

FINAL REPORT

1999 Project Abstract

For the Period Ending June 30, 2001

TITLE: Freshwater Mussel Resources in the St. Croix River

PROJECT MANAGERS: Daniel J. Hornbach and Mark C. Hove

ORGANIZATION: Macalester College

ADDRESS: Dept. Biology, 1600 Grand Ave. St. Paul, MN 55105

WEB SITE ADDRESS: <http://www.macalester.edu/~hornbach>

FUND: Funding provided by the Minnesota Environmental and Natural Resources Trust Fund as recommended by the Legislative Commission on Minnesota Resources

LEGAL CITATION: ML 1999, Ch. 231 Sec. 16, Subd. 15(b)

APPROPRIATION AMOUNT: \$58,000

Overall Project Outcome and Results

The purpose of this project is to address conservation and management needs of one of the most threatened group of organisms in Minnesota, native freshwater mussels. Project objectives include completing a mussel relocation and refugia study begun in 1997 in association with the University of Minnesota, and review the status of one of the most important mussel communities in Minnesota, that in the St. Croix River.

Refugia may become important conservation tools in protecting native mussels from invasive species such as zebra mussels. We completed monitoring of a refuge project begun in 1997 designed to provide information on the long-term impact of mussel relocations and thus provide insight into the efficacy of this conservation measure. We collected, measured, and weighed each mussel from reference and relocation sites at Wild River State Park. Survival rates among the three mussel species of interest was relatively high emphasizing the importance of handling mussels carefully (*e.g.* using the protocol described in this report) during relocation and providing the mussels with high quality habitat.

The distribution of various mussel species in the St. Croix River have been fairly well established, however, the long-term stability of these populations has not been examined. Between 1999 and 2000 we quantitatively resampled seven locations in the St. Croix River that we have periodically sampled since 1990. Analysis of these 10 years of data

shows that select locations downstream of the dam at Taylors Falls, Minnesota had significantly lower juvenile mussel densities and an increase in fine sediments. Previous studies found neither increased sedimentation nor lack of juvenile recruitment above the dam at Taylors Falls. This suggests that increased sedimentation below the dam may be influencing mussel recruitment or juvenile mussel survival.

Project Results Use and Dissemination

Project results were shared with resource professionals and are posted on the Macalester College web site (<http://www.macalester.edu/~hornbach>).

AUG 13 2001

2001 LCMR Final Work Program Report

submitted to:

Legislative Commission on Minnesota Resources
Room 65, State Office Building
100 Constitution Avenue
Saint Paul, Minnesota 55155

submitted by:

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August 2001

Date of Final Report: June 30, 2001

Date of Work Program Approval: July 1, 1999

Project Completion Date: June 1, 2001

LCMR Final Work Program Report

I. PROJECT TITLE: Freshwater Mussel Resources in the St. Croix River

Project Managers: Daniel J. Hornbach and Mark C. Hove

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Total Biennial Project Budget:

December 22, 1999

\$ LCMR:	\$58,000	\$ Match:
-\$ LCMR Amount	<u>\$18,000</u>	-\$ Match Amount
=\$ LCMR Balance:	\$40,000	=\$ Match Balance:

December 26, 2000

\$ LCMR:	\$58,000.00	\$ Match:
-\$ LCMR Amount	<u>\$46,311.90</u>	-\$ Match Amount
=\$ LCMR Balance:	\$11,688.10	=\$ Match Balance:

June 30, 2001

\$ LCMR:	\$58,000.00	\$ Match:
-\$ LCMR Amount	<u>\$58,000.00</u>	-\$ Match Amount
=\$ LCMR Balance:	\$ 0.00	=\$ Match Balance:

A. Legal Citation: Minnesota Laws 1999, Chapter 231, Section 16, Subdivision. 15(b) [ML 99, Ch. 231 Sec. 16, Subd. 15(b)]

NF05 Freshwater Mussel Resources in the St. Croix River \$58,000

Appropriation Language: Freshwater Mussel Resources in the St. Croix River \$29,000 the first year and \$29,000 the second year are from the trust fund to the commissioner of natural resources for an agreement

with Macalester College to continue refugia studies and assess populations for freshwater mussels.

B. Status of Match Requirement: None

II. and III. FINAL PROJECT SUMMARY

The purpose of this project is to address conservation and management needs of one of the most threatened group of organisms in Minnesota, native freshwater mussels. Project objectives include completing a mussel relocation and refugia study begun in 1997 in association with the University of Minnesota, and review the status of one of the most important mussel communities in Minnesota, that in the St. Croix River.

Refugia may become important conservation tools in protecting native mussels from invasive species such as zebra mussels. We completed monitoring of a refuge project begun in 1997 designed to provide information on the long-term impact of mussel relocations and thus provide insight into the efficacy of this conservation measure. We collected, measured, and weighed each mussel from reference and relocation sites at Wild River State Park. Survival rates among the three mussel species of interest was relatively high emphasizing the importance of handling mussels carefully (*e.g.* using the protocol described in this report) during relocation and providing the mussels with high quality habitat.

The distribution of various mussel species in the St. Croix River have been fairly well established, however, the long-term stability of these populations has not been examined. Between 1999 and 2000 we quantitatively resampled seven locations in the St. Croix River that we have periodically sampled since 1990. Analysis of these 10 years of data shows that select locations downstream of the dam at Taylors Falls, Minnesota had significantly lower juvenile mussel densities and an increase in fine sediments. Previous studies found neither increased sedimentation nor lack of juvenile recruitment above the dam at Taylors Falls. This suggests that increased sedimentation below the dam may be influencing mussel recruitment or juvenile mussel survival. Project results were shared with resource professionals and placed on the Macalester College website (<http://www.macalester.edu/~hornbach>).

IV. OUTLINE OF PROJECT RESULTS:

- **Result 1 - Relocation and refugia study**

LCMR Budget:	\$13,610	Match:	\$0
Balance:	\$0	Match Balance:	\$0

Project Activities in Result 1

We collected an additional two years of data for the mussel relocation study begun in 1996. The study sites, constructed in the summer of 1997, were located on the St. Croix River near Wild River State Park (near Almelund and Sunrise, Minnesota). At both reference and relocation sites we recovered mussels, and assessed growth and survivorship during the summers of 1999 and 2000. Data were analyzed and presented or discussed at scientific meetings between the winters of 1999-2001. Recovery of mussels from the study sites ranged between 42-64%. The mean annual mortality rate was between 88-100%. Results from this study suggest that mussels relocated using the protocol described in this report will not be harmed in the short term. Details on the results of this project objective are submitted under "**X. RESEARCH PROJECTS**" below. See attached draft paper submitted to the journal Conservation Biology that summarizes data collected on the three primary mussels species of interest.

