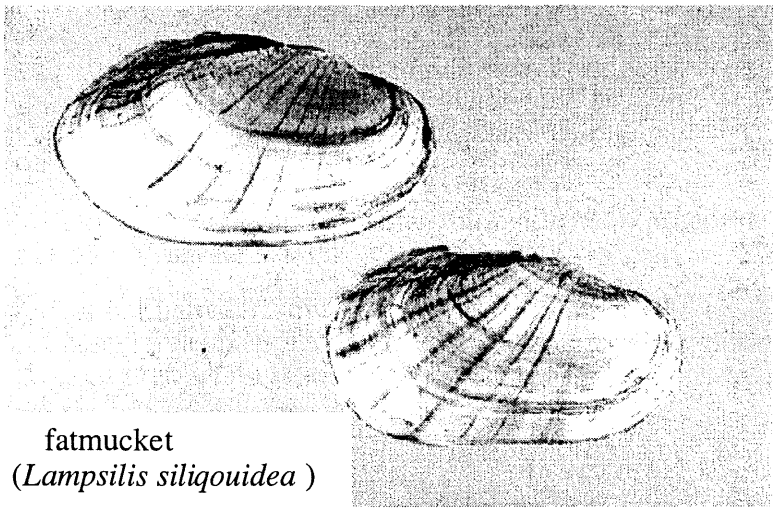
(3) St. Croix River mussels. November 1997. Environmental Journal, a radio program broadcast throughout much of Minnesota.

(4) Utility and unusual behaviors of our native freshwater mussels. December 1997. Guest lecturer for University of Minnesota undergraduate course in fisheries and wildlife management (FW 3054, Biological Conservation: An Ecosystem Approach).

(5) Unusual fish host-attracting behaviors of upper Mississippi River freshwater mussels. 1998. Guest lecturer at Saint Olaf College.

(6) Utility and unusual behaviors of our native freshwater mussels. December 1998. Guest lecturer for University of Minnesota undergraduate course in fisheries and wildlife management (FW 3054, Biological Conservation).

Several of the articles described above will also be submitted to peer-reviewed journals.



fatmucket
(*Lampsilis siliqouidea*)

VI. Context:

A. Significance: According to a recent study, freshwater mussels are the most imperiled group of all U.S. flora and fauna (Dicke 1996). Water quality managers have to plan for both mixed use (fishing, water sports) and conservation of healthy aquatic communities. To meet these goals, water managers need essential data on distribution, abundance, and environmental needs of aquatic species. The St. Croix River holds a nationally unique and diverse mussel community including two federally endangered species, two species under review for federal listing, thirteen species listed by Wisconsin and nineteen listed by Minnesota. Among their dramatic ecological impacts, zebra mussels have reduced the diversity and abundance of native mussel communities (Hebert *et al.* 1991). Boat traffic has inadvertently spread zebra mussels to the upper Mississippi River; adults have been found on boats in the lower St. Croix River at Stillwater. Resource managers have been requesting information on effective methods for relocating freshwater mussels to refugia where potential threat from zebra mussel infestation is reduced. In conjunction with the NPS and NBS study, this project was designed to help these

managers by evaluating the effectiveness of two refugia in the upstream portions of the St. Croix River, and developing recommendations for other refugium programs.

For most freshwater mussels, the larval stage must briefly attach to one or more specific fish species ("host") in order to complete the life cycle. Identification of host fishes is the highest priority item listed under the basic biology goal of the national strategy for freshwater mussel conservation (Biggins *et al.* 1995). Host requirements of three rare mussels living in Minnesota (spectaclecase, pistolgrip, and ellipse) are unknown, making it nearly impossible to determine viability of imperiled mussel populations either in degraded habitats, where they now occur, or in habitats being considered for translocation to rescue them from spread of zebra mussels or other adverse environmental effects. Identification of the hosts for these three rare mussels will enable managers to more effectively manage and conserve their populations.

B. Time: This project was completed within the expected time period (7/97-6/99).

C. Budget Context:

	July 1995 - June 1997	July 1997 - June 1999	July 1999 - June 2001
	Prior expenditures on this project	Project expenditures	Anticipated future expenditures on this project
1. LCMR	$0	$91,000	$0
2. Other State*	$7288 ($1200 anticipated)	$11,200 ($6300 anticipated)	$6300 anticipated
3. Non State cash	$0	$0	$8400**
Total	$7288	$112,200	$14,700

* - We regularly obtain funding from the University of Minnesota Undergraduate Research Opportunities Program for undergraduate research assistantships.
** - The U.S. Fish and Wildlife Service is supporting work on life history of the federally endangered winged mapleleaf.

BUDGET:

Personnel	$71,820
Equipment	$5150
Acquisition	n/a
Development	n/a
Other (Supplies)	$14,030
Total	$91,000

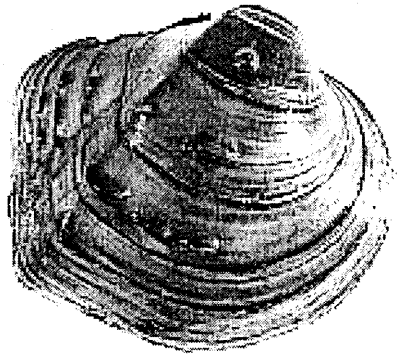
VII. Cooperation:

This project was completed with the assistance of several local, state, and federal agencies. Via coordination with the Macalester College, US Geological Survey (USGS), USFWS, and National Park Service (NPS), we developed and tested techniques for establishing refugia as a means of conserving freshwater mussel biodiversity in the St. Croix River. Dr. Daniel Hornbach, coordinator at Macalester College, received $8440 in LCMR project dollars (20%

time) to assist with the creation and monitoring of the mussel refuge. Dan Hornbach provided most of the field crew for the relocation project, analyzed the data, and composed most of the text project results. Diane Waller coordinated participation by the USGS, Chuck Kjos coordinated participation by the USFWS, and Susan Jennings, and Randy Ferrin coordinated participation by the NPS.

Determination of the required fish hosts of three rare Minnesota mussels was completed with help from the NPS, Minnesota Department of Natural Resources (MN DNR), Wisconsin Department of Natural Resources (WI DNR), and University of Minnesota, Undergraduate Research Opportunities Program (UROP) and Summer Science Program (SSP). James Straka, Macalester College, provided invaluable advice on developing molecular markers for mussels and shared several restriction enzymes enabling completion of a diagnostic suite of DNA cutting sites. Loren Miller, Willy Eldridge, and Wansuk Senanan, University of Minnesota, also assisted with optimizing laboratory methods and interpreting data. Susan Jennings, Byron Karns, and Randy Ferrin of the NPS assisted with collection of fish from the St. Croix River. Karl Koller and Chris Kavanaugh of the MN DNR provided several fish for use in the host fish laboratory studies. Ron Benjamin, Mark Endris, and Rhonda Kenyon provided a great deal of assistance through provision of gravid mussels and test fishes for this project. The UROP program provided 6 student assistants (value $6500) and SSP provided 1 student assistant (value $3000) in support of this work.

Mussel and fish surveys of the Little Fork and Big Fork rivers were conducted with assistance from the National Forest Service (NFS). The NFS was very helpful and saved the project money with their contribution of field technicians, and transportation and lodging support during the survey. Chantel Cook and Jeremy Cable coordinated participation by the NFS. Nancy Berlin and Brenda Stauffer provided valuable assistance with some of the field work. Results from each project were shared with cooperators. The UROP program provided 3 student assistants (value $3700) and SSP provided 2 student assistant (value $6000) in support of this work.



wartyback
(*Quadrula nodulata*)

VIII. Location:

Figures 1-3 illustrate locations of project sites.

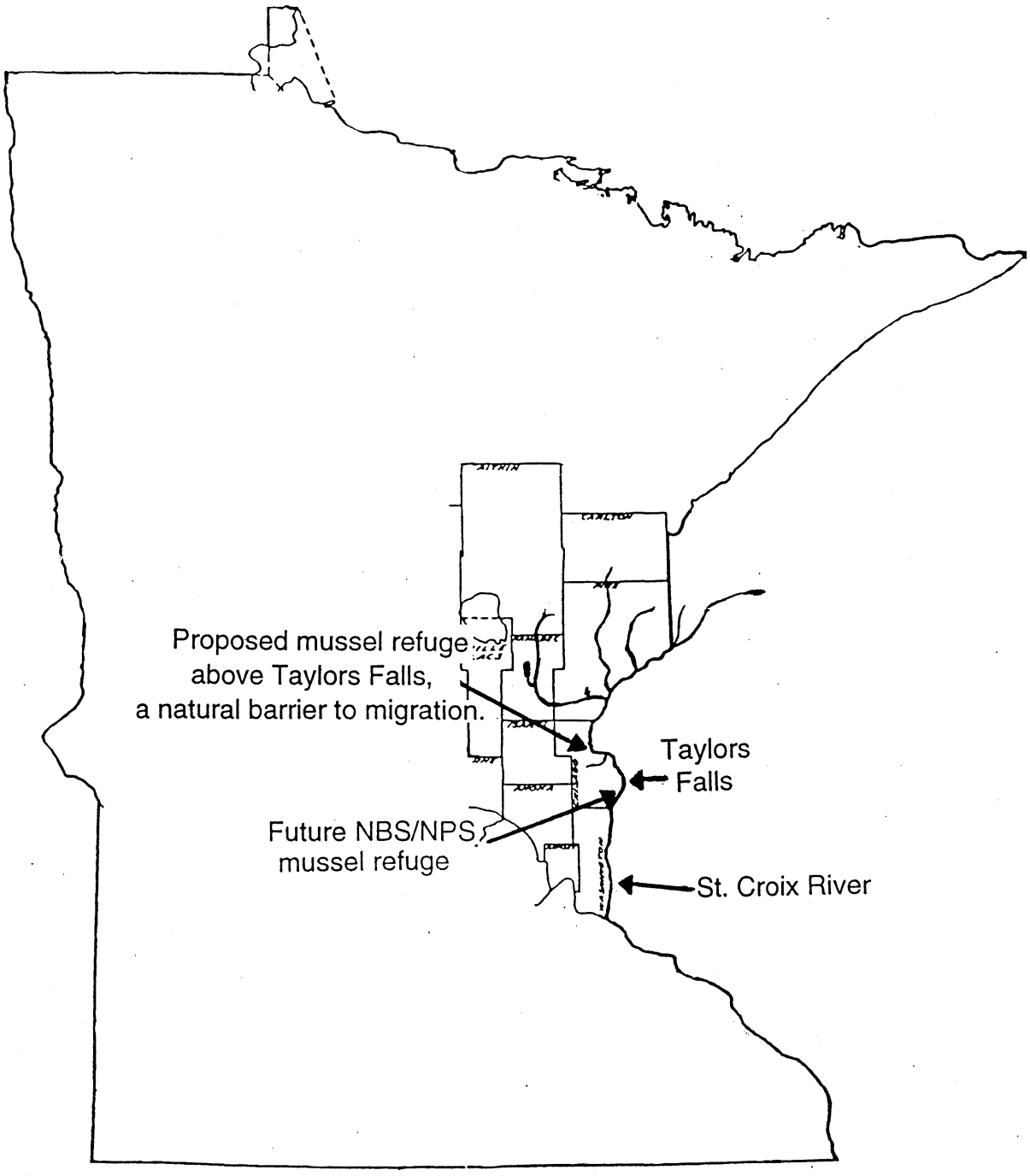


Figure 1. St. Croix River native mussel study refuges for protection from encroaching zebra mussels.

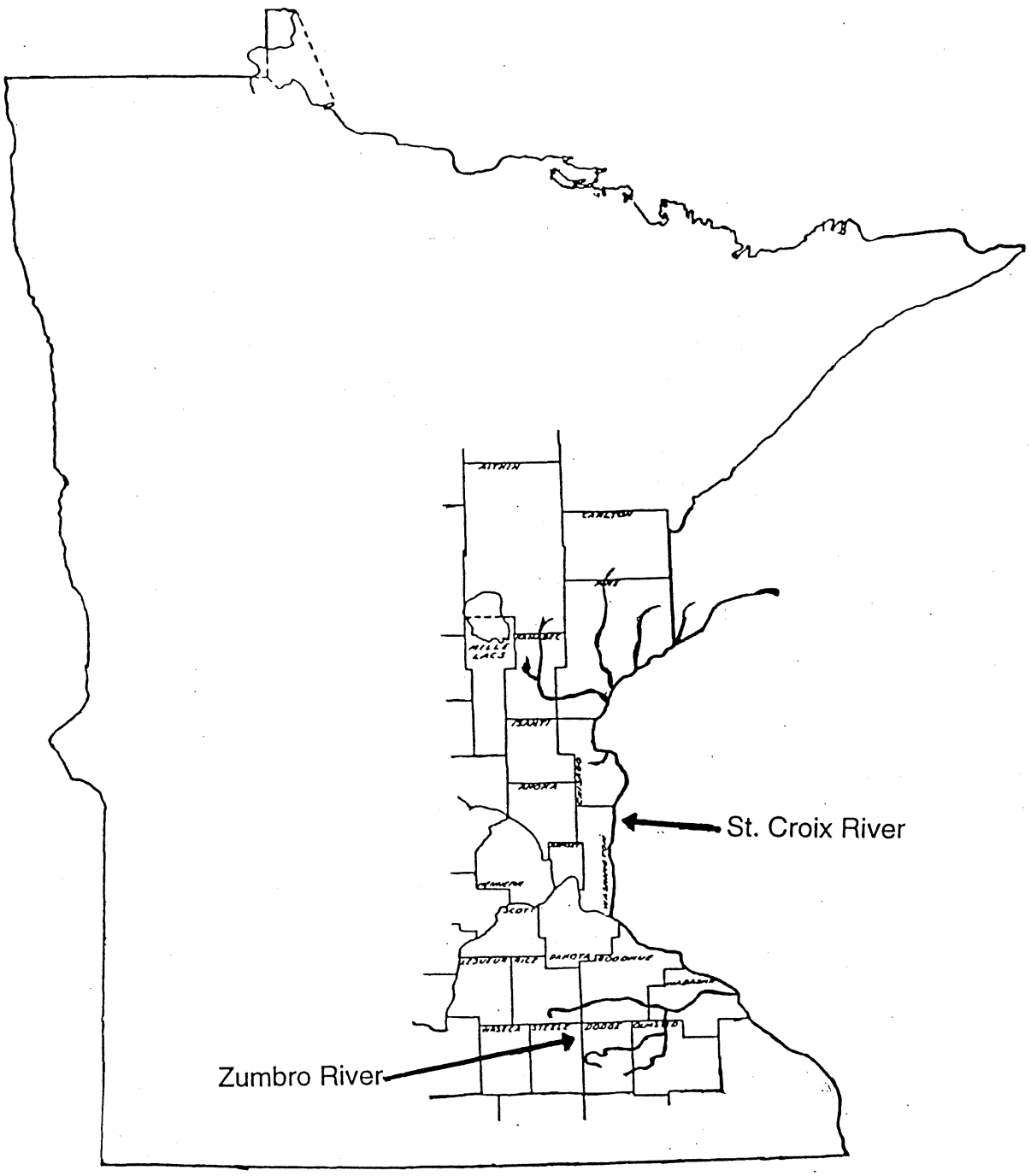


Figure 2. Rare mussel life history study sites: a) St. Croix River, and b) Zumbro River.

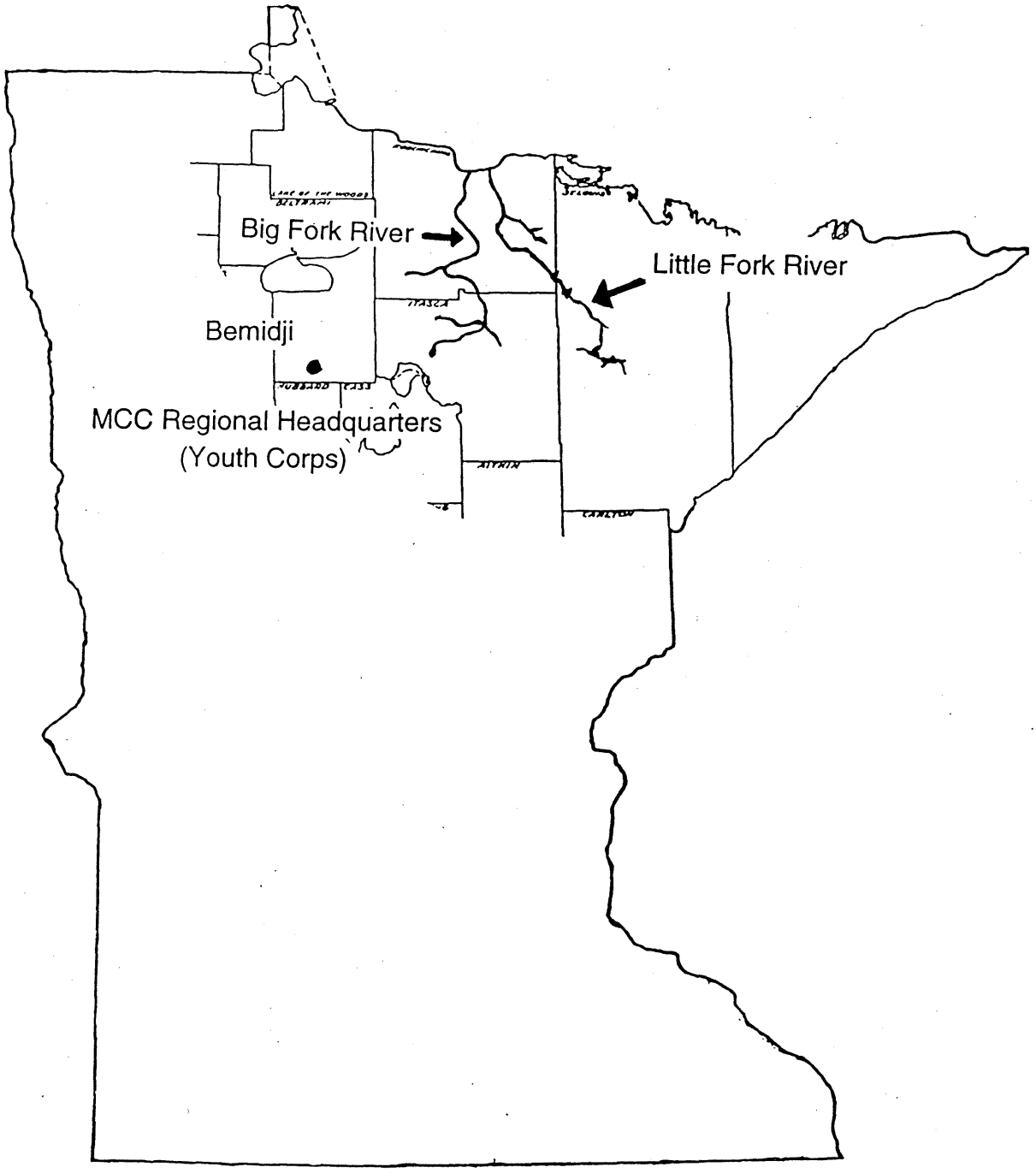


Figure 3. Little Fork and Big Fork River basins. Study areas for fish and mussel surveys.