Project Review of Result 1

We were satisfied with results from this project but would change a few aspects of the study design in light of the observations made. We did not expect mussels to be as mobile as we observed. Many mussels appeared to move between cells in the study grid and hundreds of mussels moved in and out of the study grid. Movement may have been due to our disturbing the animals as we removed them briefly from the riverbed for identification and measurement. If we had divided each cell within the study grid into an inner, center section of the cell and an outer section of the cell we would have been better able to identify mussels that moved at least 1 meter from those that moved less than 1 meter. If we had only marked and tracked the three primary study species we would have saved a considerable amount of time. A lot of time was spent recording the presence of new mussels immigrating into the study grids and tracking

the growth and survival of the non-target species. However, if we had not marked the other mussel species we wouldn't have appreciated the magnitude of movement that mussels demonstrated throughout the project. Although we did not include data collected from the non-target mussel species in our recent paper we submitted for publication we plan to review these additional data in the future.

Project Activities	Completion Date
1. Return to refuge areas and measure mussel growth, survival, population density, and diversity at refuges	August 1999
2. Analyze data and prepare interim report	September 1999
3. Present interim results at St. Croix River Rendezvous meeting	October 1999
4. Post interim results and educational information on mussel relocations on the internet	May 2000
5. Return to refuge areas and measure mussel growth, survival, population density, and diversity at refuges	August 2000
6. Analyze data	September 2000
7. Present interim results at St. Croix River Rendezvous meeting	October 2000
8. Post interim results and educational information on mussel relocations on the internet	May 2001
9. Submit Final Report to LCMR and MN DNR	August 2001

• **Result 2 - Monitoring mussel populations in the St. Croix River**

LCMR Budget: \$44,390 Match: \$0
Balance: \$0 Match Balance: \$0

Project Activities in Result 2

We quantitatively sampled mussel communities and habitat at seven locations on the St. Croix River: Wild River State Park, Interstate State Park, Franconia, Osceola, Lakeland, Bayport, and Prescott. At these sites

we collected at least 100 0.25 m² samples of the substrate and removed all of the mussels by sieving. We identified and measured the length of each live mussel collected. All dead mussels were identified and counted. From these measurements we estimated population density, community diversity, and age structure of the mussel populations. The greatest diversity of native mussel species was observed at Interstate State Park with 26 species and the lowest at Bayport with 13 species. The two federally endangered mussels living in the St. Croix River were collected during the study. Higgins eye (*Lampsilis higginsii*) were observed at Interstate State Park, Osceola, Lakeland, and Prescott, and winged mapleleaf (*Quadrula fragosa*) at Interstate State Park. Zebra mussels and Asian clams were observed at Lakeland and Prescott. Juvenile mussel density (mussels < 30 mm), at Interstate State Park and Franconia has declined significantly over the last ten years. There was a decrease in the size of average river bottom substrates over time at locations downstream of the Taylors Falls dam. This suggests that increased sedimentation below the dam may be influencing mussel recruitment or juvenile mussel survival. Details on the results and analysis of these project objectives are submitted under "**X. RESEARCH PROJECTS**" below.

Project Review of Result 2

Only a few ideas come to mind that would have improved this project. Safety is very important while conducting work under water. In retrospect we should have asked for additional money to facilitate conversion of our old equipment to newer, safer equipment and enabled our purchase of underwater communication system and emergency oxygen equipment. Also, we underestimated our travel costs due in part to the increase in the price of gas.

Project Activities	Completion Date
1. Visit 3 of 7 sites and quantitatively sample the mussel community	August 1999
2. Analyze data and prepare interim report	September - December 1999
3. Present interim results at St. Croix River Rendezvous meeting	October 1999
4. Post interim results and educational information on mussel populations of the St. Croix River on the internet	May 2000
5. Visit final 4 of 7 sites and quantitatively sample the mussel community	August 2000
6. Analyze data and prepare interim report	September - December 2000
7. Present interim results at St. Croix River Rendezvous meeting	October 2000
8. Post interim results and educational information on mussel populations of the St. Croix River on the internet	May 2001
9. Final Report to LCMR and MN DNR	August 2001

V. DISSEMINATION

We disseminated project results in several ways. Results from work completed in 1999 were presented at the following professional meetings: 1999 St. Croix River Rendezvous, Marine on St. Croix, Minnesota, 1999 PEW Midstates Science and Mathematics Consortium, St. Louis, Missouri, 2000 Annual Meeting of the Minnesota chapters of the American Fisheries Society and The Wildlife Society, St. Cloud, Minnesota, and 2000 Mississippi Research Consortium meeting, La Crosse, Wisconsin. Project results from data collected during summer 2000 were presented at the 2000 National meeting of the American Fisheries Society, St. Louis, Missouri, 2000 Summer Sciences Undergraduate Research Program, Macalester College, St. Paul, Minnesota, 2000 St. Croix River Rendezvous, Marine on St. Croix, 2001 Minnesota, Minnesota and Dakota chapters of the American Fisheries Society, Fargo, North Dakota, 2001 national meeting of the Freshwater Mollusk Conservation Society, Pittsburgh, Pennsylvania, and 2001 national meeting of the North American Benthological Society,

La Crosse, Wisconsin. The St. Croix River Rendezvous meetings are attended by a wide variety of stakeholders. These include individuals from Minnesota state agencies, a number of federal agencies including the Fish and Wildlife Service and the National Park Service. Individuals from academia, media, and the general public often attend these meetings. We have worked with project cooperators to submit results from the mussel relocation project to a refereed scientific journal (Conservation Biology) and the observation of the federally endangered winged mapleleaf upstream of Taylors Falls, Minnesota was published in the newsletter of the Freshwater Mollusk Conservation Society. All data collected was provided to the Minnesota DNR for inclusion in their Heritage database. As indicated in the outline of project results, students have assisted in collecting and analyzing data, and developing a web page describing the results of this project. Our web page is available at <http://www.macalester.edu/~hornbach>.