IX. Reporting Requirements:

Periodic work program progress reports were submitted December 1997 and December 1998. A final work program report and associated products were submitted prior to July 1, 1999.

X. Research projects:

I. Abstract

Native mussels are one of the most threatened groups of animals in Minnesota. To assist natural resource managers with conservation of rare species we researched the following objectives: 1) establish and monitor study refugia in the St. Croix River to protect freshwater mussels from invading zebra mussels, 2) determine the suitable fish hosts for three threatened Minnesota mussels, and 3) survey co-occurring mussel and fish resources in northern Minnesota's Little Fork and Big Fork rivers, providing crucial baseline data for water resource managers. Via coordination with the Macalester College, US Geological Survey (USGS), and National Park Service (NPS), we developed and tested a technique for establishing refugia as a means of conserving freshwater mussel biodiversity in the St. Croix River. This involved: relocating mussels; identifying possible refugia sites; and testing the effectiveness of these refugia. In summary it appears that there were few adverse impacts of relocation of mussels on either the mussels relocated or on the receiving mussel community. This study has all of the criteria suggested by Cope and Waller (1995) for an optimal relocation study. Dan Hornbach, Macalester College, and I will continue this monitoring effort for at least two more years.

We determined suitable host fish requirements of several rare mussels living in the St. Croix and Mississippi rivers, and the host fish requirements of a rare mussel found in the Zumbro, Cannon, and Root rivers. Laboratory studies revealed the mussel larvae of three rare, large-river mussels appear to have specific hosts in the catfish family. The stream-dwelling ellipse has more general host requirements. This study has identified previously unknown suitable hosts for four rare Minnesota mussels.

A survey of co-occurring mussels and fish was conducted at fifty sites in the Little Fork and Big Fork rivers of St. Louis, Koochiching, and Itasca counties. We generated species diversity lists, abundance lists, general habitat and water quality measurements for all sites. Voucher specimens were identified, preserved, and deposited at the Bell Museum of Natural History (state repository) and collection records were shared with the MN DNR's Natural Heritage and Nongame Reserch Program. A new mussel species to the Rainy River watershed was collected and voucher material was collected for two species that were previously known only from accounts in the literature. Statistical analysis of mussel catch rate and fish relative abundance revealed different mussel species appear to utilize different host fishes in the Big Fork and Little Fork river drainages.

Project results were disseminated to natural resource managers, scientists, and the public via presentations, the Internet, and various publications with the goal of improving water resource management.

II. Background

According to a recent study, freshwater mussels are the most imperiled group of all U.S. flora and fauna (Dicke 1996). Water quality managers have to plan for both mixed use (fishing, water sports) and conservation of healthy aquatic communities. To meet these goals, water managers need essential data on distribution, abundance, and environmental needs of aquatic species.

Native mussel refuge from zebra mussels

The St. Croix River holds a nationally unique and diverse mussel community including two federally endangered species, two species under review for federal listing, thirteen species listed by Wisconsin and twenty-three listed by Minnesota. Among their dramatic ecological impacts, zebra mussels have reduced the diversity and abundance of native mussel communities (Hebert *et al.* 1991). Boat traffic has inadvertently spread zebra mussels to the upper Mississippi River; adults have been found on boats in the lower St. Croix River at Stillwater.

Resource managers have been requesting information on effective methods for relocating freshwater mussels to refugia from zebra mussel infestations, but this information is currently unavailable (Cope and Waller 1995). *In situ* refugia offer several advantages over other relocation methods. They ensure similarity of water quality and habitat characteristics between the origin and relocation site and availability of necessary fish hosts. Also, they allow retention of existing genetic diversity of the mussel and host fish populations.

Natural resource managers in Minnesota, Wisconsin and various federal agencies have expressed their urgent need for effective means of protecting the biodiversity of freshwater mussels in the St. Croix River. Few studies, however, have adequately examined relocation as a viable means of protecting populations from either zebra mussels or as a mitigation technique associated with human influences on river habitats (Cope and Waller, 1995). Among the most obvious locations to relocate threatened mussels, are areas that harbor rich and diverse mussel assemblages including, if possible, the species that are being relocated. However, if mussels are being moved into a dense mussel bed, the question arises concerning the impact of increasing the mussel density in the relocation area.

In summary, the major question examined was:

1. How effective is in-situ relocation as an effective means to protect native mussels? What is the impact of relocation on the relocated mussels and on the mussel populations that receive the relocated mussels? Is there a negative effect of increasing mussel density in areas receiving relocated mussels?

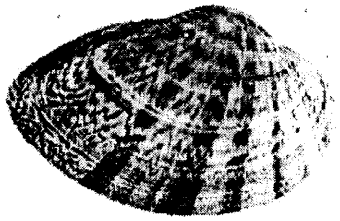
Rare mussel life history studies

For most freshwater mussels, the larval stage must briefly attach to a specific fish species ("host") or small group of fish species to complete their life cycle. Identification of fish hosts is the highest priority item listed under the basic biology goal of the national strategy for freshwater mussel conservation (Biggins *et al.* 1995). Host requirements of three rare mussels living in Minnesota (spectaclecase, pistolgrip, and ellipse) are unknown, making it nearly impossible to determine viability of imperiled mussel populations either in degraded habitats, where they now occur, or in habitats being considered for translocation to rescue them from spread of zebra mussels or other adverse environmental effects. Project results on fish hosts of three rare mussels in Minnesota will enable managers to more effectively manage and conserve some of the state's most imperiled mussel fauna.

Fish and mussel surveys in northern Minnesota

Natural resource managers need information on co-occurring mussels and their fish hosts in order to plan for both mixed use (tourism, forestry, agriculture) and conservation of healthy aquatic communities, including native mussels in their native habitats. Persistence of mussel

populations in rivers depends on co-occurrence of their fish hosts. A major gap in Minnesota's information on co-occurring mussel and fish species is the Rainy River drainage. The last surveys of mussel and fish resources of the northern Minnesota Rainy River drainage were conducted 50 and 20 years, respectively. Meanwhile, human population, timber harvest, and farmland development have increased in the area. We will survey the Big Fork and Little Fork rivers to determine if fish and mussel communities have changed with these changes in land use. Based on historical records, two mussel species proposed for state listing and three state listed fish species may live in the Rainy River drainage. Our survey will determine if and where these species occur in the Little Fork and Big Fork Rivers. The state's County Biological Survey does not have sufficient funds to collect data on aquatic species at other than highest priority sites; thus the data we generate will be of interest both to the Natural Heritage and Nongame Research Program and the Minnesota County Biological Survey.



fawnsfoot
(*Truncilla donaciformis*)

III. Methodology

Result 1: Native mussel refuge from zebra mussels

Work towards this result was a cooperative effort between Macalester College, US Geological Survey (USGS), and National Park Service (NPS). Except for the native mussel collection and monitoring sites the protocol for establishing and monitoring the refugia in this project was the same as those for the USGS/NPS refugia study (Cope 1996). Following these procedures will facilitate comparisons between the different refugia, strengthen recommendations for future relocation projects, and improve our assessment of potential refugia for freshwater mussels in the St. Croix River. The methods for this part of the work program (Cope 1996) were reviewed by employees at the U.S. Fish and Wildlife Service and four independent peer reviewers (Dr. Greg Cope, US Geological Service, La Crosse Research Station, personal communication).

We are conducting a relocation of mussels into in situ refugia in the St. Croix River, located upstream of a natural barrier to zebra mussel migration (Taylors Falls). All refuge sites lie within National Park Service or State Park-managed zones, thus facilitating their maintenance and monitoring. Divers collected three species of unionid mussels, two representing the subfamily Ambleminae (*Quadrula pustulosa* and *Elliptio dilatata*) and one representing the subfamily Lampsilinae (*Lampsilis cardium*), from the upper St. Croix River at Wild River State Park near Almelund, Minnesota. Mussels were collected from a study grid established at the upstream boat launch in the park and relocated to a study grid at the downstream boat launch. Collected mussels were identified according to Cummings and Mayer (1992). Recent surveys of the mussel fauna in this area of the St. Croix River have indicated the density of

mussels at Wild River State Park will provide a valuable comparison with the USGS refugia at Franconia, Minnesota. The site near Wild River State Park revealed a mean mussel density of $32/m^2$, whereas sites sampled near Franconia, Minnesota and Hudson, Wisconsin had average mussel densities of $11/m^2$ and $19/m^2$ respectively (Dr. Daniel Hornbach, Macalester College, St. Paul, Minnesota, personal communication). A comparison between the sites will reveal if there are significant differences in transplanted mussel survival and growth at differing mussel densities of the receiving mussel bed.

Boundaries of each relocation site were delineated and site limits were recorded from permanent land marks using standard surveying techniques and by a military-grade Global Positioning System (GPS). A random (PROC Plan in SAS) nested block design (Waller *et al.* 1993) was used to monitor mussel survival and the potential effects of physical habitat on unionids. A grid (5 x 5 m) composed of $1\text{-}m^2$ cells was placed at the source site near the upstream boat launch at Wild River State Park, Minnesota and at the destination site near the downstream boat launch at the same park (Figure 4). The grid location was permanently marked underwater and its location above water recorded with standard surveying methods and GPS.

The experimental design of the grids (Figure 4) consisted of five randomly selected $1\text{-}m^2$ cells, that serve as undisturbed (non-handled) resident controls. The resident mussels were removed from an additional five randomly selected cells within the grids and mussel density, species richness, and ratio of live to dead shell was recorded and the mussels were returned to their respective cells, serving as handling controls. Future conservation and recovery efforts will assess the effects of increased mussel density on overall survival and growth (an increase in length or weight). Therefore, the five handling control cells in each grid received additional mussels collected from the area immediately surrounding the grid to effectively double the natural density in the cells and additionally serve as a density-doubling control for the treatment (Ambleminae and Lampsilinae) cells. Each of the three species representing the subfamilies Ambleminae and Lampsilinae has five replicates (grid cells) with 10 mussels per replicate for a total mussel density of $10/m^2$ plus the natural density in each cell (Figure 4).

Ten $1/4\text{-}m^2$ quadrats were established at fixed points from the area immediately surrounding (within 1 m) the grid at both the source (near upstream boat launch, Wild River State Park, Minnesota) and destination (near downstream boat launch, Wild River State Park, Minnesota) sites (Figure 4) and analyzed for mussel density, species richness, live:dead ratio, and substrate characteristics.

Divers collected mussels at the source site until the total number of mussels needed (300) for the study was obtained. These mussels were held in a delineated area in the river at the source site until they were processed and relocated. At the time of processing, mussels were measured (total length to the nearest mm), weighed (total weight to the nearest g), and uniquely marked (given sequential numbers) by etching the periostracum (numbers at least 1 cm in height and width) with a rotary grinding tool (Waller *et al.* 1993). Mussels were transported over water (single trip with travel time limited to interval necessary for point to point travel) from the source site to the destination sites in ice-chests. Mussels inside the ice-chests were placed in no more than four horizontal layers; each layer draped in damp burlap or similar cloth material to retain moisture. The mussels inside each ice-chest were kept to within $\pm 2\ ^{\circ}C$ of river water at the time of mussel removal by adding ice to trays placed above the mussels. There was no direct contact of ice with mussels. Mussels used in the reference control grid were also be given similar transportation exposure conditions and duration.

Refuge Site 1
Wild River State Park, MN

	1	2	3		
	QP	RC	RC	DDC	QP
10	LS	LS	DDC	ED	QP	4
	ED	LS	RC	ED	LS
9	LS	QP	RC	DDC	QP	5
	DDC	RC	ED	ED	DDC
	8	7	6		

Refuge Site 2
Wild River State Park, MN

	1	2	3		
	QP	ED	QP	ED	DDC
10	LS	DDC	ED	RC	DDC	4
	DDC	LS	RC	RC	QP
9	ED	QP	RC	RC	ED	5
	QP	LS	LS	LS	DDC
	8	7	6		

Reference Site
Franconia or Interstate Park, MN

	1	2	3		
	DDC	QP	QP	RC	LS
10	ED	LS	QP	RC	DDC	4
	DDC	DDC	ED	RC	DDC
9	LS	QP	QP	ED	ED	5
	RC	RC	ED	LS	LS
	8	7	6		

Key for each grid

RC - Resident control

DDC - Density-doubling control

QP - *Quadrula pustulosa*
(5 cells x 10 mussels/cell=50)

ED - *Elliptio dilatata*
(5 cells x 10 mussels/cell=50)

LS - *Lampsilis siliquoidea*
(5 cells x 10 mussels/cell=50)

Grand total mussels required

Quadrula pustulosa = 150

Elliptio dilatata = 150

Lampsilis siliquoidea = 150

Figure 4. Experimental design for collection of substrate samples (□) and for random placement of mussels in the St. Croix River mussel refuge study.

Different species of mussels require different types of substrate (Cummings and Mayer 1992). Because the selected refuge sites likely differ in physical habitat, such as substrate characteristics, from the source site, the proposed research will characterize differences in physical habitat. Substrate samples were collected from the source and destination sites in the area immediately surrounding each of the grids by hand excavating substrate in a 1/4 m^2 quadrat to a depth of 15 cm (Dunn 1994). Ten samples were collected from each of the source and destination sites. The whole sediment samples was weighed on site and passed through a set of three sequential sieves (mesh size openings of 12, 6, and 3 mm) to obtain sediment particle fractions. Before sieving, a representative subsample (minimum wet weight of 25 g) was taken from the weighed bucket, placed in sealable storage bags, stored on ice, and transported to the laboratory for analysis of wet weight:dry weight ratio. After sieving, each particle size fraction was recovered from the respective sieve and weighed on site to determine the percent relative composition. After weighing, a representative subsample (minimum wet weight of 25 g) from each fraction was placed in sealable storage bags, stored on ice, and transported within 48 h to the laboratory for analysis of weight:dry weight ratio.

A quantitative assessment of mussel survival, growth, and substrate characteristics was made in 1998. During the evaluation, mussels within all cells of each of the grids were collected by divers, placed into numbered dive bags, identified, measured (total length to the nearest mm), weighed (total weight to the nearest g), and replaced into their respective cells. Control cells were sampled to assess natural mortality, growth and population structure in both the undisturbed resident mussels (non-handled controls) and in the handled, density-doubled controls. Substrate and mussels population samples (n=10) was taken annually from the area immediately surrounding the grids (Figure 4) at the source and refuge sites to assess potential changes in physical characteristics or resident mussel density after the relocation.

All methods used for determination of wet weight:dry weight ratio of sediment are published, standard analytical procedures (APHA *et al.* 1992). For all sample analyses, an analytical blank and replicate sample was analyzed with every 20 samples analyzed.

Extreme caution was exercised during all phases of this project to ensure that the exotic zebra mussel were not inadvertently transported to the currently uninfested waters of the St. Croix River. We closely coordinated our project with the National Park Service, who monitored for the presence of zebra mussels in the St. Croix River biweekly throughout the ice-free season of 1997 and 1998. The project would have been halted if the perceived risk was greater than the derived benefit, however, the risk of inadvertantly transporting zebra mussels during the project was deemed very low and project activities were completed. Statistical analysis were conducted with JMP version 3.2.2 for the Macintosh (SAS 1994).

Result 2: Rare mussel life history studies

Determination of the fish host(s) for glochidia requires documentation of: 1) glochidia transformation on the suspected host fish, and 2) glochidial infestation of the same fish species under natural conditions. We completed artificial infestation of fish with glochidia to observe if transformation occurred (Neves *et al.* 1985). To ensure that suitable fish hosts identified in the laboratory were indeed functional hosts under natural conditions, we searched for evidence of the same fish-mussel relationships under field conditions. This objective has two parts: 1) determine glochidial host suitability, and 2) determine natural glochidial infestation of fish.

Determine Suitable Fish Hosts

Suitable fish hosts were determined using a protocol similar to that described in Neves *et al.* (1985). Fishes for glochidia host suitability tests were collected with a seine, angling gear,

and electrofishing equipment. Test fish are held in holding tanks (40 L or 400 L) at least 20 d prior to glochidia infestation, at temperatures between 18-23 °C. Test fish were held in holding tanks (40 L or 400 L) at least 20 d prior to glochidia infestation, at temperatures between 12-23 °C.

Gravid female mussels were collected from a variety of different sources and held at the University of Minnesota. Spectaclecase (*Cumberlandia monodonta*), pistolgrip (*Tritogonia verrucosa*), snuffbox (*Epioblasma triquetra*), purple wartyback (*Cyclonaias tuberculata*), and winged mapleleaf (*Quadrula fragosa*) were collected from the St. Croix River, and ellipse (*Venustaconcha ellipsiformis*) were obtained from the Zumbro River. The date, water temperature, number of marsupia used to brood glochidia, and marsupia color was recorded as gravid female mussels under investigation were observed. Most gravid female mussels were held in beakers in aquaria until they release glochidia naturally. Glochidia were pipetted from the gills of gravid ellipse in 1998. To determine the health of collected glochidia, a subsample was exposed to a 0.1-1% sodium chloride solution. For each trial ≥ 70% of the glochidia closed their valves upon exposure to salt and were used for host tests. After collection of glochidia, female mussels were returned to the collection site.

To infest fish with glochidia, we placed them in a 1-2 L bath with several hundred to several thousand glochidia under vigorous aeration. Fish were exposed to glochidia for roughly 1-3 hours, depending on susceptibility of the species to infestation. The state of infestation was assessed every one to two hours. After we observed that a treated fish had 2-20 glochidia on its gills, we transfered the fish to a clean aquarium. Infested fish were held in aquaria at 11-22°C and fed three times a week. Fathead minnows (*Pimephales promelas*) were given to piscivorous fish once a week and removed from aquaria 5-10 minutes after introduction to minimize the possibility of their consuming glochidia or juvenile mussels lying on the aquarium floor. Small fishes (*e.g.*, cyprinids, etheostomids, catostomids, etc.) were held in suspended nets to prevent them from eating juvenile mussels on the aquarium floor. Aquaria were siphoned and siphonate checked for presence of glochidia and juveniles three times a week. Each search for juveniles was terminated after three consecutive searches failed to reveal a glochidium or juvenile mussel. At this termination point, each fish was anesthetized and searched for attached glochidia using a dissecting microscope. If a glochidium was found, the fish was revived and the experiment continued until glochidia are no longer attached to the fish. A mussel was considered a juvenile when foot movement or valve closure is observed. A fish was considered a suitable host when glochidia encystment and metamorphosis to the juvenile stage occurred. Fish, mussel, and amphibian nomenclature follows Robins *et al.* (1991), Turgeon *et al.* (1988), and Oldfield and Moriarty (1994) respectively.

Determine Natural Glochidial Infestation of Fish

To date, two techniques have been used to identify the species of glochidia that naturally infest fish. Traditionally, glochidia were identified using simple morphological features (*e.g.*, outline shape, un/spined), and length and width measurements (Surber 1915). However, morphological differences frequently cannot identify glochidia below the level of subfamily or genus (Neves and Widlak 1988, Weiss and Layzer 1993). A promising technique under development involves screening glochidia with naturally occurring DNA markers which have first been shown to clearly distinguish different species. This process involves isolating DNA from a glochidium collected from a naturally infested fish and screening the isolated DNA with a battery of diagnostic markers to determine which species of mussel it is. This molecular genetic approach has been successfully used to identify the species of glochidia infesting fish in Pennsylvania (White *et al.* 1994).

Between 4-20% of the fish community is infested with glochidia during the glochidia release period (Scruggs 1960, Kitchel 1985, Hove and Neves 1994). Pistolgrip and spectaclecase are short-term brooders and release their young in the early spring (Oesch 1984). We collaborated with the NPS to collect a large number of fish over mussel beds every other week during May and June, 1997-98, in order to collect glochidia from naturally infested fish.

We measured juvenile mussel morphological characters in order to identify the species of glochidia found on naturally infested fish. In the early spring many species of freshwater mussels release glochidia into the St. Croix River. However, only five species 9fawnsfoot (*Truncilla donaciformis*), deertoe (*T. truncata*), fragile papershell (*Leptodea fragilis*), spectaclecase, and pistolgrip) release glochidia with length or width dimensions less than 100 microns (Surber 1913, Oesch 1984). Preliminary identification of juvenile mussels was based morphologic characters described in Surber (1915) and Oesch (1984). Of these five species, only spectaclecase and pistolgrip are rare in the state of Minnesota and are the focus of this study. Therefore, we will identified species-specific DNA markers for these five species, and then attempted to use these markers on field-collected glochidia with valve length less than 100 microns.

Methods for identification and application of species-specific DNA markers follow those described in White (1994). This technique involves screening the DNA from field-collected glochidia via restriction fragment length polymorphism (RFLP) analysis of polymerase chain reaction (PCR) products. The PCR technique generates large numbers of copies (*i.e.*, amplification) of specific regions of DNA from a very small tissue source (*e.g.*, a single glochidium, sliver of adult mussel mantle tissue). First, we isolated DNA from a non-lethal sliver of mantle tissue collected from adults of known species. Via RFLP analysis of this adult DNA, we determined which combinations of DNA-cutting restriction enzymes sites are diagnostic for each mussel species of interest. We then attempted to identify the species of glochidia collected from naturally infested host fishes based on the restriction sites (or RFLP patterns) found in their DNA.

To create a diagnostic suite of restriction sites, we conducted tests using DNA isolated from spectaclecase and pistolgrip glochidia and tissue samples from deertoe, fawnsfoot, and fragile papershell. We amplified the first internal transcribed spacer (ITS-1) region of the nuclear ribosomal DNA using PCR. This region was selected for two important reasons. First, the ITS-1 region has highly conserved DNA sequences in its flanking regions. These conserved sequences can be used to develop a single set of primers for the PCR amplification of DNA from many species. Second, the length of the ITS-1 region varies considerably across taxa. White *et al.* (1994) found that the ITS-1 regions of fishes examined are markedly different in length from freshwater mussels. Therefore any host fish DNA that was inadvertently amplified by PCR was not confused with mussel DNA. Amplified mussel DNA was cut using several restriction enzymes. We used gel electrophoresis to determine which enzymes produce bands (cut PCR products) unique to a given mussel species. We developed a protocol to discriminate among the five species of mussels, and attempted to test glochidia.

Pistolgrip and spectaclecase are short-term brooders and release their young in the early spring (Oesch 1984). Via collaboration with the National Park Service, we collected fish naturally infested with glochidia adjacent to a St. Croix River mussel bed at Interstate Park, Minnesota during springs of 1997 and 1998. Fish collected from the St. Croix River were transported to and held at the University of Minnesota, Wet Laboratory. Fish holding and juvenile mussel collection procedures were the same as those described above for the host suitability study. Preliminary identification of juvenile mussels was based morphologic characters described in Surber (1915) and Oesch (1984).

Result 3: Fish and mussel surveys in northern Minnesota

The mussel and fish surveys using standard protocols were conducted jointly for efficient use of field personnel. Methods for the fish and mussel surveys are presented below.

Study Area

The Little Fork and Big Fork rivers flow through St. Louis, Koochiching, and Itasca counties of northern Minnesota. These rivers are part of the Rainy River watershed that drains into Lake of the Woods and is part of the Hudson Bay drainage. The Big Fork River basin is 2,063 mi^2 is size. The lower two-thirds of the watershed flow through the relatively flat terrain of the bed of Glacial Lake Agassiz in Koochiching County, while the upper one-third drains the morainic region of lakes and ridges in Itasca County. The Little Fork River basin is 1,849 mi^2 is size, nearly all of it flowing through the bed of Glacial Lake Agassiz (Waters 1977).

The mussel and fish communities were examined and voucher specimens collected from 50 sites on the Little Fork and Big Fork rivers and their tributaries. Surveys were conducted in late summer and early fall. At this time water levels are generally low and fish populations are high, since winter mortality to the year's cohort has not yet occurred. Voucher specimens were deposited at the Bell Museum of Natural History, University of Minnesota.

Correlation analysis of data was conducted. Data analyzed included fishes identified to species, and live mussels collected by Hove during timed searches. The following comparisons were generated: (1) Relative abundance of each fish species with catch per unit effort (CUE) for each mussel, (2) Relative abundance of each fish species with CUE for Anodontinae and Lampsilinae, (3) Mussel diversity with fish diversity at each site, (4) Mussel CUE with fish families Centrarchidae, Catostomidae, Percidae, & Cyprinidae, and (5) CUE of mussels subfamilies with same four fish families.

Mussel survey

Mussel survey methodology was similar to that developed by Dr. Robert Bright at the Bell Museum of Natural History (Bright *et al.* 1995). Each site was examined using a combination of SCUBA, snorkeling, or wading. Two quantitative sampling techniques were used at each site. The first method involved establishing a starting point near the edge of the river and several rows of quadrats that result in a rectangular grid system having the starting point as a corner. Rows of quadrats were spaced 4-10 m apart. At each quadrat, a 1/8 m^2 steel frame was placed on the bottom and the substrate inside the frame was sampled for mussels to a depth of about 10-15 cm (wrist depth). The number of quadrats examined at each site depended on the width of the river. In the upper reaches of the rivers, the number of quadrats ranged from 20-30, and in the lower reaches 30-40 quadrats were sampled. Extra quadrats were sampled at the first three upper and lower sites to determine if more quadrats added to the number of species found and to determine a reasonable number of quadrats given time constraints. After quadrats were examined, a timed search was conducted. This procedure involved the project manager searching as many habitats as possible for 30 minutes. Searching prime mussel habitat was emphasized in the timed search. To better describe the extant mussel community live mussels were collected preferentially to dead shells. We collected dead shells when no live animals were observed.

Habitat information recorded included the riverine type (pool, glide, run, or riffle), substrate particle size, depth, and water temperature. Substrate particle sizes were given in the standard APHA system (APHA *et al.* 1992). We noted the substrate preferences of each mussel collected during the grid sampling. Water temperature was measured using a high-quality thermometer at a depth of 10 cm at a shaded portion of the stream.

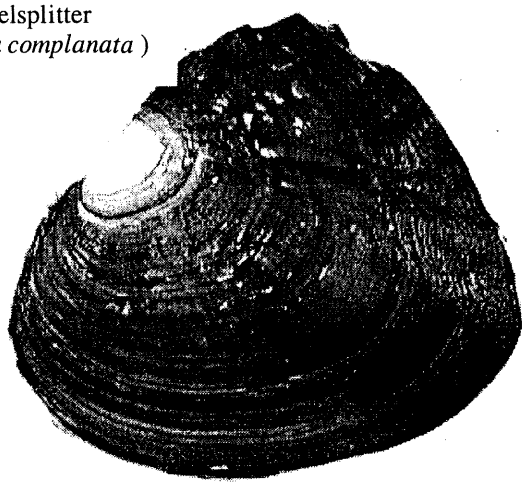
All live mussels were aged, sexed when possible, and measured to the nearest tenth of a millimeter using a caliper. Length was measured parallel to the hinge. Height was taken as the maximum distance perpendicular to the length. Width was measured perpendicular to the commisure. Age was determined by counting the external annuli. We determined gravidity of a subsample of each mussel species by opening a mussel's valves with a pair of sharpened carpet pliers or butter knife to determine if the marsupia were inflated with glochidia. All specimens were identified on site with the aid of a "mobile" reference collection provided by the Bell Museum and mussel identification key (Stern 1990). Voucher specimens were placed in the Bell Museum of Natural History, University of Minnesota.

Fish survey

Fish were collected with bag seines, one of which had a 9.5 mm chain attached to its lead line; Erickson nets (Erickson 1980); and three types of electrofishers. Selection of gear type depended on the size of the body of water. Electrofishing gear was used in small streams to medium-sized rivers. Electrofishing equipment or small mesh seines were used in lakes and large rivers. Some stations required using seines in combination with electrofishing gear.

Collection sites were selected mainly on historical records and habitat diversity, and secondarily on the distance between stations and accessibility. The length of a sampling station ranged between 60-100 m. The survey was conducted in the fall to take advantage of generally low water levels and fish populations are high, since winter mortality to the year's cohort has not yet occurred. Becker (1983) was used to identify fishes. Voucher specimens were placed in 10% formalin on site and transported to the Bell Museum of Natural History. Standard length and weight was measured to the nearest 1 mm and 0.1 g respectively.

white heelsplitter
(*Lasmigona complanata*)



IV. Results and Discussion

Objective 1: Native mussel refuge from zebra mussels

Based on the 0.25 m^2 quadrats taken, the average density of mussels was approximately 77 mussels/m^2 at each location (Figure 5). An analysis of variance indicated there was no significant difference between locations or years at the two sites. Thus in 1997 77 mussels were added for the double density treatments. These results also indicate that there were not significant changes in mussel density at either site outside the relocation grids.

These average densities were much higher than that actually found in the experimental grid (Figure 6). On average there were 38.8 mussels/m^2 in the grid (standard deviation = 20.1). These results indicate that the 0.25 m^2 may be more efficient in obtaining all mussels. Clearly, since the substrate is excavated and sieved a greater number of small mussels could be found. It is possible that the "double density" treatments were actually very dense treatments. An analysis of variance indicated there was no significant difference in density among treatments or between locations. These results show that the two locations were in fact good "replicates" to be used for a relocation study.

Similar numbers of mussels were taken at the two sites, 2344 from Sunrise and 2118 from Wild River (Table 1). A total of 18 species of mussels were found at both the Sunrise River and Wild River sites. *Cumberlandia monodonta* was found at the Sunrise River site but not the Wild River site, while *Obliquaria reflexa* was taken at Wild River but not the Sunrise River site.

Figures 7 and 8 show the proportions of mussels marked in 1997 that were recovered, found dead or were missing in 1998. A nominal logistic regression (status as dependent and location and treatment as independent variables) indicate that status varies significantly only by location ($p<0.0001$) although treatment is close to having a significant influence ($p=0.08$). The main difference between locations is the greater percentage of mussels found dead at the reference site (Wild River) ranging from about 8-10% while at the relocation site the percentage of mussels found dead was only about 3-7%. The fact that there was no difference among treatments and that there were a lower number of mussels dying at the relocation site indicates that, at least for a one-year period, relocation appears to have little negative impacts on mussel survivorship.

While there was a fair number of mussels marked in 1997 that were missing in 1998 (501 - 11%), there was a large number of new mussels that entered the relocation grids between 1997 and 1998 (810 at the Sunrise River site and 625 at the Wild River site - Table 1). Figure 9 shows the distribution of new mussels among treatments. There was a significant difference among treatment and between locations (c^2 analysis). There was greater proportion of mussels that moved into the *Quadrula pustulosa* treatment and a lower proportion that moved into the *Elliptio dilatata* at the Wild River (reference) site compared to the Sunrise site. These data indicate that mussels seem to be quite mobile, although it is possible that the apparently large amount of immigration and emigration from the grids could be in response to the disturbance resulting from the relocation process.

Paired t-tests indicated there were significant increases in both shell length and wet weight between 1997 and 1998 at both the Sunrise and Wild River sites (Figure 10). Average shell length increases were 1.54 and 1.75 mm at the Sunrise and Wild River sites, respectively. Weight changes were 5.01 and 6.98 mg at Sunrise and Wild River sites, respectively (Figure 10). Analysis of variance indicated that both species (when examining the top six species at each location - Table 1) and treatment had significant impacts on increases in both shell length and wet weight. The treatment effects are difficult to interpret because of the unequal distribution of species among treatments. There were clear differences among species the most obvious in the thin-shelled *Lampsilis cardium*. The wet weight increased 15.4 and 9.2 mg at the Wild River and Sunrise sites respectively (Figure 11). These weight gains corresponded to 1.97 and 1.84 mm in shell length at the Wild River and Sunrise sites (Figure 11). While the weight gains for other species were not as dramatic for other species, the trend of greater weight gains at Wild River compared to the Sunrise site was consistent for 5 other common species (Figure 11).

To examine the change in growth more closely we examined growth of mussels in the three treatments involving the addition of specific species, *Lampsilis cardium, Elliptio dilatata* and *Quadrula pustulosa*. A series of t-tests indicated a significantly higher change in wet-weight among all three species at the relocation site (Wild River) compared to the reference site (Sunrise) (Figure 12). There was only a significant difference in the change in shell length for *Lampsilis cardium* between sites (Figure 12). These results indicate that the relocation of these species did not have a significant negative impact on their growth. In fact growth was greater at the relocation site.

Cope and Waller (1995) prepared an extensive review of mussel relocations and evaluated their relative success as a conservation and management strategy. They found that for the studies which reported mortality data, the average mortality was 49% based on an average recovery rate of 43%. In our study the recovery rate (recovered + dead) was much higher (~74-80% at Wild River and ~78-85% at Sunrise) with mortality ranging from 2.7-10% depending on location and treatment. They indicated that mussel relocations should be monitored on a long-term and quantitative basis. They also indicated that sublethal indicators of relative condition should also be measured. We hope to continue this monitoring effort for at least two more years. It has all of the criteria suggested by Cope and Waller (1995) for an optimal relocation study.

In summary it appears that there were few adverse impacts of relocation of mussels on either the mussels relocated or on the receiving mussel community. Mussel survivorship was high and there was measurable growth in most species. Continued monitoring of these treatments would allow for the examination of the longer-term success of in-situ relocation as a means to protect mussels from a number of threats including the introduction of zebra mussels. Dr. Hornbach will monitor this relocation project for the next two years to further examine the efficacy of this procedure for populations threatened either by zebra mussel infestations or human interference.

Objective 2: Rare mussel life history studies

Determination of Suitable Fish Hosts - test for evidence of metamorphosis of glochidia into juvenile mussels

Host suitability tests were conducted on a variety of rare Minnesota mussel species including: pistolgrip, spectaclecase, snuffbox, purple wartback, ellipse, and winged mapleleaf glochidia. Yellow bullhead and brown bullhead facilitated pistolgrip metamorphosis of glochidia (Tables 2 and 3). Juveniles grew 2-3 times in length while attached to hosts. Although no juveniles were collected from black bullheads and creek chubs, growth was observed in pistolgrip glochidia collected from these fishes. None of the twenty-five fish species or mudpuppies exposed to spectaclecase glochidia facilitated metamorphosis of glochidia (Table 2). Snuffbox glochidia were exposed to four fish species. Blackside darters and logperch served as hosts (Tables 2 and 3). Four of seven Ictalurids were found to be suitable hosts for purple wartyback glochidia (Tables 2 and 3). Several fishes are suitable hosts for ellipse glochidia include: brook stickleback, mottled and slimy sculpin, banded, blackside, fantail, Iowa, Johnny, mud, and rainbow darters, and logperch (Tables 2 and 3).

None of the 81 trials conducted on 53 fish species or mudpuppies facilitated glochidia metamorphosis of winged mapleleaf glochidia (Table 2). Although no juveniles were collected from black and brown bullheads, and flathead catfish, growth was observed in glochidia collected from these fishes. No glochidia were observed on the gill lamellae of the lamprey ammocoete on the 57th day of the experiment.

Premature deaths of test fish may have prevented identification of suitable hosts that would have otherwise been identified. Growth of glochidia was observed during each of three trials using flathead catfish. Unfortunately, all but one of these fish died from "Ich" (*Ichthyophtherius multifilis*) before the study was completed. During previous host suitability studies in our laboratory, flathead and channel catfishes exposed to purple wartyback (*Cyclonaias tuberculata*) glochidia frequently contract Ich and die prior to excystment of the juveniles. Future host suitability studies should include catfishes, especially flathead catfish, among the species to be tested.

This study has identified previously unknown suitable hosts for several mussels. We showed for the first time that yellow bullheads and brown bullheads are suitable hosts for pistolgrip glochidia (Watters 1994). It was unusual to observe the growth by glochidia while attached to hosts. To our knowledge this is the first time Amblemine glochidia have been shown to double or triple in size while attached to the host. We verified that logperch are suitable hosts for snuffbox (Watters 1994, Sherman 1998) and found that banded darters also facilitate glochidial transformation. Channel catfish have been shown to be suitable hosts for purple wartyback glochidia (Hove *et al.* 1994). We found that flathead catfish and black bullhead also serve as suitable hosts. Five fishes have been shown to be suitable hosts for creek heelsplitter glochidia (Hove *et al.* 1995, Watters 1994). This study adds pumpkinseed to the list of suitable hosts. Prior to this study Johnny darters and slimy sculpin were shown to facilitate ellipse glochidia transformation (Hove *et al.* 1996). We observed brook stickleback, banded darter, blackside darter, fantail darter, Iowa darter, mud darter, rainbow darter, logperch and mottled sculpin are also suitable hosts.