VI. CONTEXT

A. Significance: Freshwater mussels are among the most endangered organisms on earth (Abramovitz 1996). Recently the state of Minnesota has acknowledged the threats posed to this group of organisms by increasing the number species of mussels recognized as endangered or threatened. The major reasons for listing of a species as threatened or endangered are reduction in the geographical distribution of a species or a reduction in the population size of a species. Most of the work that has been completed on the mussel fauna of Minnesota has been distributional in nature: describing which species are found in various lakes and streams. Little work has been done to quantify the population sizes of mussels and those few studies that have been conducted generally lack a temporal component. To better manage mussel resources an indication of the change in populations over time is required. Also information on changes in age-structure and species composition is crucial in developing adequate management strategies for this resource.

The St. Croix River is designated as a Wild and Scenic River. One of the most important resources in the St. Croix River are its freshwater mussels. Recent studies indicate freshwater mussels are the most imperiled group of organisms in the United States (Master *et al.* 1998). At least 40 species of mussels live in the St. Croix River. Two of these species are on the federal endangered species list and 14 species are

state endangered or threatened in Minnesota. Many of the state-listed species have been placed on this list because of limited distribution and declining populations. The St. Croix River provides a refuge for many of these threatened species since population levels remain high. There have been a number of projects that managers must evaluate that may influence mussel habitats in the St. Croix watershed. These include the Stillwater Bridge project, NSP power line projects, water release from the reservoir above the dam at St. Croix Falls, Wisconsin and a number of smaller less public projects (gas pipelines, video cables, emergency fire hydrants, etc.). Resource managers must have both data upon which to base recommendations and mitigation options in order to effectively manage natural resources.

The decline in freshwater mussels is being accelerated by the introduction of the exotic species especially the zebra mussel, *Dreissena polymorpha*. Zebra mussels have recently been found in the lower portion of the St. Croix River. A number of agencies are examining techniques to protect the native mussel fauna from the zebra mussel invasion. There have been few studies that have examined the efficacy of relocation of mussels into refugia as a conservation strategy for these organisms (Cope and Waller 1995). Of these relatively few studies, most have followed the growth and survivorship over a fairly short time period (1-2 years). We began an examination of the efficacy of relocation strategies in the summer of 1997 as part of an LCMR program. This project was completed in the summer of 1998 with only a single year of data being provided. To more fully understand the long-term success of this relocation experiment we monitored the mussels that were relocated in 1997 through 2000. Since mussels are long-lived and slow growing, this longer time horizon is needed to provide insight for management activities.

In addition, this project will have a significant educational component for it will involve undergraduate students in an applied scientific project and presentation of results.

B. Time: This project began on July 1, 1999 and the final report was provided to the LCMR by August 2001. Both activities in this project have the potential to be on-going projects. The relocation project could be continued beyond 2000, assuming low mortality and low out-migration of mussels. Certainly there is a need for a continued long-term mussel monitoring program on the St. Croix River. It is possible that I will

request additional funds from the LCMR to continue these projects in the future.

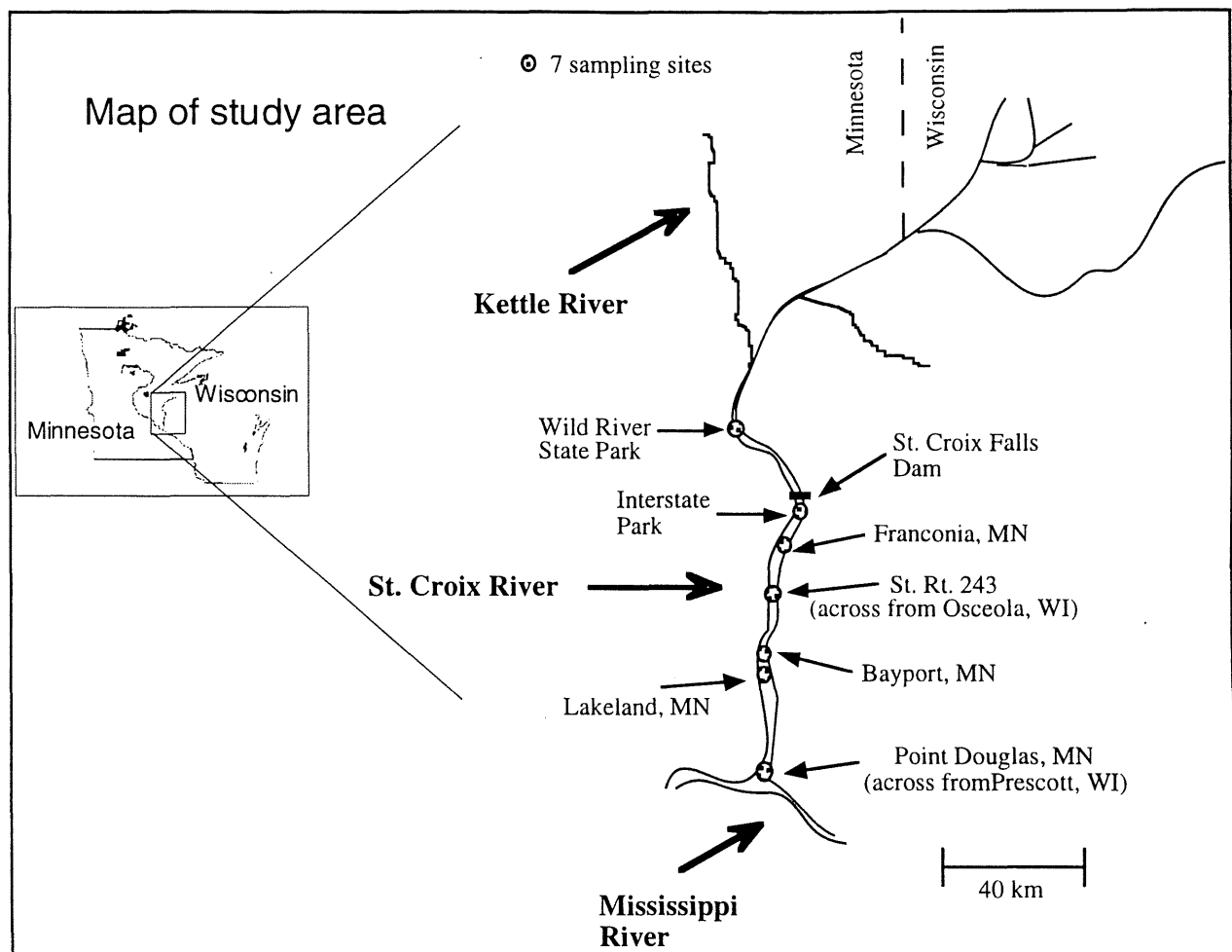
C. Budget Context: There are a number of agencies that have supplied past funding for a variety of projects that have provided baseline data upon which to base the current project. The relocation activity was funded from 1997-1999 through the LCMR to the University of Minnesota (\$21,795). Our monitoring program is based on comparing population sizes assessed in 1999 and 2000 with past estimates. The past estimates have been obtained from 1990 to the present. Sponsors that have supported this past research include the MN DNR Non-game Wildlife program, the US Environmental Protection Agency, the US Fish and Wildlife Service, the US National Park Service, and the WI DNR. Additional support was provided by the Blandin Foundation, Howard Hughes Medical Institute, and Macalester College. Often the population assessments were made as part of a larger project and it is difficult to estimate the amount of the awards used specifically for population assessments.