Determination of Natural Glochidial Infestation of Fish

Fish likely to be naturally infested with glochidia were collected from the St. Croix River this summer. Approximately one hundred fish (nine species) were collected from Interstate State Park, Minnesota and transported to the University of Minnesota. Juvenile mussels were not collected from smallmouth bass, river darters, or western sand darters (Table 4). Juvenile mussels were collected from emerald and mimic shiners, freshwater drum, redhorse, and logperch (Table 5). Relatively common juvenile mussels (Anodontine) were collected from mimic and emerald shiners. Anodontine juveniles were collected from Moxostomids. Juvenile mussels collected from logperch were difficult to identify but appear to be either threeridge (*Amblema plicata*) or spike (*Elliptio dilatata*). Juveniles collected from freshwater drum are likely: pink heelsplitter (*Potamilus alatus*), butterfly (*Ellipsaria lineolata*), and deertoe.

Preliminary methods for identification of glochidia by application of species-specific DNA markers have been customized for use in our laboratory. We optimized methods in White *et al.* (1994) for our laboratory and were able to reproduce results published in White (1994). Using several restriction enzymes we identified a diagnostic suite of species-specific banding patterns for the five mussels of interest. Uncut ITS-1 region for all five mussels was approximately 560 bp. Hae III cuts pistolgrip and spectaclecase DNA in fewer places than deertoe, fawnsfoot, and fragile papershell DNA (Figure 13). Amplified DNA from spectaclecase and pistolgrip is cut differently using both Msp I and Sau 96, and Mse I (Figure 14). Fragile papershell DNA is cut into smaller pieces using Msp I than fawnsfoot and deertoe DNA, and deertoe DNA is cut in more places using Sau 96 than fawnsfoot DNA (Figure 15).

DNA banding patterns identified in this study should be verified. We used a small number of mussels in conducting these tests. Additional individuals from each species from a variety of locations should be collected and tested to determine if the markers we identified are consistent within a species and between drainages. Molecular markers for the other St. Croix River

mussels should be identified to fully realize the utility of this technique in identifying natural hosts of rare Minnesota mussels.

Objective 3: Fish and mussel surveys in northern Minnesota

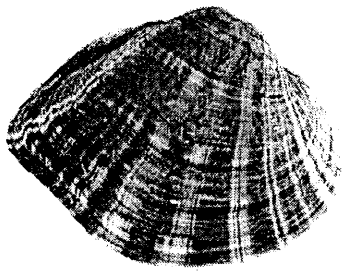
The mussel and fish fauna of the Big Fork and Little Fork rivers were surveyed during August - September, 1997 and 1998 respectively at twenty five sites distributed throughout each watershed (Table 6). Ten mussel species (4,685 individuals) were collected between the two river basins, including creek heelsplitter, flutedshell, and black sandshell which are special concern species in Minnesota (Table 7). Average live mussel density in mainstem Big Fork and Little Fork rivers was 2.8 /m^2, range 0-32/m^2. 48 fish species among 6,666 specimens were collected. Minnesota listed lake sturgeon (threatened) and northern brook lamprey (special-concern) were observed.

Mussels and fishes were collected at nearly every site in the Big Fork River basin. Ten mussel species (1,712 live individuals) observed include (in order of abundance): fatmucket, plain pocketbook, giant floater, cylindrical papershell, white heelsplitter, black sandshell, creek heelsplitter, creeper, paper pondshell, and flutedshell (Table 8). In the Big Fork River average mussel density was 3.7 mussels/m^2 and ranged from 0 at some headwater sites to 31.5 mussels/m^2 at Harrison boat landing (Big Fork River mile 154.3). Highest densities were generally highest in the headwaters with a gradual decline in density to nearly 0 at the confluence with the Rainy River (Figure 16). We collected 3,576 fishes (42 species) during the survey (Table 9). In the Big Fork River, habitat and water quality appeared good at most sites. Silty substrate and turbid water were observed at only three sites: Bear River (Site 4), Reilly Brook (Site 11), and Bowstring River (Site 25).

Mussels and fishes were collected at every site in the Little Fork River basin. Eight mussel species (2,973 live individuals) observed include (in order of abundance): fatmucket, plain pocketbook, black sandshell, giant floater, cylindrical papershell, white heelsplitter, creek heelsplitter, and creeper (Table 10). In the Little Fork River average mussel density was 2.0 mussels/m^2 and ranged from 0 to 7.1 mussels/m^2 (Figure 17). We collected 3,090 fishes (38 species) during the survey (Table 11). In the Little Fork River, habitat and water quality appeared fair to good at most sites.

Several new observations of mussels were made for the first time. Flutedshell has not been previously observed from the Hudson Bay drainage before (Graf 1997). The fact that a single flutedshell shell was collected from an atypical marsh habitat raises the question of whether the mussel was introduced as a glochidium attached to a stocked sunfish or bass. Paper pondshell was sighted for the first time in the Lake of the Woods basin. We also collected voucher specimens of creeper and cylindrical papershell that were only known previously from the Lake of the Woods basin from accounts in the literature.

Strong linear relationships between mussel and fish abundance were rare. Of 1590 comparisons only 16 had coefficients of determination (R^2) > 0.5 and p-values < 0.05 (Table 12). Because most mussels depend on host fishes for transformation from glochidium to juvenile, there must be a relationship between their distributions on some level. Strong relationships may be few because the relationships are more complicated. For example, the host requirements for these mussels may be far broader than we realize. In addition, fishes are more mobile than mussels and most species are shorter lived. It may be that the persistence of certain components of a fish community over decades is a more important independent variable. Future data analysis will incorporate cluster analysis and fish data from the 1970s and the 1980s.



deertoe
(*Truncilla truncata*)

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IV. Results and Products

The overall goal of this program was to improve freshwater mussel conservation and water resource management, by filling critical information gaps on the life-cycle requirements and ecology of freshwater mussels. Specific results or outcomes are:

- Analyzed effectiveness of in-situ relocation as an effective means to protect native mussels.
- Described the impact of relocation on the relocated mussels and on the mussel populations that receive the relocated mussels.
- Determined if there is a negative effect of increasing mussel density in areas receiving relocated mussels.
- Determined the suitability of potential refugia for native freshwater mussels in the St. Croix River.
- Identified fish hosts for spectacle case, pistogrip, *Cumberlandia monodonta*, *Tritogonia verrucosa*, and *Venustaconcha ellipsiformis*.
- Developed species-specific molecular markers for *C. monodonta*, *T. verrucosa*, *Truncilla truncata, T. donaciformis*, and *Leptodea fragilis* that can be used to identify glochidia, juvenile, and adult life stages.
- Described the composition and population health of the mussel and fish communities in the Little Fork and Big Fork rivers.
- Evaluated the influence of fish species distribution on mussel species distribution.
- Addition of fish and mussel site locality records from the Little Fork and Big Fork rivers to the Minnesota County Biological Survey.
- Created slide shows, posters, scientific publications, and World Wide Web Page describing survey project results.

Additional products developed with matching grant funding

- Identified suitable fish hosts for purple wartyback (state listed), and snuffbox (state listed). Funding sources – Minnesota Department of Natural Resources, Natural Heritage and Nongame Research Program, and University of Minnesota, Undergraduate Research Opportunity Program.

- Identified potential fish hosts for the federally endangered winged mapleleaf. Funding source – federal aid under Section 6 of the Endangered Species Act of 1973 with matching funds from the Wisconsin Department of Natural Resources.
- Preliminary investigation of whether brooding mussels release chemical fish attractants. Funding source – University of Minnesota, Undergraduate Research Opportunity Program.
- Influence of heat on development time of glochidia. Funding source – University of Minnesota, Undergraduate Research Opportunity Program.
- Well-fed host fishes produce 'healthier' juvenile mussels than poorly fed host fishes. Funding source – University of Minnesota, Undergraduate Research Opportunity Program.
- Accession of 145 lots of freshwater mussels at the University of Minnesota, Bell Museum of Natural History. Funding source – University of Minnesota, Bell Museum of Natural History.

V. Timetable

Result 1: Native mussel refuge from zebra mussels

Project Activities	Completion Date
1) Evaluate first refuge sites, improve design if necessary	August 1997
2) Construct refuges in upper & lower St. Croix River	August 1997
3) Collect & measure mussels, and move them to refuges	August 1997
4) Measure mussel growth, survival, population density and diversity at refuges	September 1998
5) Analyze data and develop general recommendations for future relocation efforts	April 1999
6) Prepare final report; give public presentations on findings	June 1999
After grant period	
1) Combine findings with those of NPS & NBS (their study ends Sept. '99) in scientific publications	1999/2000
2) Pursue additional funding to continue monitoring the refuges beyond grant period	

Result 2: Rare mussel life history studies

Project Activities	Completion Date
1) Collect fish for first year host studies	August 1997
2) Spectaclecase and pistolgrip host studies and collection of naturally infested fish	August 1997
3) Collect additional fish for ellipse host studies	November 1997
4) Ellipse host studies	May 1998
5) Development & testing of spectaclecase and pistolgrip genetic markers	April 1998
6) Collect fish for second year host studies	September 1998
7) Spectaclecase and pistolgrip host studies and collection of naturally infested fish	August 1998
8) Collect additional fish for ellipse host studies	October 1998
9) Ellipse host studies	March 1999
10) Continued development & testing of spectaclecase and pistolgrip genetic markers	May 1999
11) Application of genetic markers to identify juvenile mussels collected from naturally infested fish	May 1999
12) Submit results for publication and post on Internet	June 1999

Result 3: Fish and mussel surveys in northern Minnesota

Project Activities	Completion Date
1) Fish and mussel survey of the Little Fork River	September 1997
2) Catalogue material from Little Fork River into the Bell Museum	June 1998
3) Fish and mussel survey of the Big Fork River	October 1998
4) Catalogue material from Big Fork River into the Bell Museum and update specimen database	June 1999
5) Submit survey results for publication and presentations, and submit results for use by MN DNR County Biological Survey and National Forest Service	June 1999

VI. Budget Requirements

Four organizations are providing in kind funds for this project. The National Biological Service (NBS) and National Park Service (NPS) have a $210,000 grant to establish and initiate a monitoring program for a native freshwater mussel refuge. This project involves studying the effectiveness of a mussel refuge as a sanctuary from the invading exotic zebra mussel and will involve establishing one refuge below Taylors Falls (Figure 1). Due to the possibility of zebra mussels moving upstream to the natural barrier provided by Taylors Falls, we propose establishing and monitoring a second experimental refuge upstream of the falls and then compare the effectiveness of the two refuge sites. Thus, the in-kind support from NBS and NPS will make the comparison possible. The University of Minnesota and Macalester College will also provide in kind support.

Existing grant programs relating to this LCMR project include funding from the Minnesota Department of Natural Resources, Natural Heritage and Nongame Research Program which is providing $9788 (FY96-FY98) for the determination of host species for the three rare mussels to be studied in this project. Also, a proposal is pending before the University of Minnesota, Undergraduate Research Opportunities Program for $3150 (FY96) to study fish hosts. These projects will allow us to develop important techniques that we will then apply in the LCMR project.

Expenditure allocation

	July 1997-June 1999 Total LCMR requested amount distributed to:
Personnel	
University of Minnesota	$63,980
Macalester College	$7840
Total	$71,820
Equipment	
University of Minnesota	$4600
Macalester College	$550
Total	$5150
Acquisition	n/a
Development	n/a
Other (Supplies)	$12,980
University of Minnesota	$1050
Macalester College	$14,030
Total	$91,000

COST FOR RESULTS:

Result 1 — Native mussel refuge from zebra mussels

Project Manager salary	$12,355
Macalester College: 1 UG* salary for 2 summers	$7840
Macalester College: equipment-SCUBA, lab	$550
Macalester College: SCUBA, lab	$1050
Total	$21,795

Result 2 — Rare mussel life history studies

Project Manager salary	$26,083
University of Minnesota: equipment	$2560
University of Minnesota: supplies	$10,880
Total	$39,523

Result 3 — Fish and mussel surveys in northern Minnesota

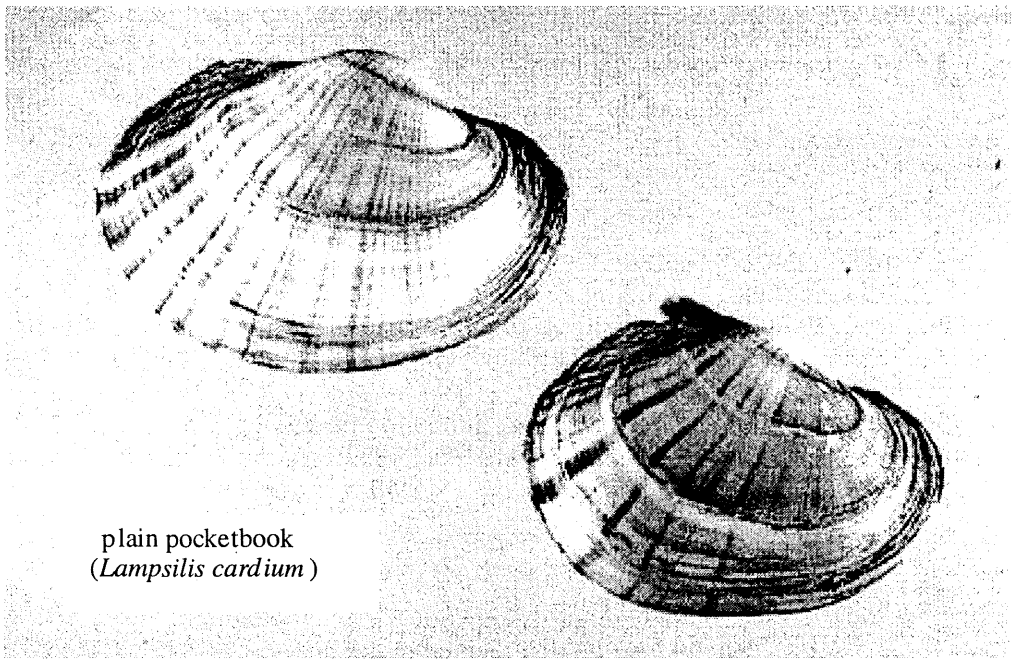
Project Manager salary	$13,042
Bell Museum 1 UG for 2 summer-fall periods	$12,500
University of Minnesota: equipment	$2040
University of Minnesota: supplies	$2100
Total	$29,682

Total $91,000

* UG = undergraduate research assistant

VII. Investigator/Cooperator Background

Please see resumes attached to grant proposal that describe the experiences of Mark Hove, Dr. Daniel Hornbach, Dr. Anne Kapuscinski, and Dr. Jay Hatch in conducting natural resource studies. Sue Jennings, Resource Management Specialist-NPS, and her staff are experienced with boat handling and fish collection in the St. Croix River. Dr. Greg Cope, Research Biologist-NBS, and his staff are experienced with boat handling, and refuge design and construction in the St. Croix River. Taylor Polomis, Assistant Area Supervisor-MN DNR, and his staff are experienced with collecting fish from Twin Cities lakes and rivers. Ron Benjamin, Area Supervisor, WI DNR, and his staff are experienced with collecting fish and mussels from MN-WI boundry waters.



plain pocketbook
(*Lampsilis cardium*)

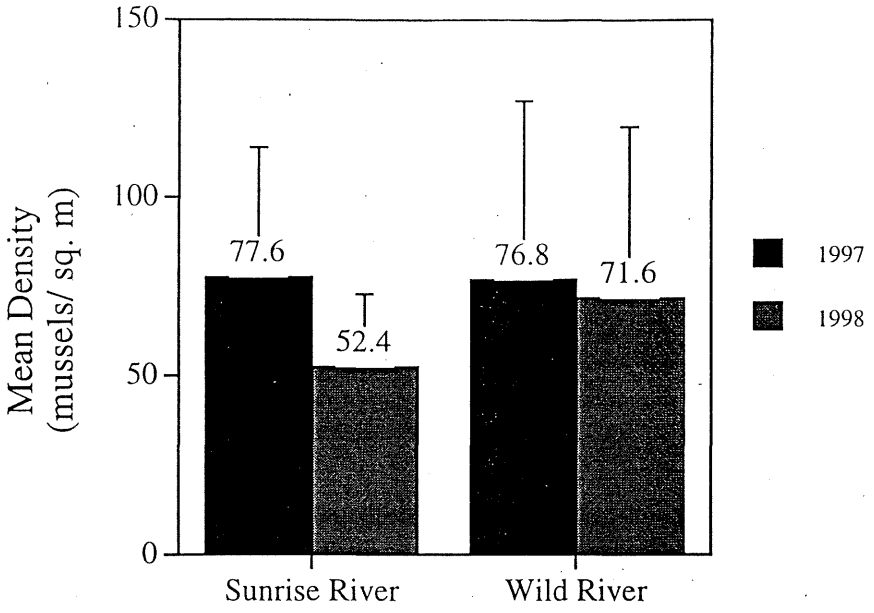


Figure 5. Mussel density at reference (Sunrise River) and relocation (Wild River) sites. Average mussel density based on 10 0.25 m^2 quadrats taken outside the study grids.

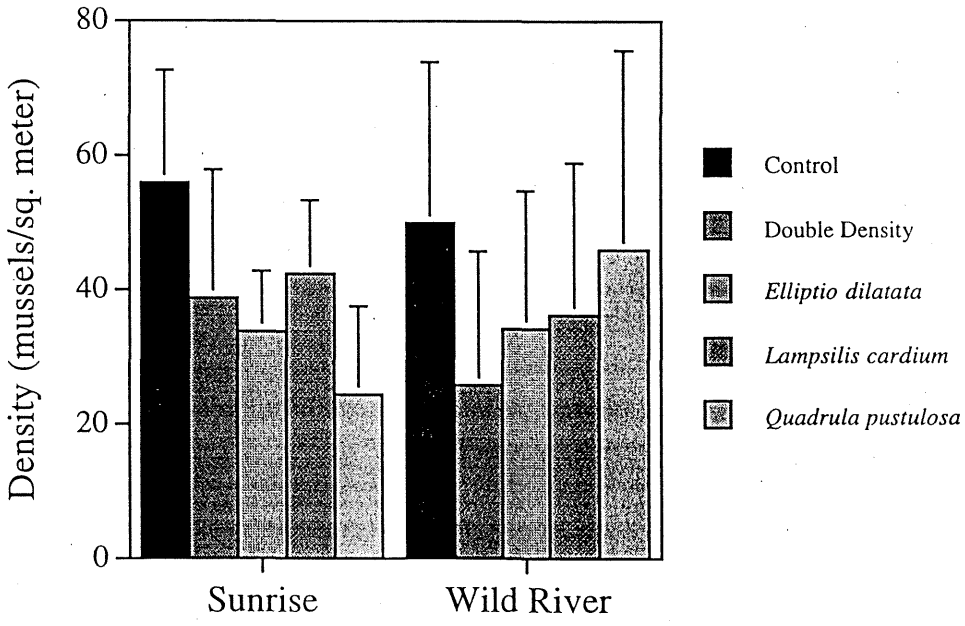


Figure 6. Average mussel density in the 5 treatments.