Budget:

	Total	Relocation activity	Population monitoring
Personnel			
Project Manager	\$20,400	\$5,100	\$15,300
Summer Students	\$18,100	\$3,960	\$14,140
Web page development	\$3,600	\$1,800	\$1,800
Field equipment	\$3,000		\$3,000
SCUBA equipment	\$3,400	\$1,000	\$2,400
Other			
Travel	\$3,500	\$750	\$2,750
Supplies	\$6,000	\$1,000	\$5,000
Acquisition	\$0	\$0	\$0
Development	\$0	\$0	\$0
Total	\$58,000	\$13,610	\$44,390

VII. COOPERATION: We cooperated with the Minnesota DNR, WI DNR, US Fish and Wildlife Service, and National Park Service on this project. All of these agencies have responsibility for overseeing the mussel resources of inland waters. We are required to obtain scientific collecting permits from all of these agencies. In addition, the National Park Service and the states (WI and MN) have the responsibility of managing the mussel resources in the St. Croix River. We have provided these agencies with all of the data we collected. Also, we cooperated with employees of the Biological Survey of the USGS at the Upper Midwest Environmental Science Center. They conducted a relocation project at another location in the St. Croix River, using the same methodology that we used. We are collaborating to publish the results of these studies. None of these individuals or agencies received LCMR project dollars.

VIII. LOCATION: Minnesota counties - Washington, Chisago



IX. REPORTING REQUIREMENTS: Periodic work program progress reports were submitted on December 1999 and December 2000. A final work program report and associated products was submitted before August 10, 2001.

Attachment A - Deliverable Products and Related Budget

		Result 1	Result 2	
Budget Item		Relocation activity	Population monitoring	Row Total
Wages, salaries and benefits	Project Manager	\$5,100	\$15,300	\$42,100
	Summer Students			
	Webpage development	\$3,960 [†]	\$14,140 [†]	
		\$1,800 ^{††}	\$1,800 ^{††}	
Space rental, maintenance & utilities		\$0	\$0	\$0
Printing & advertising		\$0	\$0	\$0
Communications, phone, etc.		\$0	\$0	\$0
Contracts				
Professional/technical		\$0	\$0	\$0
Other Contracts		\$0	\$0	\$0
Local automobile mileage paid		\$750	\$2,750	\$3,500
Other travel expenses in MN		\$0	\$0	\$0
Travel outside Minnesota		\$0	\$0	\$0
Office supplies		\$0	\$0	\$0
Other supplies	Gas for boat, air fills, sieves, etc.	\$1,000	\$5,000	\$6,000
Tools and equipment	SCUBA equipment & field computer	\$1,000	\$5,400	\$6,400
Office equipment & computers		\$0	\$0	\$0
Other capital equipment		\$0	\$0	\$0
Other direct operating costs				
Land acquisition		\$0	\$0	\$0
Land rights acquisition		\$0	\$0	\$0
Buildings or other land improvements		\$0	\$0	\$0
Legal fees		\$0	\$0	\$0
Column total		\$13,610	\$44,390	\$58,000

[†]3 students first summer, 2 students second summer

^{††} Work-study award for 1 student during the academic year

X. RESEARCH PROJECTS: (August 2001)

I. Abstract

The purpose of this project was to address conservation and management needs of one of the most threatened group of organisms in Minnesota, native freshwater mussels. Project objectives include completing a mussel relocation and refugia study begun in 1997 in association with the University of Minnesota, and review the status of one of the most important mussel communities in Minnesota, that in the St. Croix River.

Refugia may become important conservation tools in protecting native mussels from invasive species such as zebra mussels. We completed monitoring of a refuge project begun in 1997 designed to provide information on the long-term impact of mussel relocations and thus provide insight into the efficacy of this conservation measure. We collected, measured, and weighed each mussel from reference and relocation sites at Wild River State Park. Survival rates among the three study species was high emphasizing the importance of handling mussels carefully (*e.g.* using the protocol described in this report) during relocation and providing the mussels with high quality habitat.

The distribution of various mussel species in the St. Croix River have been fairly well established, however, the long-term stability of these populations has not been examined. Between 1999 and 2000 we quantitatively resampled seven locations in the St. Croix River that we have periodically sampled since 1990. Analysis of these 10 years of data shows that locations downstream of the dam at Taylors Falls, Minnesota had lower adult mussel density, significantly lower juvenile mussel densities at Interstate State Park and Franconia, Minnesota, and an increase in fine sediments at locations downstream of Taylors Falls. Previous studies found neither increased sedimentation nor lack or juvenile recruitment above the dam at Taylors Falls. This suggests that increased sedimentation below the dam may be influencing mussel recruitment or juvenile mussel survival. Project results were shared with resource professionals and placed on the Macalester College website (<http://www.macalester.edu/~hornbach>).

II. Background and hypothesis

Freshwater mussels (Family Unionidae) are widely distributed throughout the United States. There are 44 species of freshwater mussels currently on the federally endangered species list (Abramovitz 1996). Despite this fact there is little known concerning the factors that control the distribution of these organisms, especially in flowing water systems. Certainly factors such as surface geology, stream size, water quality, substrate type, water flow, and food availability, among others, are important in determining the community structure and population dynamics of freshwater mussels (Strayer 1983). Not only are unionids important because of their diversity in flowing water systems, but based on the river continuum concept (Vannote *et al.* 1980) large filter feeders (such as mussels) are also extremely significant components of larger river systems. They are one of the few organisms able to process the fine particulate organic matter that makes up a significant portion of the energy basis for these reaches of river systems. The recent introduction of the zebra mussel (*Dreissena polymorpha*) from Europe is likely to affect adversely populations of native mussels (Family Unionidae). There is the potential for both direct and indirect influence of *Dreissena* on the biodiversity of unionids.