Table 1. Mussels collected at Sunrise River and Wild River sites.

Species	Sunrise River Site						
	Treatments						
	Control	Double Density	*Elliptio dilatata*	*Lampsilis cardium*	*Quadrula pustulosa*	New-1998	Total
Actinonaias ligamentina	99	223	71	84	43	299	819
Alasmidonta marginata	3	2	0	1	1	11	18
Amblema plicata	1	3	0	1	1	0	6
Cumberlandia monodonta	0	0	0	1	0	0	1
Cyclonaias tuberculata	23	40	10	15	12	36	136
Elliptio dilatata	92	193	91	65	32	316	789
Fusconaia flava	11	8	3	6	6	11	45
Lampsilis cardium	11	13	6	54	1	28	113
Lampsilis siliquiodea	4	13	2	1	1	7	28
Lasmigona costata	15	26	11	16	6	22	96
Leptodea fragilis	3	5	1	1	0	8	18
Ligumia recta	5	6	0	0	2	12	25
Obovaria olivaria	2	10	4	3	2	3	24
Pleurobema coccineum	7	7	4	3	4	13	38
Potamilus alatus	0	0	0	0	1	3	4
Quadrula pustulosa	12	17	8	9	57	15	118
Strophitus undulata	4	8	3	1	0	7	23
Truncilla truncata	5	8	5	2	3	14	37
UNKNOWN ADULT	0	0	0	0	0	1	1
UNKNOWN JUVENILE	1	0	0	0	0	4	5
TOTAL	298	582	219	263	172	810	2344
	Wild River Site						
Actinonaias ligamentina	117	223	61	64	92	240	797
Alasmidonta marginata	0	1	2	2	0	6	11
Amblema plicata	1	6	0	0	0	2	9
Cyclonaias tuberculata	8	30	8	14	17	9	86
Elliptio dilatata	54	95	85	44	57	148	483
Fusconaia flava	2	6	7	2	3	8	28
Lampsilis cardium	9	8	4	54	4	16	95
Lampsilis siliquiodea	0	4	0	1	2	6	13
Lasmigona costata	3	11	3	3	7	19	46
Leptodea fragilis	7	7	1	0	0	10	25
Ligumia recta	5	9	2	1	1	23	41
Obliquaria reflexa	0	4	0	1	0	1	6
Obovaria olivaria	1	8	2	1	4	5	21
Pleurobema coccineum	4	11	3	8	8	13	47
Potamilus alatus	2	1	1	0	0	2	6
Quadrula pustulosa	22	65	16	42	55	57	257
Strophitus undulata	2	2	2	3	1	5	15
Truncilla truncata	12	29	14	11	10	36	112
UNKNOWN JUVENILE	1	0	0	0	0	19	20
TOTAL	250	520	211	251	261	625	2118

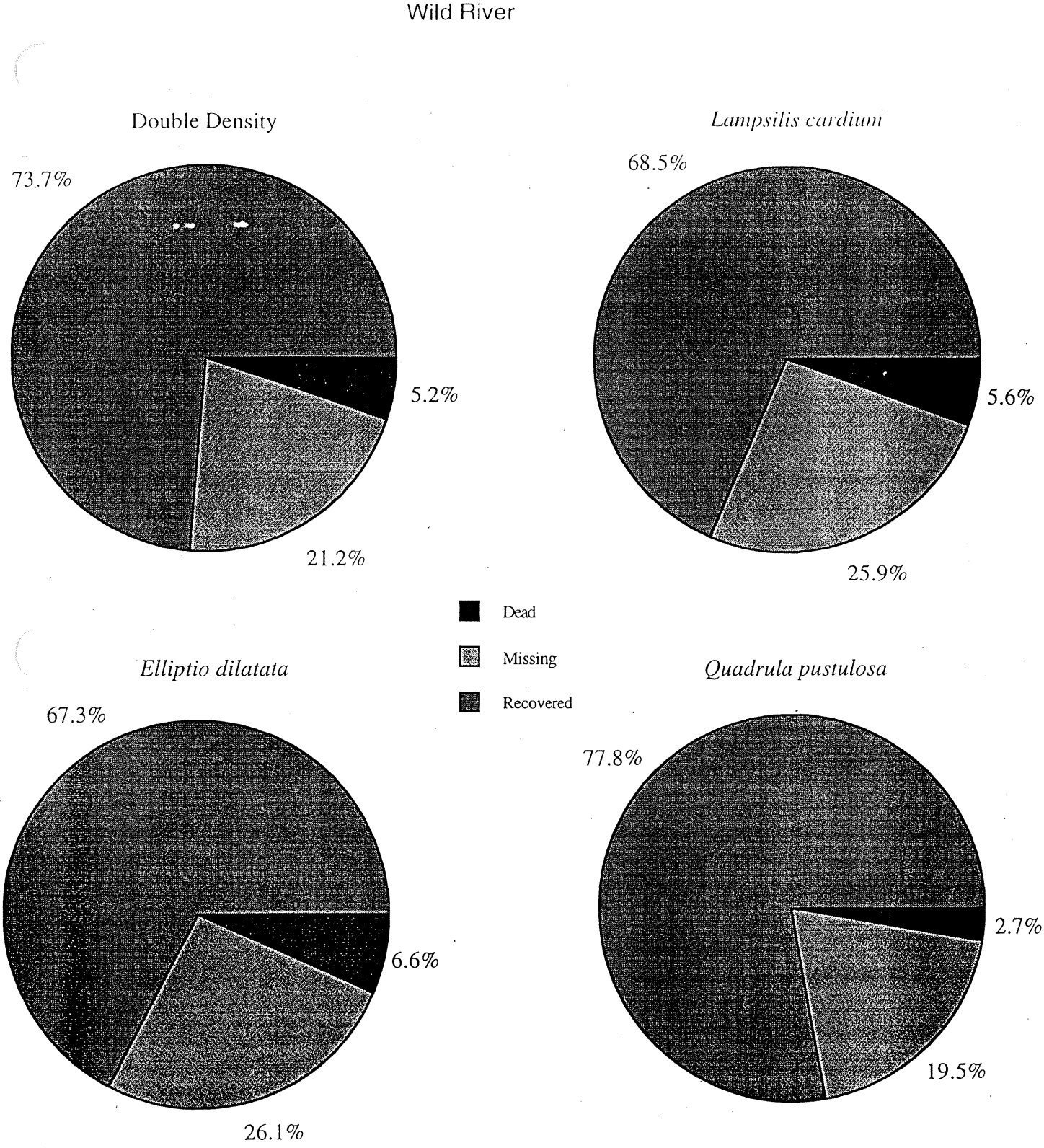


Figure 7. Percentage of mussels recovered, found dead or missing from the four treatments at the Wild River reference site.

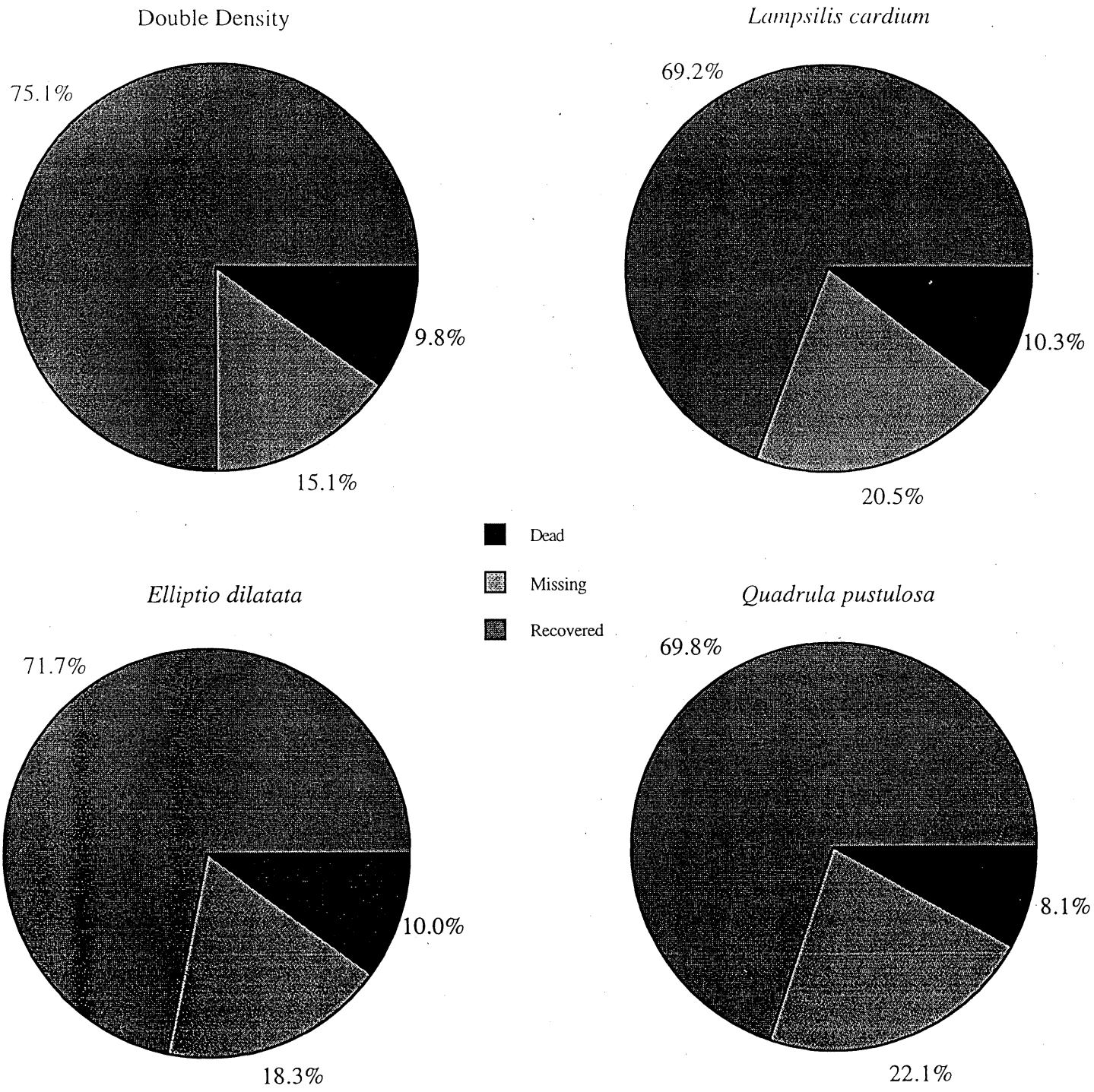


Figure 8. Percentage of mussels recovered, found dead or missing from the four treatments at the Sunrise River relocation site.

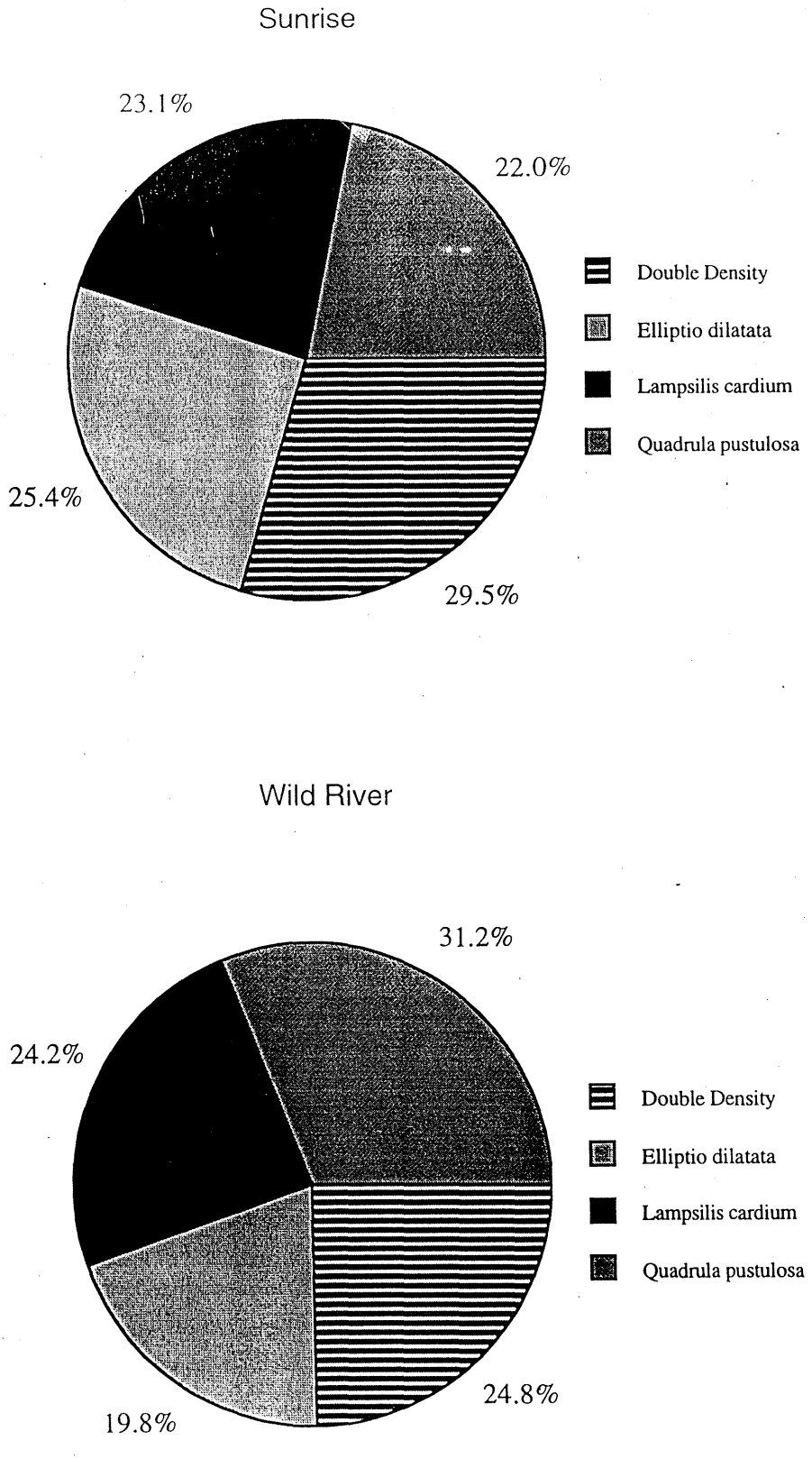


Figure 9. Distribution of new mussels found in 1998 among various treatments.

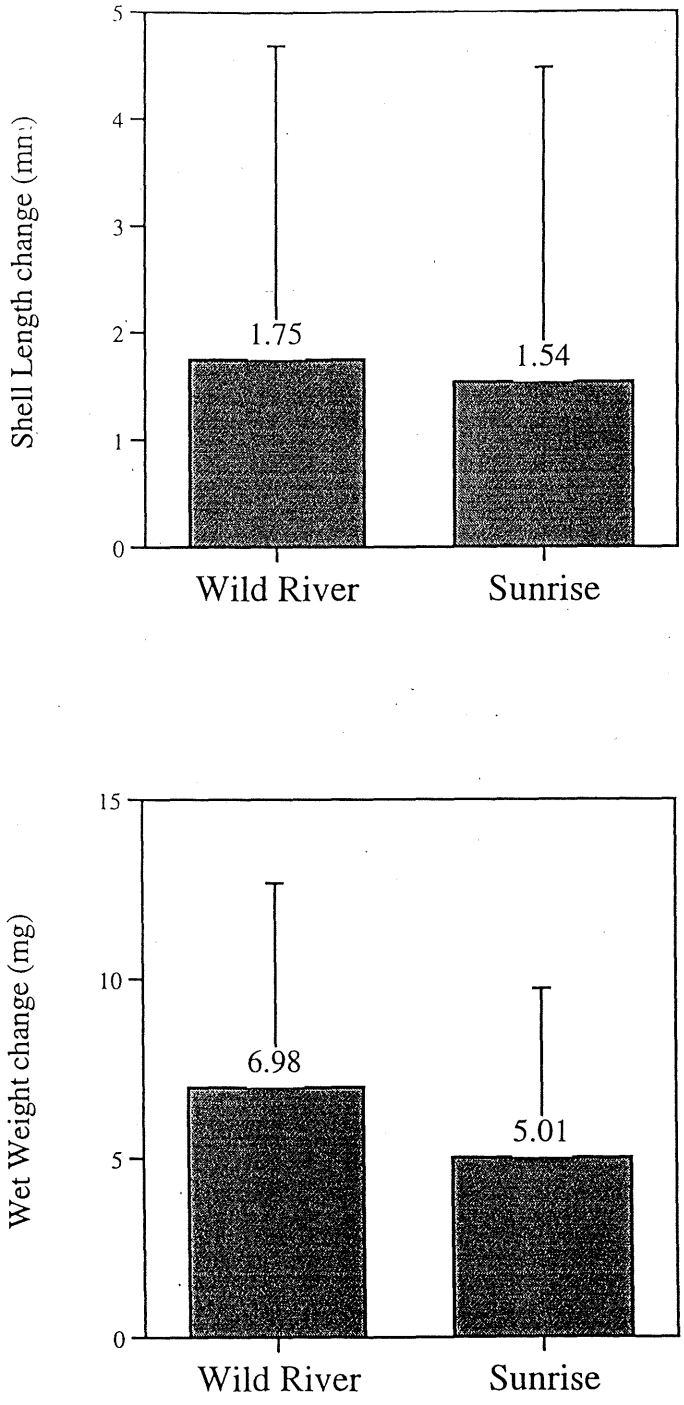


Figure 10. Changes in shell length and wet weight between 1997 and 1998.