Despite the importance of unionids in flowing water systems in North America, little is known about the population dynamics of these organisms. Mussel are relatively long-lived (10-80+ years) and have a unique reproductive cycle (McMahan, 1991). Males release sperm into the water and are taken in through the incurrent aperture by the female. Fertilization of the eggs occurs and zygotes are brooded in the water tubes of the gills by the female. Various species become sexually mature at different ages (3-9 years). Female unionids often produce a large number of eggs (500,000 - 1 million, Burky 1983). The zygotes develop into glochidia larvae that are discharged into the water column. The discharged larvae attach to a host, usually a fish, where they remain for varying periods of time (estimates range from 10-90 days for various species). Glochidia attached to a suitable host species transform to juveniles and drop off their fish host. The juveniles, upon settling on suitable habitat attach by a byssal thread, which prevents their being swept away in water currents.

Given the elaborate life cycle, and the great longevity of these organisms, understanding the population dynamics requires a long-term commitment to develop a historical data set of population size. To address this lack of data, Dan Hornbach begun what he hoped will be a long-term project to follow the population dynamics of mussels in the St. Croix River. This river has a diverse assemblage of unionids in fairly high density (Doolittle 1988, Hornbach 1997). There are 40 species of mussels that have been recorded from the St. Croix River (Hornbach 1997) with individual locations often having as many as 20 species present. There are two federally endangered species (*Lampsilis higginsii* and *Quadrula fragosa*) in the river, and for the winged mapleleaf mussel (*Quadrula fragosa*) the St. Croix River contains the only known extant population (Hornbach *et al.* 1996). Historically this species was found in at least 13 states but is now restricted to two locations, one in the St. Croix River. Additionally there are 14 species of mussel in the St. Croix River that the state of Minnesota has declared endangered or threatened. Many of these state-listed species are considered imperiled because of an historical reduction in the distribution and abundance of these organisms. However, the St. Croix River appears to be a refuge for many of these species with healthy reproducing populations. Proper management of this rare aquatic resource should be a priority. Unfortunately, many of the past studies on the St. Croix River have utilized qualitative sampling and this can provide biased population and community estimates compared to quantitative estimates (Hornbach and Deneka 1996).

As mentioned above, freshwater mussels are among the most endangered organisms in North America. Historically, declines in mussel density and changes in community structure have been attributed to habitat degradation and alteration (Fuller 1974, Cummings and Mayer 1991, McMahon 1991, Williams *et al.* 1993, Layzer *et al.* 1993). Currently, many researchers believe that the introduction of the zebra mussel from Europe in the late 1980s may prove to be even more devastating than past impacts on native mussels (Nalepa 1994, Schloesser and Nalepa 1994, Ricciardi *et al.* 1995). With these impacts there have been a number of efforts to examine techniques to protect native mussel populations. 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. We continued a relocation study that was begun in the river in the summer of 1997. In this study we examined the influence of mussel relocation on both the relocated mussels and on the mussels that existed in the location to which mussels were relocated.

In summary, the two major questions we examined were:

1. How temporally stable are mussel populations? That is how much "natural" variation is found in mussel population density, community composition and age-structure in the St. Croix River?
2. 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?

III. Description of methodology

To examine the changes in mussel population density, age-structure, and community structure over time, we returned to seven locations in the St. Croix River that Dan Hornbach had sampled in the past (Figure 1).