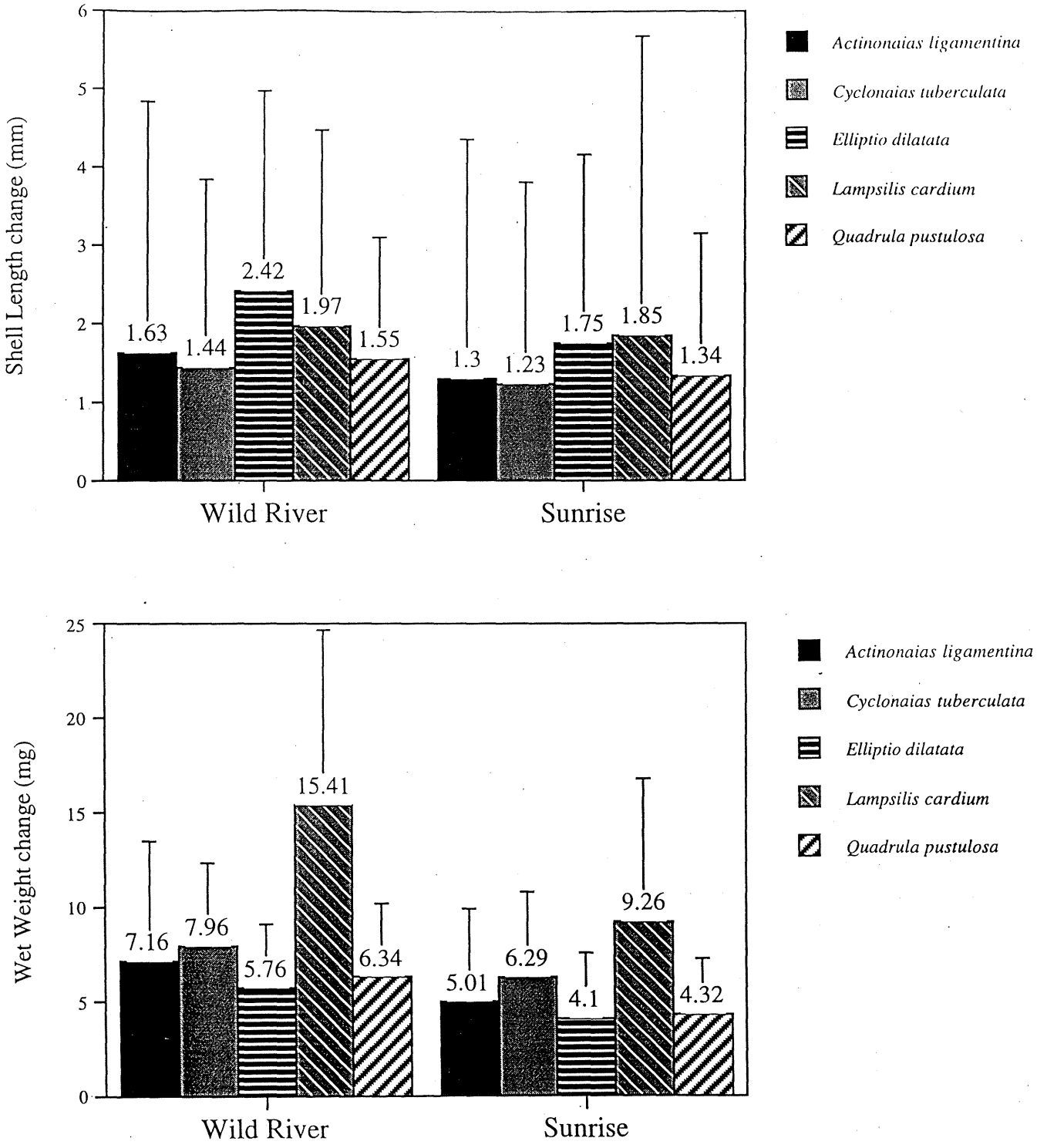
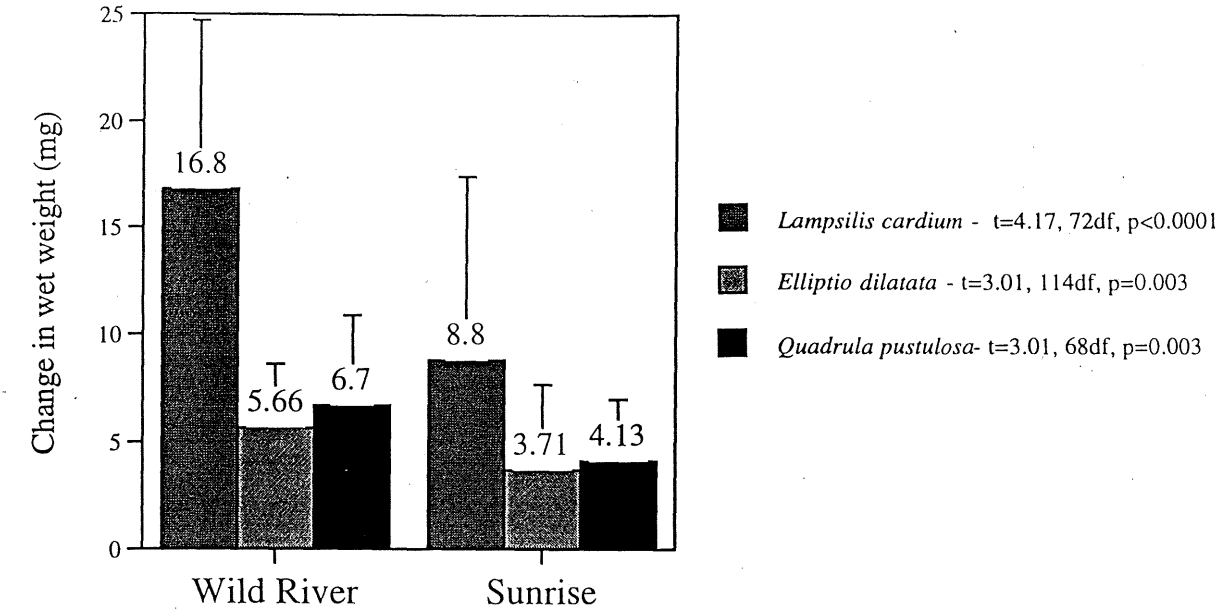


Figure 11. Changes in shell length and wet weight among dominant species between 1997 and 1998.



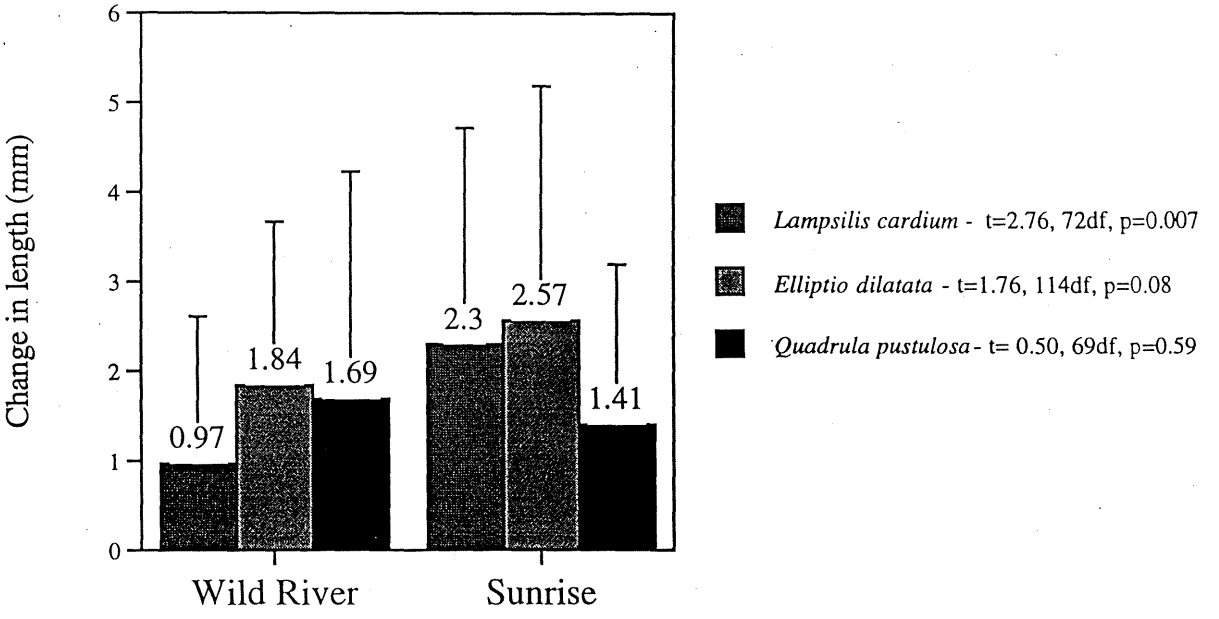


Figure 12. Changes in shell length and wet weight among the three species treatments.

Table 2. Suitable hosts for pistolgrip, ellipse, snuffbox, and purple wartyback glochidia.

	Number of fish inoculated/survived	Days to meta-morphosis	Number of juveniles recovered
pistolgrip			
yellow bullhead	3/1	15-22	11
brown bullhead	7/7	26-36	6
snuffbox			
blackside darter	4/4	23-30	5
logperch I	2/2	29-38	7
logperch II	5/5	28-51	122
purple wartyback			
black bullhead I	9/5	29-33	3
black bullhead II	6/6	12-22	5
channel catfish I	4/0	31-33	61*
channel catfish II	7/1	23-36	92
channel catfish III	3/1	17-29	119
channel catfish IV (barbels only)	4/0	17-19	2*
flathead catfish I	6/0	29-33	16*
flathead catfish II	3/3	19-27	3
yellow bullhead	6/3	24-38	87
ellipse			
brook stickleback	8/3	18-35	74
mottled sculpin I	4/0	19-36	64*
mottled sculpin II**	8/4	184-191	17
slimy sculpin**	11/7	192-197	27
banded darter**	9/0	149-176	2*
blackside darter I	5/3	51-53	1
blackside darter II	4/4	30-34	7
blackside darter III	7/7	18-21	3
blackside darter IV**	39/30	179-186	3
fantail darter	8/6	18-35	56
Iowa darter I	8/8	18-30	41
Iowa darter II**	16/13	184-192	4
Johnny darter**	65/0	129-137	1*
logperch I	6/0	25-32	3*
logperch II**	38/0	129-137	1*
mud darter**	17/17	141-149	1
rainbow darter**	21/17	186-194	15

* - Incomplete trial, test subjects died before completion of study.
** - Fish held at 11 °C throughout most of the trial.

Table 3. Trials where glochidial metamorphosis was not observed.

Common name	Number of individuals inoculated	Number of survivors	Glochidia attachment period (days)
spectaclecase			
chestnut lamprey	5	5	6-9
bowfin	4	4	1-4
carp	1	1	1-4
common shiner	1	1	1-4
fathead minnow	10	10	1-4
goldfish	5	4	1-4
longnose dace	9	7	20-22
mimic shiner	4	0	*
northern redbelly dace	8	3	20-22
spotfin shiner	10	10	1-4
white sucker	6	6	**
channel catfish I	1	1	1-4
channel catfish II	6	1	6-11
channel catfish III	4	1	5-9
flathead catfish I	4	3	11-13
flathead catfish II	4	4	**
stonecat I	3	3	1-4
stonecat II	3	3	1-4
tadpole madtom	3	3	1-4
yellow bullhead I	7	5	1-4
yellow bullhead II	1	1	3-6
central mudminnow	10	10	1-4
burbot I	3	3	1-4
burbot II	2	2	1-4
burbot III	6	5	3-6
banded killifish	8	0	*
mottled sculpin	6	6	6-8
black crappie	8	8	1-4
green sunfish	10	10	1-4
pumpkinseed	7	7	1-4
rock bass	10	10	1-4
blackside darter	6	6	1-4
fantail darter	4	4	1-4
Iowa darter I	10	10	4-6
Iowa darter II	2	2	17-20
Johnny darter	11	11	1-4
logperch	6	6	1-4
yellow perch	12	8	1-4
freshwater drum	3	3	1-6
mudpuppy I	4	4	13-15
mudpuppy II	3	3	1-4
tiger salamander	7	7	14-16

* - Incomplete trial, test subjects died before completion of the study.
** - Unsuccessful inoculation.

Table 3. Trials where glochidial metamorphosis was not observed. (Continued.)

Common name	Number of individuals inoculated	Number of survivors	Glochidia attachment period (days)
pistolgrip			
bowfin I	4	4	2-5
bowfin II	4	4	9-12
northern pike	10	10	7-10
central mudminnow	15	13	5-8
carp	5	5	2-5
longnose dace	8	8	15-17
northern redbelly dace	10	10	1-4
spotfin shiner	7	5	15-17
creek chub	6	6	22-24
quillback	3	3	5-9
white sucker I	5	2	8
white sucker II	16	16	2-5
tadpole madtom I	6	5	19-21
tadpole madtom II	3	3	8-12
black bullhead I	6	6	10-13
black bullhead II	7	7	16-18
black bullhead III	3	3	18-21
black bullhead IV	11	11	13-16
black bullhead V	14	14	25-26
black bullhead VI	9	9	8-12
yellow bullhead I	4	4	21-23
yellow bullhead II	3	3	18-20
channel catfish I	5	2	8
channel catfish II	6	6	1-6
channel catfish III	7	7	1-4
flathead catfish	6	0	*
trout-perch	2	1	1-4
burbot	4	3	4-8
banded killifish I	8	0	*
banded killifish II	14	14	2-5
brook stickleback I	10	10	9-11
brook stickleback II	4	4	4-7
mottled sculpin	6	6	6-8
bluegill	6	6	8
largemouth bass	6	5	2-5
pumpkinseed	5	5	8
rock bass	6	6	14-16
yellow perch	9	9	4-8
blackside darter	6	6	4-7
fantail darter	9	9	4-7
Iowa darter I	5	3	22-24
Iowa darter II	8	8	4-7
Johnny darter	13	13	1-4
logperch	7	7	4-7

* - Incomplete trial, test subjects died before completion of the study.

Table 3. Trials where glochidial metamorphosis was not observed. (Continued.)

Common name	Number of individuals inoculated	Number of survivors	Glochidia attachment period (days)
ellipse			
lake sturgeon	4	4	1-7
shovel sturgeon	2	2	1-7
central mudminnow	1	1	21-26
bigmouth shiner	23	23	1-5
bluntnose minnow I	8	8	2-5
bluntnose minnow II	8	8	1-4
bluntnose minnow III	11	11	1-5
bluntnose minnow IV	6	6	1-5
common shiner	4	4	1-5
emerald shiner I	6	5	1-4
emerald shiner II	12	12	1-5
fathead minnow	16	16	1-5
goldfish I	7	7	1-4
goldfish II	1	1	1-5
hornyhead chub I	6	6	1-3
hornyhead chub II	1	1	1-5
longnose dace	44	44	1-5
northern redbelly dace	2	2	1-5
river shiner	2	2	1-5
sand shiner	2	2	1-5
spotfin shiner	15	15	1-5
northern hognose sucker	1	1	1-5
redhorse sp.	3	3	5-9
white sucker	16	16	1-5
channel catfish	6	0	*
flathead catfish	15	15	3-10
tadpole madtom I	5	5	1-5
tadpole madtom II	6	6	1-5
mottled sculpin	6	0	*
slimy sculpins I	4	0	*
slimy sculpins II	6	0	*
burbot	4	4	37-39
banded killifish	1	0	*
brook stickleback I	1	0	*
brook stickleback II	1	1	40-141
black crappie	1	1	15-21
bluegill	1	1	54-75
green sunfish I	8	8	17-20
green sunfish II	2	2	26-40
largemouth bass	1	0	15-21
orangespotted sunfish	2	2	9-21
pumpkinseed I	8	6	13-15
pumpkinseed II	2	2	26-40
crystal darter	1	1	1-8
gilt darter	27	27	10-16
logperch I	3	2	8-11
logperch II	8	8	8-18
river darter I	1	1	23-25
river darter II***	27	27	1-5
slenderhead darter***	19	17	142-182
yellow perch	13	13	40-75

* - Incomplete trial, test subjects died before completion of the study.

** - Only barbels were infested with glochidia. *** - Fish held at 11 °C throughout most of the trial.

Table 3. Trials where glochidial metamorphosis was not observed. (Continued.)

Common name	Number of individuals inoculated	Number of survivors	Glochidia attachment period (days)
butterfly			
common shiner	4	0	*
longnose dace	6	5	1-6
spotfin shiner I	6	4	1-3
spotfin shiner II	6	5	8-11
banded killifish	6	5	8-11
black bullhead	6	6	3-6
mottled sculpin	8	0	*
burbot	6	5	3-6
bluegill	6	5	20-22
green sunfish	8	8	3-6
blackside darter	6	4	3-6
logperch	9	9	3-6
walleye	5	2	6-9
snuffbox			
channel catfish	5	0	*
yellow perch	8	8	17-19
blackside darter	5	5	36-38
purple wartyback			
brown bullhead	7	0	*
black bullhead I	1	0	*
black bullhead II**	7	0	*
black bullhead III	9	5	17-19
stonecat	8	8	17
tadpole madtom	8	8	22-24

* - Incomplete trial, test subjects died before completion of the study.

Table 3. Trials where glochidial metamorphosis was not observed. (Continued.)

Common name	Number of individuals inoculated	Number of survivors	Glochidia attachment period (days)
winged mapleleaf			
brown bullhead	6	3	27-29
burbot (Trial I)	4	4	5-8
burbot (Trial II)	1	1	2-11
burbot (Trial III)	2	2	1-2
channel catfish (Trial I)	16	1	36-39
channel catfish (Trial II)	36	0*	29-34
common shiner	4	4	1-2
creek chub	4	3	2-5
fathead minnow (Trial I)	1	1	1-2
fathead minnow (Trial II)	26	26	1-3
Johnny darter (Trial I)	38	38	2-5
Johnny darter (Trial II)	9	9	1-3
Johnny darter (Trial III)	2	2	1-2
lake sturgeon	4	4	1-2
lamprey sp. (ammocoete)	1	1	unclear
mimic shiner	14	14	1-2
mudpuppy	2	2	1-2
n. hognose sucker	1	1	1-3
northern pike	8	7	2-8
orange-spotted sunfish	2	2	1-2
pumpkinseed	4	4	1-2
rainbow darter	1	1	1-3
river shiner (Trial I)	7	7	1-2
river shiner (Trial II)	1	1	1-3
rock bass	17	17	1-2
sauger	4	4	1-2
shorthead redhorse	22	21	1-3
shovelnose sturgeon	2	2	1-2
slimy sculpin	13	13	2-8
smallmouth bass	12	12	1-2
spotfin shiner (Trial I)	7	7	1-2
spotfin shiner (Trial II)	11	11	1-3
stonecat	1	1	1-11
stoneroller	16	16	8-11
walleye	10	9	1-2
white bass	22	0*	8-11
white sucker	14	14	1-3
yellow perch	17	17	5-8

* - Incomplete trial, test subjects died before completion of the study.

Table 3. Trials where glochidial metamorphosis was not observed. (Continued.)