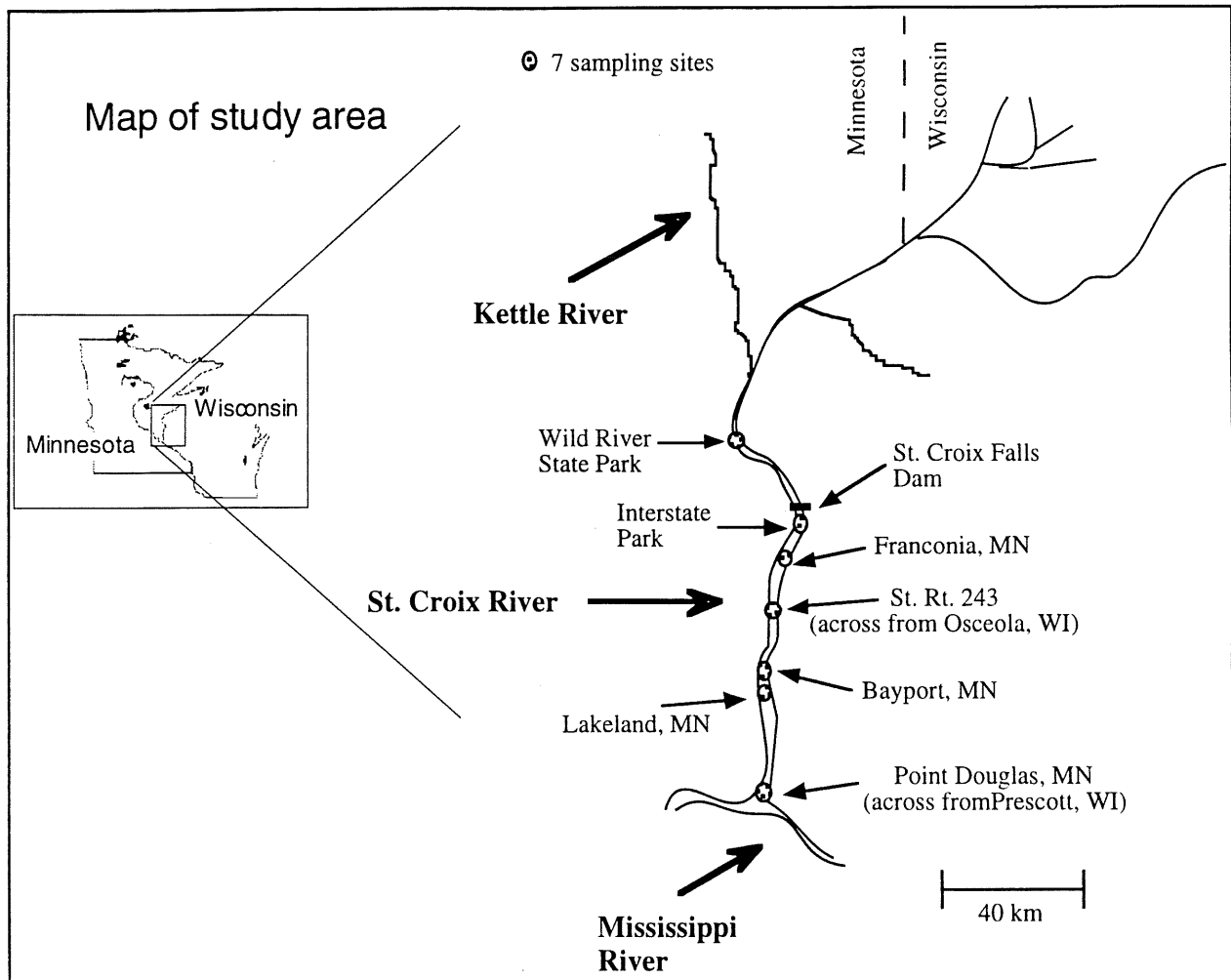


Figure 1. Seven mussel communities studied in the St. Croix River.

At each of these locations stratified quantitative sampling was used to collect data from ten sampling sites. Five of these sites were from the middle of the channel and five near the shore. At each of these ten sampling sites, ten 0.25 m² quadrats were taken with SCUBA. The entire contents (mussels and substrate) were removed from the quadrat and sieved to ensure that all sizes of mussels were collected. Mussels were identified and their shells measured (length, width, and height). In my

previous work, Dan Hornbach found that this sampling regime of taking approximately 100 0.25-m² quadrats is sufficient to characterize the mussel community at a location (Hornbach and Deneka 1996).

From the quantitative samples, measures of community structure, age-distribution, and population density were ascertained and compared to previous sampling data. In addition, at each site we examined those factors likely to have a major impact on the distribution and abundance of unionids. Changes in these factors could be used to explain any observed differences in mussel population density, or community or age structure. These factors include water depth, location in the river, flow rates, and substrate type. Water depth was determined with a sonar unit, location in the river with a GPS unit, and flow rate with a Marsh-McBirney Model 201D flowmeter. Substrate type was determined by wet sieving the substrate that is taken with the mussel samples through four sieves and then weighing each fraction (Lewis 1984). For data analysis of mussel densities all density values were log₁₀ transformed. Mean values were tested for significant differences using Tukey's HSD test (Zar 1984).

At the present time, very few self-sustaining populations of zebra mussels have been identified in the St. Croix River. There have been isolated individuals found attached to native mussels or small clumps of zebra mussels attached to boat hauls in the river below Stillwater. We inspected all native mussels that were collected for the presence of zebra mussels. Care was taken to disinfect our sampling boat and gear as we move from location to location in the river. We also began sampling upstream and moved downstream to minimize the potential of accidentally moving zebra mussels into areas not previously infected.

To examine the efficacy of *in-situ* mussel relocations as a conservation technique, we continued the study design approved by the LCMR for implementation in 1997/98. This design included constructing a site in the St. Croix River near the confluence with the Sunrise River as a "reference" site and a site near Wild River State Park as a "relocation" site. At each site a 5 m x 5 m grid has been demarcated on the bottom of the river. At each grid we randomly selected five 1 m x 1 m quadrats and assigned them to one of the following treatments:

Control - in 1997 these quadrats were not disturbed

Quadrula pustulosa - in 1997 each of these quadrats received an additional 10 *Quadrula pustulosa*.

Lampsilis cardium - in 1997 each of these quadrats received an additional 10 *Lampsilis cardium*.

Elliptio dilatata - in 1997 each of these quadrats received an additional 10 *Elliptio dilatata*.

Double density - in 1997 each of these quadrats received double the "normal" density. This was calculated by taking 10 0.25 m² quadrats outside the relocation grid and averaging the number of mussels collected. This amounted to adding 78 mussels to each of the double density treatment areas.

For all quadrats (except controls in 1997) we removed all mussels, identified them, measured their shell length and weighed them. Except for 2000 we also etched a unique number on the shell of each mussel we collected. Mussels were replaced in the quadrats from which they were removed. At the reference site the *Q. pustulosa*, *L. cardium* and *E. dilatata* were collected from around the relocation grid while at the relocation area these species were taken from the reference site and move to the relocation area. Thus these 3 species are the "test" or relocated mussels. Figure 2 gives an overview of the relocation design. For the 10 0.25 m² quadrats taken to estimate the population density, we removed all of the sediment and wet sieved it through four sieves, weighing each fraction (Lewis 1984) in order to determine sediment size. This procedure will allow us to examine changes in mussel mortality and growth in the various treatments. Analysis of variance was used to assess differences in growth and survivorship and similar analysis was used to detect differences in mortality among treatments (SAS Institute 1994).

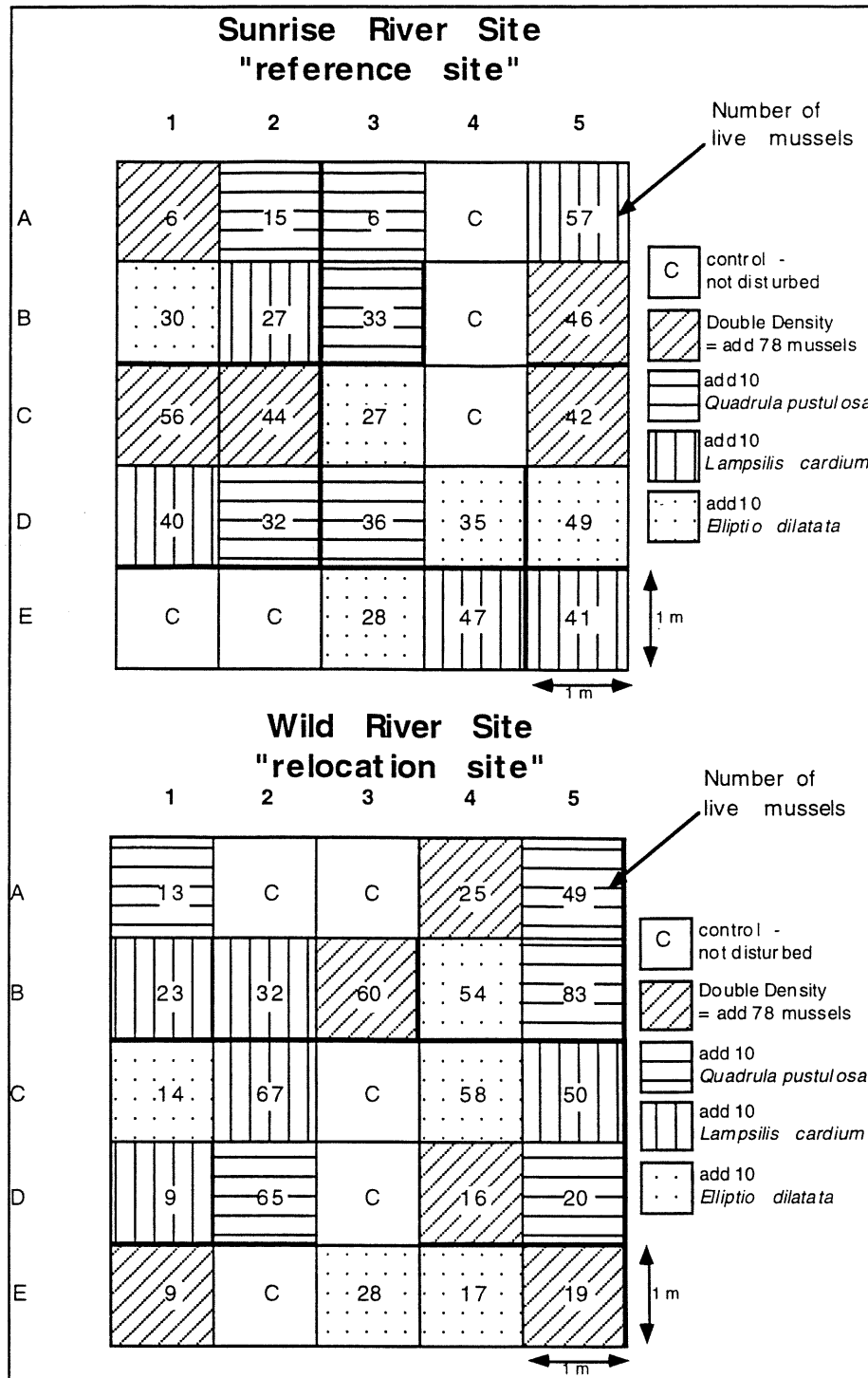


Figure 2. Study design in mussel relocation project.

IV. Description of Results

Result 1 - Relocation and refugia study

Mussel relocation was successful considering the recovery and survivorship of the target mussel species (50 mussels of each - *Quadrula pustulosa*, *Elliptio dilatata*, *Lampsilis cardium*) The number of recovered mussels and percent survivorship moved to the relocation site was not statistically different from that of the mussels maintained at the reference site (Table 1). Mussel recovery dropped approximately 50% over the study period. We believe this was due in part to the movement of mussels into and out of the study grids.

Table 1. Recovery and percent mortality of relocated mussels from the reference and relocation sites.

	<i>Elliptio dilatata</i>		<i>Lampsilis cardium</i>		<i>Quadrula pustulosa</i>	
Site	No.recovered	% dead	No.recovered	% dead	No. recovered	% dead
1998						
Reference	32	12	32	8	32	8
Relocation	32	8	35	2	28	8
1999						
Reference	28	2	30	8	25	4
Relocation	27	2	25	2	20	8
2000						
Reference	22	6	22	4	23	0
Relocation	18	6	16	2	20	2

*Two additional mussels were inadvertently added in 1997.

The mean length of relocated mussels was similar among reference and relocation sites. The average length of *Q. pustulosa* ranged from 55 to 59 mm. *Elliptio dilatata* average length was 74 mm and *L. cardium* ranged from 89 to 93 mm (Table 2).

Table 2. Mean length \pm 1 standard deviation and age (range in parentheses) of mussels relocated to reference and relocation sites on the St. Croix River in 1997.

	Mean length (mm)
<i>Elliptio dilatata</i>	
Reference	74 \pm 12 (54-94)
Relocation	74 \pm 15 (38-106)
<i>Lampsilis cardium</i>	
Reference	93 \pm 9 (76-115)
Relocation	89 \pm 10 (68-111)
<i>Quadrula pustulosa</i>	
Reference	55 \pm 13 (22-80)
Relocation	59 \pm 11 (31-77)

The mean natural density of mussels varied over time. The mean natural density of mussels (based on 10-1/4 m² quadrats from the periphery cells of each grid for each year) appeared to decline between 1997 and 2000, although the only statistically significant decrease occurred between 1997 and 1998 at the reference site (Table 3).

Table 3. Mean density \pm 1 standard deviation (range in parentheses) of live mussels from the natural population, non-handled, resident control cells at the reference and relocation sites on the St. Croix River from 1997 to 2000.

	Natural population ^a			
	Year			
	1997	1998	1999	2000
Reference	78 \pm 36 (32-136)	52 \pm 20 (24-88)	50 \pm 21 (20-84)	44 \pm 17 (24-72)
Relocation	77 \pm 50 (20-168)	72 \pm 48 (20-152)	51 \pm 33 (24-116)	49 \pm 34 (20-124)

^aEstimated from 10 m² quadrats taken from the periphery cells of each grid.

The doubling of mussel density at the reference and relocation sites, as measured by the DDC, had no effect on mussel recovery or survival. Mussel density in the five DDC cells did not change significantly over time (Table 4). The cumulative number of unmarked mussels increased from 805 in 1998, to 1201 in 1999, and to 1372 in 2000 at the reference site. A similar increase in the cumulative number of unmarked mussels was recorded at the relocation site where 605 mussels were collected in 1998, 1094 in 1999, and 1266 in 2000.

Table 4. Mean density \pm 1 standard deviation (range in parentheses) of live mussels from the density-doubled control cells at the reference and relocation sites on the St. Croix River from 1997 to 2000.