Common name	Number of individuals inoculated	Number of survivors	Glochidia attachment period (days)
winged mapleleaf			
bigmouth shiner	22	21	1-3
black bullhead (Trial I)	11	3	27-29
black bullhead (Trial II)	17	13	22-25
black bullhead (Trial III)	1	1	34-35
black crappie (Trial I)	9	9	1-2
black crappie (Trial II)	1	1	1-3
bluegill (Trial I)	7	7	1-2
bluegill (Trial II)	1	1	1-3
bluegill (Trial III)	1	1	1-11
bluntnose minnow (Trial I)	18	18	1-2
bluntnose minnow (Trial II)	1	1	1-3
brook stickleback (Trial I)	1	1	8-11
brook stickleback (Trial II)	1	1	26-35
central mudminnow	9	9	11-13
crystal darter	2	2	1-3
emerald shiner (Trial I)	17	17	1-2
emerald shiner (Trial II)	6	6	1-3
flathead catfish (Trial I)	15	1	50-54
flathead catfish (Trial II)	39	0*	50-54
flathead catfish (Trial III)	25	0*	69-75
green sunfish (Trial I)	16	15	5-8
green sunfish (Trial II)	1	1	1-3
guppy (Trial I)	4	4	20-22
guppy (Trial II)	5	1	3-8
hornyhead chub (Trial I)	6	6	1-2
hornyhead chub (Trial II)	2	2	1-2
Iowa darter	12	12	2-5
largemouth bass	10	10	1-2
logperch (Trial I)	1	1	1-2
logperch (Trial II)	21	21	1-3
longnose dace (Trial I)	3	3	1-2
longnose dace (Trial II)	39	39	1-3
mottled sculpin (Trial I)	1	1	2-5
mottled sculpin (Trial II)	12	12	2-8
n. redbelly dace	19	19	1-2
river darter	11	11	1-3
slenderhead darter	2	2	2-5
tadpole madtom (Trial I)	3	3	1-2
tadpole madtom (Trial II)	11	11	2-11
western sand darter	22	22	1-3
yellow bullhead (Trial I)	3	3	5-8
yellow bullhead (Trial II)	20	17	22-25
yellow bullhead (Trial III)	1	1	34-35

* - Incomplete trial, test subjects died before completion of the study.

Table 4. St. Croix River fishes that facilitated glochidia metamorphosis.

Common name	Number of fish collected	Number of juveniles recovered	Mussel subfamily or species
1997			
emerald shiner	25	*	Anodontinae
mimic shiner	70	*	Anodontinae
redhorse	5	*	Anodontinae
logperch	5	*	Ambleminae
freshwater drum	6	*	Pink heelsplitter , & other species
1998			
bluntnose minnow	13	4	Unknown
mimic shiner	64	3	Unknown
spotfin shiner	48	9	Unknown
logperch	35	738	Unknown
river darter	27	9	Unknown
yellow perch	2	3	Unknown
walleye	11	4	Unknown
western sand darter	16	5	Unknown
freshwater drum	13	4254	Pink heelsplitter , & other species

* - Not recorded.

Table 5. St. Croix River fishes and amphibians that did not produce juvenile mussels.

Common name	Approximate Number collected	Number of individuals less than 1 yr old
1997		
smallmouth bass	1	0
river darter	2	0
western sand darter	3	0
1998		
emerald shiner	32	0
white sucker	9	9
redhorse sp.	1	1
smallmouth bass	10	10
Johnny darter	5	0
mudpuppy	1	0

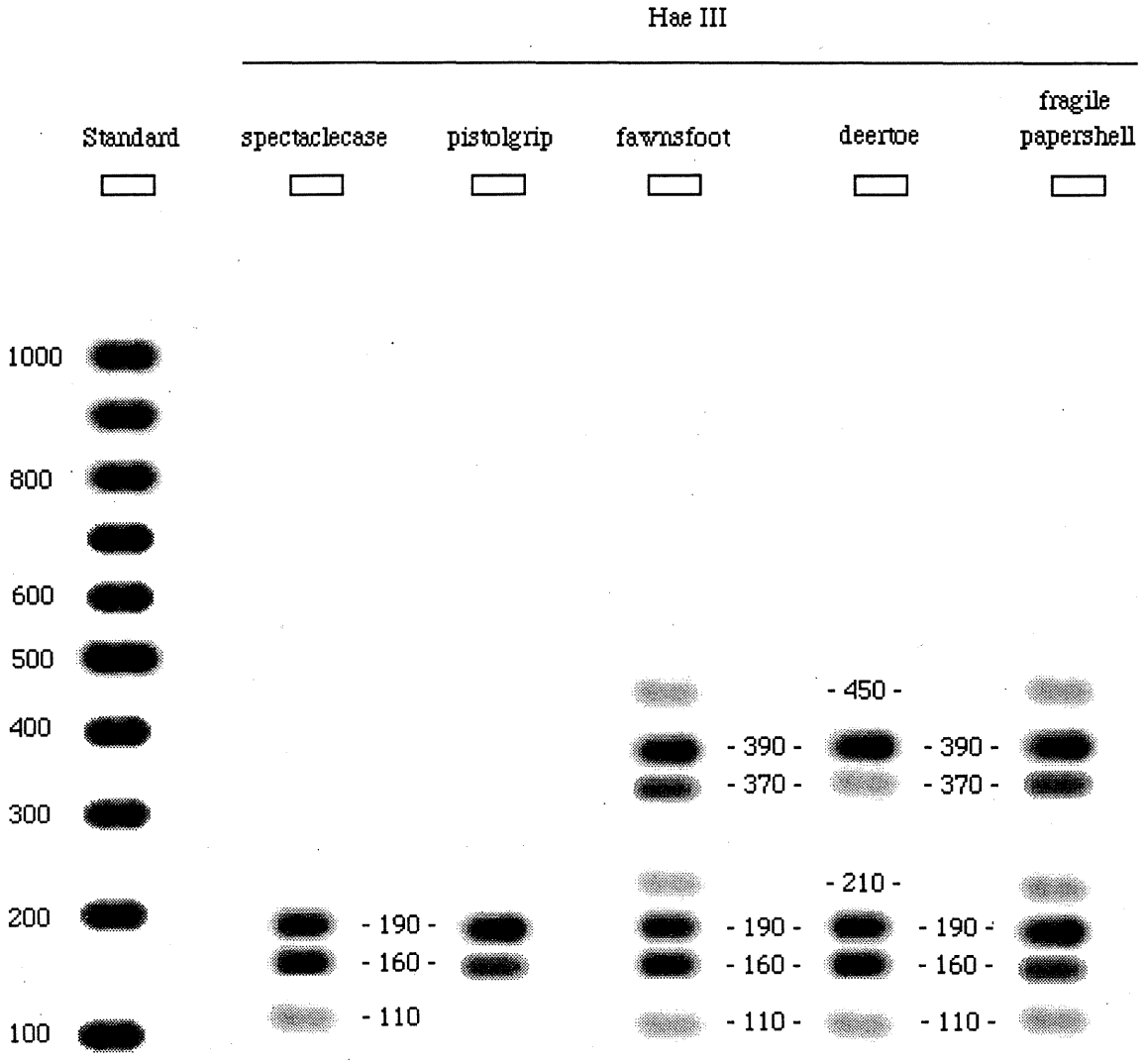


Figure 13. Stylized gel of spectaclecase, pistolgrip, fawnsfoot, deertoe, and fragile papershell ITS-1 region DNA cut with restriction enzyme Hae III.

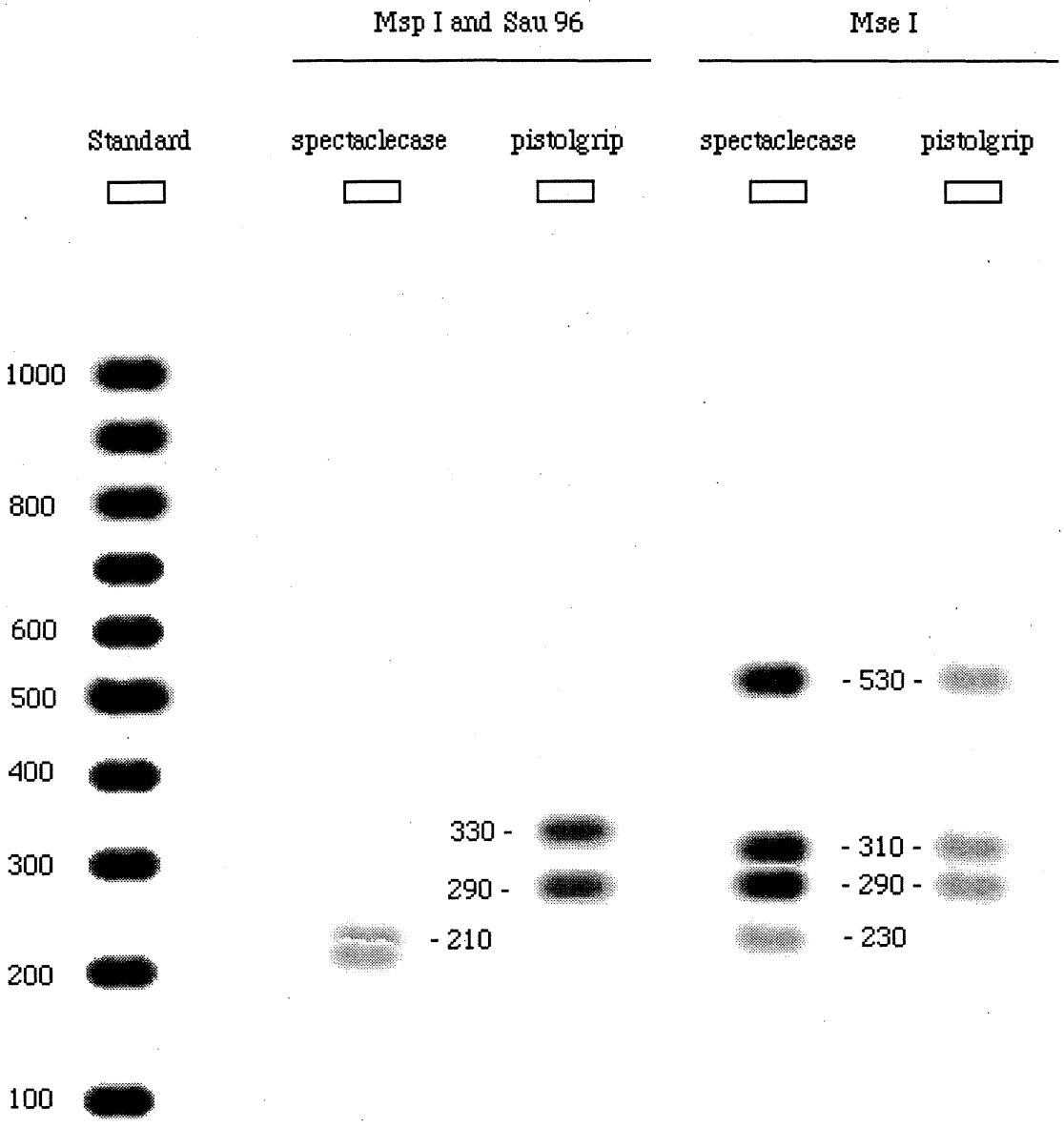


Figure 14. Stylized gel of spectaclecase and pistolgrip ITS-1 region DNA cut with both Msp I and Sau 96, and Mse I restriction enzymes.

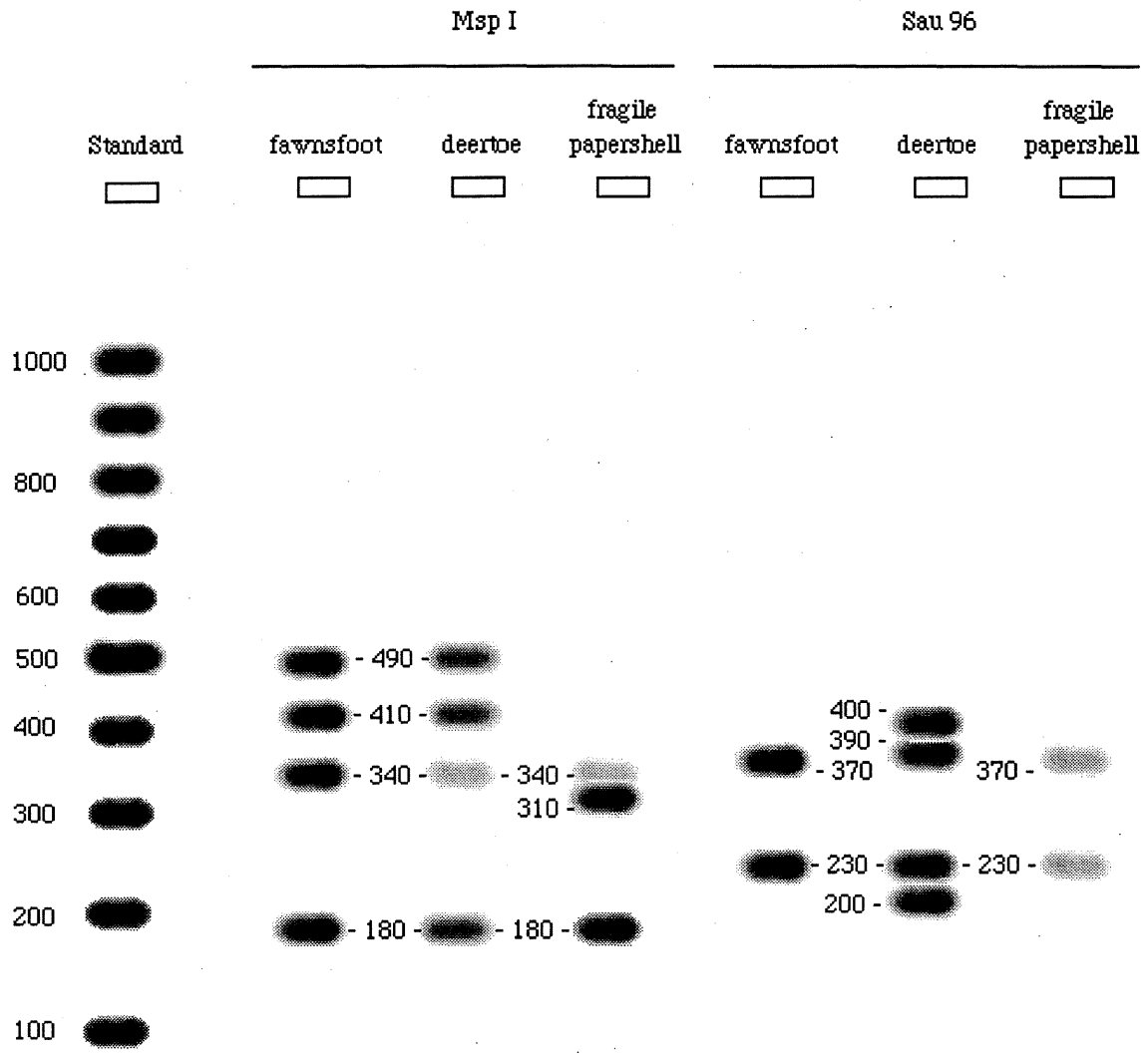


Figure 15. Stylized gel of fawnsfoot, deertoe, and fragile papershell ITS-1 region DNA cut with Msp I and Sau 96 restriction enzymes.

Table 6. Sites surveyed in the Big Fork and Little Fork rivers.

Big Fork River drainage

Site	Water body	County	Township	Range	Section	Site description
1	Big Fork River	Koochiching	T70N	R26W	32	MN Highway 11 bridge
2	Big Fork River	Koochiching	T69N	R26W	20	Confluence with Bear River
3	Big Fork River	Koochiching	T157N	R25W	13, 24	County Route 1 bridge
4	Bear River	Koochiching	T68N	R26W	4, 9	County Route 1 bridge
5	Big Fork River	Koochiching	T156N	R25W	27	Gowdy Landing, approximately 11 km NNE of Big Falls
6	Sturgeon River	Koochiching	T155N	R26W	26	County Route 30 bridge
7	Big Fork River	Koochiching	T155N	R25W	36	Big Falls, upstream of MN Highway 71
8	Big Fork River	Koochiching	T64N	R27W	13	County Route 6 bridge
9	Big Fork River	Koochiching	T63N	R27W	14	County Route 30 bridge
10	Caldwell Brook	Koochiching	T151N	R26W	2	County Route 54 bridge
11	Reilly Brook	Koochiching	T63N	R25W	7	County Route 62 bridge
12	Big Fork River	Koochiching	T63N	R26W	36	County Route 5 bridge
13	Big Fork River	Itasca	T62N	R25W	23, 26	County Route 1 bridge
14	Deer Creek	Itasca	T62N	R24W	17, 18	County Route 525 bridge
15	Moose Brook	Itasca	T150N	R27W	35	County Route 26 bridge
16	Big Fork River	Itasca	T149N	R26W	1	County Route 14 bridge
17	Big Fork River	Itasca	T61N	R26W	27	County Route 38 bridge, Big Fork
18	South Fork Coon Creek	Itasca	T60N	R25W	3, 4	County Route 344 bridge
19	Rice Creek	Itasca	T60N	R26W	16, 21	County Route 254 bridge
20	Bowstring River	Itasca	T149N	R27W	26	Shogren Dam, upstream of County Route 145 bridge
21	Popple River	Itasca	T148N	R27W	20	County Route 46 bridge
22	Bowstring River	Itasca	T147N	R26W	16	County Route 35 bridge
23	Bowstring River	Itasca	T147N	R25W	23, 24	County Route 6 bridge
24	North Star Lake	Itasca	T59N	R26W	28	Eastern shore of north lake extension
25	Bowstring River	Itasca	T58N	R27W	23	County Route 253 bridge

Table 6. Sites surveyed in the Big Fork and Little Fork rivers. (Continued.)

Little Fork River drainage

Site	Water body	County	Township	Range	Section	Site description
1	Little Fork River	Koochiching	T69N	R26W	12	End of UT 238
2	Little Fork River	Koochiching	T68N	R25W	9	Lofgren Park, Little Fork
3	Beaver Brook	Koochiching	T68N	R24W	19, 20	End of UT 79
4	Cross River	Koochiching	T68N	R25W	36	Near end of County Route 73
5	Little Fork River	Koochiching	T68N	R24W	31	End of County Route 73
6	Little Fork River	Koochiching	T66N	R24W	7, 18	End of UT 36
7	Nett River	Koochiching	T66N	R24W	8	County Route 8 bridge
8	Little Fork River	Koochiching	T65N	R23W	31	Forest Service Road, western edge of Nett Lake Indian Reservation
9	Prairie Creek	Koochiching	T64N	R22W	23, 24	County Route 65 bridge
10	Valley River	Koochiching	T63N	R22W	7	County Route 57 bridge
11	Willow River	St. Louis	T63N	R21W	9, 10	County Route 75 bridge
12	Willow River	St. Louis	T63N	R20W	10, 11	County Route 406 bridge
13	Little Fork River	St. Louis	T63N	R21W	33	County Route 114 bridge
14	Flint Creek	St. Louis	T62N	R19W	8, 17	County Route 1 bridge
15	Sturgeon River	St. Louis	T62N	R21W	27, 34	County Route 107 bridge
16	Little Fork River	St. Louis	T62N	R20W	24	County Route 481 bridge
17	Rice River	St. Louis	T62N	R18W	31, 32	U.S. 53 bridge
18	Little Fork River	St. Louis	T62N	R18W	16, 17	County Route 600 bridge
19	Sturgeon River	St. Louis	T61N	R20W	9	County Route 22 bridge
20	Rice River	St. Louis	T61N	R18W	9	County Route 22 bridge
21	Bear River	Itasca	T61N	R22W	29, 30	MN Highway 65
22	Sturgeon River	St. Louis	T61N	R20W	26, 27	County Route 73 bridge
23	Dark River	St. Louis	T60N	R20W	3, 10	County Route 688 bridge
24	East Branch Sturgeon River	St. Louis	T60N	R20W	33, 34	County Route 73 bridge
25	Shannon River	St. Louis	T59N	R21W	2, 11	Forest Service Road south of Shannon Lake

Table 7. Live mussels observed during surveys of Big Fork and Little Fork rivers.

N	Mussel species	N	Mussel species
3252	fatmucket (*Lampsilis siliquoidea*)	728	plain pocketbook (*Lampsilis cardium*)
146	cylindrical papershell (*Anodontoides ferussacianus*)	228	giant floater (*Pyganodon grandis*)
195	black sandshell[1] (*Ligumia recta*)	98	white heelsplitter (*Lasmigona complanata*)
35	creek heelsplitter[1] (*Lasmigona compressa*)	3	creeper (*Strophitus undulatus*)
0	paper pondshell[2] (*Utterbackia imbecillis*)	0	flutedshell[1, 2] (*Lasmigona costata*)

[1] MN special-concern species, [2] Observed as empty shells

Table 8. Mussels collected during the Big Fork River survey. Slashes separate data collected at each site. The first number denotes the number of individuals collected during the quantitative survey. The second number is the number of individuals collected during the qualitative survey. The last number is the total number of mussels collected.

Site	cylindrical papershell	plain pocketbook	fatmucket	white heelsplitter	creek heelsplitter
1			1/23/24	0/4/4	
2		0/4/4	1/36/37	0/3/3	1/1/2
3			2/30/32		
4	0/14/14				
5		1/2/3	1/23/24		0/1/1
6	0/9/9	1/3/4	1/29/30	0/1/1	
7		0/2/2	1/19/20	0/13/13	
8		0/3/3	1/45/46		1/0/1
9	0/1/1	2/9/11	7/54/61	0/2/2	
10	0/1/1		3/148/151		
11	6/4/10				
12	0/1/1	0/2/2	8/150/158	1/4/5	0/7/7
13		1/1/2	3/8/11	0/1/1	
14	1/6/7	0/10/10	0/77/77	0/1/1	0/6/6
15	0/4/4		0/107/107		
16	0/1/1	0/5/5	6/110/116	4/14/18	
17	0/1/1	2/22/24	13/138/151		
18			0/1/1		
19	0/2/2	0/1/1	10/69/79	0/3/3	0/1/1
20	1/0/1		17/93/110	0/1/1	
21			1/16/17		
22	1/0/1	0/1/1	4/21/25		
23	0/1/1		4/60/64	0/2/2	0/3/3
24	0/7/7		2/15/17		
25					

Table 8. Live mussels collected during the Big Fork River survey. (Continued.)

Site	flutedshell*	black sandshell	giant floater	creeper	paper pondshell*
1		0/1/1			
2		0/1/1			
3		0/1/1			
4			0/1/1		
5		1/0/1			
6		0/3/3	0/19/19		
7		0/1/1			
8		0/2/2			
9		0/3/3			
10					
11			0/1/1		
12		0/1/1			
13		0/1/1			
14		0/2/2	0/8/8		
15			0/15/15		
16		0/2/2	4/6/10		
17		0/2/2			
18	0/1/1				
19				0/1/1	
20			3/10/13		0/1/1
21			2/5/7		
22			3/23/26		0/2/2
23			0/1/1		
24			0/23/23		
25					

* Collected only as empty shells.

Table 9. Fishes collected during the Big Fork River survey.

Species	1	2	3	4	5	6	7	8	9	10	11	12	13
Ambloplites rupestris	4	17	11		7		2	7	6			24	4
Ameirus (Ictalurus) melas													
Ameirus (Ictalurus) nebulosus													
Ameirus (Ictalurus) sp.													
Ammocoetes		7	4		1			1					
Catostomus commersoni	1			9			7	4	2	8	6	5	
Cottus bairdi		1		6									
Culaea inconstans										16	6		
Esox lucius	4			4	5			2	3			5	
Esox masquinongy							2		1			3	
Esox sp.													
Etheostoma exile													
Etheostoma nigrum	15	1	1	9	3	5	1	1	1	8	45		2
Hybopsis (Notropis) dorsalis	1												1
Ichthyomyzon unicuspis	2												
Lepomis cyanellus											1		
Lepomis gibbosus													
Lota lota													
Luxilus (Notropis) cornutus	51	19	2	348	86	30	2	5	1	78	160	3	8
Margariscus (Semotilus) margarita										11			
Micropterus dolomieu	13	10	4		2								
Micropterus salmoides							1		1			1	
Moxostoma anisurum	1						1						
Moxostoma macrolepidotum	3						9	2		1		2	
Moxostoma sp.													
Nocomis biguttatus		1	1	2		1	3	17	15	7	3	3	3
Notemigonus crysoleucas				1	3								
Notropis atherinoides	78	1			2								
Notropis blennius	6												
Notropis heterolepis													
Notropis hudsonius	2				44								3
Notropis volucellus					7								20
Notrus gyrinus					1		1	1					
Perca flavescens	13	2							2			14	
Percina caprodes	52	29	21		5		1						
Percina maculata	6	23	17	11	31	10	28	10	19	13	4	5	14
Percina shumardi	43	55	23	1	10								
Percopsis omnicomaycus	20	20		23			10			1			
Phoxinus eos										10	7		
Pimephales promelas				2	1					1	11		
Pomoxis nigromaculatus					1		1						
Rhinichthys atratulus									1	22	7		
Rhinichthys cataractae		1		9		1			2	35			7
Semotilus atromaculatus				13	1			1			81		
Stizostedion vitreum	1						1	1	1				
Umbra limi								1		6	6	8	

Table 9. Fishes collected during the Big Fork River survey. (Continued.)

Species	14	15	16	17	18	19	20	21	22	23	24	25	Total
Ambloplites rupestris				1		4	1						88
Ameirus (Ictalurus) melas			181				5					1	187
Ameirus (Ictalurus) nebulosus								1				1	2
Ameirus (Ictalurus) sp.							38						38
Ammocoetes													13
Catostomus commersoni	2			1		2			1			4	52
Cottus bairdi													7
Culaea inconstans							1					1	24
Esox lucius			3		3	1		1				1	32
Esox masquinongy													6
Esox sp.									1				1
Etheostoma exile			1		1						1		3
Etheostoma nigrum	17					8					2		119
Hybopsis (Notropis) dorsalis													2
Ichthyomyzon unicuspis													2
Lepomis cyanellus													1
Lepomis gibbosus							3					1	4
Lota lota										1			1
Luxilus (Notropis) cornutus	52			2		335							1182
Margariscus(S.) margarita													11
Micropterus dolomieu											1		30
Micropterus salmoides	6		2			7	6		1	2		11	38
Moxostoma anisurum									1				3
Moxostoma macrolepidotum				3					1				21
Moxostoma sp.									2				2
Nocomis biguttatus	6			1		80						1	144
Notemigonus crysoleucas	1					1		2			1	2	11
Notropis atherinoides													81
Notropis blennius													6
Notropis heterolepis	2		26			1						1	30
Notropis hudsonius	2							1	15			7	74
Notropis volucellus													27
Notrus gyrinus			3									2	8
Perca flavescens	1		7	8	90	1	81	30	133	1	15	64	462
Percina caprodes													108
Percina maculata				1									192
Percina shumardi													132
Percopsis omnicomaycus													74
Phoxinus eos													17
Pimephales promelas	1					3							19
Pomoxis nigromaculatus		5		1					18				26
Rhinichthys atratulus	2					8							40
Rhinichthys cataractae	5					4							64
Semotilus atromaculatus						34							130
Stizostedion vitreum								2	2				8
Umbra limi		2	12		2							17	54

Table 10. Live mussels collected during the Little Fork River survey. Slashes separate data collected at each site. The first number denotes the number of live individuals collected during the quantitative survey. The second number is the number of live individuals collected during the qualitative survey. The last number is the total number of live mussels collected.

Site	*A. ferussacianus*	*L. cardium*	*L. siliquoidea*	*L. complanata*
1		2/40/42	3/239/242	
2	0/1/1	2/60/62	4/125/129	
3	0/8/8	0/2/2	0/14/14	
4	1/14/15		0/4/4	
5		9/110/119	2/159/161	
6		1/19/20	0/8/8	
7	0/20/20	0/7/7	1/22/23	0/3/3
8		17/144/161	9/121/130	
9	1/15/16			
10			1/6/7	
11				
12				
13		2/61/63	0/21/21	
14	0/9/9		1/19/20	
15		2/93/95	1/140/141	0/2/2
16			12/39/51	2/8/10
17	1/0/1	0/54/54	19/528/547	2/24/26
18			0/3/3	
19	0/1/1	7/16/23	16/95/111	
20	0/1/1		6/91/97	0/2/2
21	1/8/9		4/23/27	
22	0/1/1	0/8/8	3/35/38	0/1/1
23			0/1/1	
24	0/2/2		1/98/99	
25	0/1/1		0/20/20	

Table 10. Live mussels collected during the Little Fork River survey. (Continued.)

Site	*L. compressa*	*L. recta*	*P. grandis*	*S. undulatus*
1		1/2/3		
2		0/25/25		
3			1/13/14	
4			1/24/25	
5	0/2/2	0/40/40		
6		1/7/8		
7	1/6/7	0/7/7		
8		6/44/50	0/1/1	
9				
10				
11			1/3/4	
12			0/3/3	
13		0/15/15		
14			0/8/8	
15		0/21/21		0/1/1
16	0/1/1		0/6/6	
17			0/13/13	
18			1/3/4	
19	0/1/1	0/3/3		
20			0/4/4	
21			2/17/19	
22	0/1/1	0/2/2		0/1/1
23				
24	0/2/2			
25			0/3/3	

Table 11. Fishes collected during the Little Fork River survey.

Species	1	2	3	4	5	6	7	8	9	10	11	12	13
Acipenser fulvescens	1												
Ambloplites rupestris		1	19		1	1	4	4		1			
Catostomus commersoni				7			7			4		5	
Cottus bairdi													
Culaea inconstans				1					84		2	25	
Esox lucius													
Etheostoma nigrum	8		8	18	4	2	7	2	4	14	10	1	1
Hybopsis (Notropis) dorsalis	8			1	2	6	43			10	1		
Ichthyomyzon fossor				6			1	1					
Ichthyomyzon (ammocoetes)													
Lepomis gibbosus													
Lepomis macrochirus													
Luxilus (Notropis) cornutus	10	2	11	17	56		125	4	40	31	51		
Margariscus (Semotilus) margarita			1						26			1	
Micropterus dolomieu					1	1							5
Moxostoma anisurum				1	1		4						
Moxostoma erythrurum													
Moxostoma macrolepidotum							4						4
Moxostoma sp.													
Nocomis biguttatus							3						
Notemigonus crysoleucas			13										1
Notropis atherinoides	160	37			44	4							
Notropis heterolepis													
Notropis hudsonius	2												
Notropis volucellus					5								
Notrus gyrinus	1												
Perca flavescens													
Percina caprodes	2		1	1	21		25						4
Percina maculata		1	5	25	4	16	22	1	3	20	8		10
Percina shumardi	117	46		1	9	41	2	1					
Percopsis omnicomaycus		3	8	16		13	130	1		28	17		4
Phoxinus eos	1								129	1	3	14	
Phoxinus neogaeus									3				
Pimephales notatus													
Pimephales promelas	1			1					5		1	12	
Rhinichthys atratulus				2						6			
Rhinichthys cataractae						4	3			23			4
Semotilus atromaculatus	1			19	1		3	2	5	17	3	6	
Stizostedion vitreum		1											
Umbra limi				3					2			13	

Table 11. Fishes collected during the Little Fork River survey. (Continued.)

Species	14	15	16	17	18	19	20	21	22	23	24	25	Total
Acipenser fulvescens													
Ambloplites rupestris		10	10	4		2	4		2				
Catostomus commersoni	12	5			2	2		10		32		3	
Cottus bairdi						1		2		4			
Culaea inconstans													
Esox lucius	1		2	2	3	2			2		1	2	
Etheostoma nigrum	11	3		5	3	23	12	112	2	5	6		
Hybopsis (Notropis) dorsalis													
Ichthyomyzon fossor		1							4	5			
Ichthyomyzon sp. (ammocoetes)		1											
Lepomis gibbosus			14				3				1		
Lepomis macrochirus			1		26								
Luxilus (Notropis) cornutus	71		1	1	43		3	3	1		288	1	
Margariscus (Semotilus) margarita													
Micropterus dolomieu	1	2							1				
Moxostoma anisurum		2				2			1				
Moxostoma erythrurum		4				1			12				
Moxostoma macrolepidotum						1	1		7				
Moxostoma sp.									5				
Nocomis biguttatus													
Notemigonus crysoleucas	5		1	1	38		1	2				1	
Notropis atherinoides			6										
Notropis heterolepis											1		
Notropis hudsonius													
Notropis volucellus			6	6		1	1		1				
Notrus gyrinus				3	4								
Perca flavescens						1			1			9	
Percina caprodes													
Percina maculata	6	12	2	2	1	35	13	23	26	8	1	3	
Percina shumardi													
Percopsis omnicomaycus		12						4	5		1		
Phoxinus eos													
Phoxinus neogaeus			1										
Pimephales notatus			2	13									
Pimephales promelas							2	16					
Rhinichthys atratulus													
Rhinichthys cataractae						3	11	1	3	4	1		
Semotilus atromaculatus	3	3				9		1		57	9	1	
Stizostedion vitreum		2	4	1			1						
Umbra limi							2		1				

Table 12. Linear correlations between relative abundance of fishes and mussel catch rate.

Comparison	R^2	P-value	N
Little Fork River			
fatmucket and emerald shiner	0.88	0.019	5
Pocketbook and white sucker	0.97	0.015	4
fatmucket and pumpkinseed	0.99	0.047	3
Anodontinae and mimic shiner	0.82	0.012	6
Anodontinae and river darter	0.95	0.028	4
Anodontinae and golden redhorse	0.99	0.038	3
Lampsilinae and pumpkinseed	0.99	0.047	4
Cylindrical papershell & blackside darter	0.5	0.03	10
Big Fork River			
Lampsilinae and longnose dace	0.96	0.004	5
Lampsilinae and blacknose dace	0.99	0.045	3
Anodontinae and black crappie	0.79	0.045	5
fatmucket and longnose dace	0.95	0.0045	5
Cylindrical papershell & common shiner	0.58	0.027	8
creek heelsplitter and rock bass	0.95	0.028	4
fatmucket and river darter	0.91	0.047	4
Black sandshell and Percidae	0.68	0.011	8

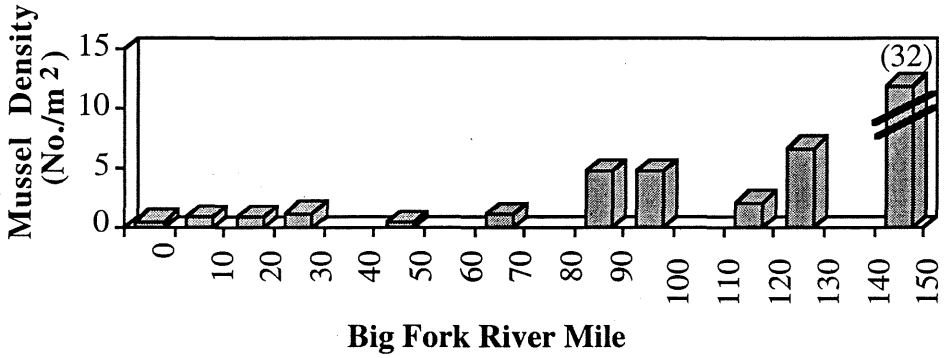


Figure 16. Live mussel densities in the Big Fork River, Minnesota.

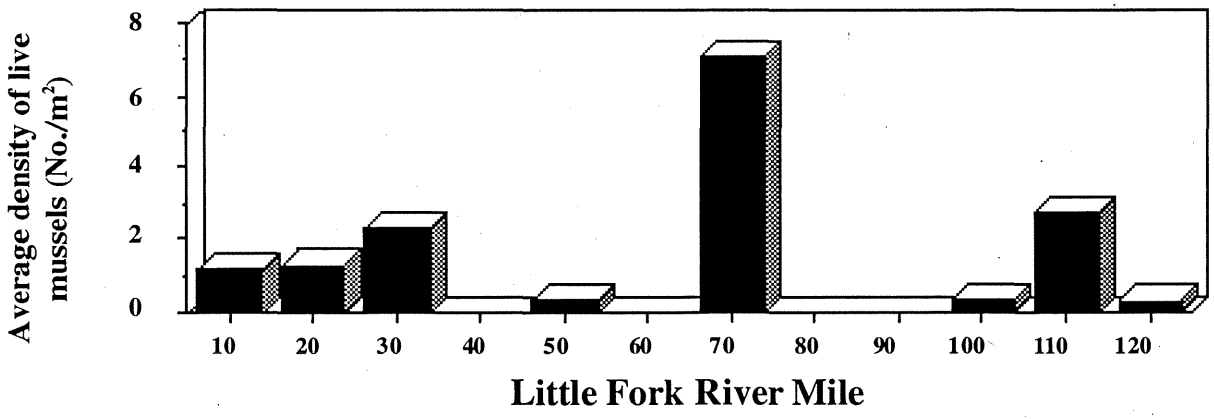


Figure 17. Live mussel densities in the Little Fork River, Minnesota.