	1997	1998	1999	2000
Reference	117 \pm 19 (84-134)	126 \pm 19 (101-153)	113 \pm 17 (84-128)	107 \pm 4 0 (64-161)
Relocation	103 \pm 20 (86-137)	99 \pm 27 (73-141)	84 \pm 24 (48-108)	78 \pm 24 (51-112)

During the course of the study, the species richness of the natural mussel populations peripheral to the sites did not change significantly. There were 10 to 15 species at the reference site and 13 to 16 species at the relocation site between 1997 and 2000. The average number of species per quadrat did not change significantly over this time period. The two dominant species at both study grids were *A. ligamentina* and *E. dilatata*. A total of 19 different species were found, 9 (47%) of which were listed (Table 5).

Table 5. Species name and conservation status of unionid mussels sampled at the reference and relocation sites on the St. Croix River from 1997 to 2000.

<i>Scientific name</i>	<i>Common name</i>	<i>Conservation status^a</i>
<i>Actinonaias ligamentina</i> (Lamarck, 1819)	mucket	MNT
<i>Alasmidonta marginata</i> Say, 1818	elktoe	MNT, WISC
<i>Amblema plicata</i> (Say, 1817)	threeridge	
<i>Cyclonaias tuberculata</i> (Rafinesque, 1820)	purple wartyback	MNT, WIE
<i>Elliptio dilatata</i> (Rafinesque, 1820)	spike	MNSC
<i>Fusconaia flava</i> (Rafinesque, 1820)	Wabash pigtoe	
<i>Lampsilis cardium</i> Rafinesque, 1820	plain pocketbook	
<i>Lampsilis siliquoidea</i> (Barnes, 1823)	fatmucket	
<i>Lasmigona costata</i> (Rafinesque, 1820)	flutedshell	MNSC
<i>Leptodea fragilis</i> (Rafinesque, 1820)	fragile papershell	
<i>Ligumia recta</i> (Rafinesque, 1819)	black sandshell	MNSC
<i>Obliquaria reflexa</i> Rafinesque, 1820	threehorn wartyback	
<i>Obovaria olivaria</i> (Rafinesque, 1820)	hickorynut	MNSC
<i>Pleurobema sintoxia</i> (Rafinesque, 1820)	round pigtoe	MNT, WISC
<i>Potamilus alatus</i> (Say, 1817)	pink heelsplitter	
<i>Pyganodon grandis</i> (Say, 1829)	giant floater	
<i>Quadrula fragosa</i> (Conrad, 1835)	winged mapleleaf	FE, MNE, WIE
<i>Quadrula pustulosa</i> (L. Lea, 1831)	pimpleback	
<i>Quadrula quadrula</i> (Rafinesque, 1820)	mapleleaf	
<i>Strophitus undulatus</i> (Say, 1817)	creeper	
<i>Truncilla truncata</i> Rafinesque, 1820	deertoe	

^aAbbreviation for conservation status: FE = federally endangered, MNE = Minnesota endangered, MNT = Minnesota threatened, MNSC = Minnesota special concern, WIE = Wisconsin endangered, WISC = Wisconsin special concern.

The textural characteristics (cobble-gravel, sand, and silt-clay fractions) of the sediment changed significantly over the course of the study. Size fractions for substrate samples collected between 1997-2000, were categorized as follows: (1) boulder and cobble, (2) gravel, and (3) fine gravel, sand, and silt. The mean percentage of boulder and cobble at the reference site changed significantly between 1997 and 1999, and significant differences were observed at the relocation site between 1997 and 2000, and 1999 and 2000 (Table 6). The mean percentage of the gravel fraction did not differ within sites over the study (ANOVA, $p > 0.05$). There was a significant difference in the mean percentage of the fine gravel and sand fraction at the reference site between 1997 and 1999, and 1997 and 2000, and at the relocation site between 1997 and 2000, and 1999 and 2000.

Table 6. Mean particle size characteristics (SD in parentheses) of sediment taken from around the periphery ($n = 10$ locations) of each grid at the reference and relocation sites on the St. Croix River from 1997 to 2000.

	<i>Mean particle fraction (%)</i>											
	<i>Boulder & cobble</i>				<i>Gravel</i>				<i>Fine gravel & sand</i>			
	<i>Year</i>				<i>Year</i>				<i>Year</i>			
	'97	'98	'99	'00	'97	'98	'99	'00	'97	'98	'99	'00
Reference	54 ^c (21)	41 ^{c,d} (22)	32 ^d (17)	34 ^{c,d} (12)	19 ^c (6)	19 ^c (6)	21 ^c (6)	22 ^c (7)	27 ^c (17)	40 ^{c,d} (18)	47 ^d (14)	44 ^d (6)
Relocation	58 ^c (18)	49 ^{c,d} (10)	57 ^c (15)	35 ^d (14)	17 ^c (7)	15 ^c (6)	16 ^c (6)	14 ^c (6)	25 ^c (15)	36 ^{c,d} (8)	27 ^c (10)	51 ^d (19)

For a given particle fraction and year, any two means not accompanied by a common letter were judged to be significantly different ($\alpha = 0.05$) based on a Tukey's hsd test.

This study demonstrates the utility of *in situ* refugia for mussel conservation. Based on mussel survival the relocation of mussels to the refugium is considered successful. Overall mean survival of recaptured mussels was 95%. Survival rate was relatively high compared to 37 other mussel relocations summarized in Cope and Waller (1995) where the average survivorship was 51%. More recently *in situ* improved relocation techniques have been resulted in higher mussel survivorship (Waller *et al.* 1995, Havlik 1997, Dunn & Sietman 1997, Dunn *et al.* 2000). Mussel handling techniques used in these projects was similar to the procedure

followed in our study. These successful examples of mussel relocations illustrate the importance of handling and transport procedures and selection of suitable relocation habitat.

Several factors influence the success of a mussel relocation effort. Some of the variables that have been studied include effects of air and water temperature, emersion duration, and collection, handling, and transportation techniques. Survival of *Amblema plicata* and *Obliquaria reflexa* has been shown to be > 90% after aerial exposure at air temperatures ranging from 15-29 °C (Waller *et al.* 1995). In the laboratory *L. cardium*, *Q. pustulosa*, and *E. dilatata* were exposed to air for 15-60 min at temperatures 15-35 °C and did not significantly change their survival, behavior, or physiological condition (Greseth 1998, Bartsch *et al.* 2000). In field studies emersion for 40 min at air temperatures 21-29 °C did not change the survivorship or growth of a variety of mussel species (Dunn *et al.* 2000). The recommendations made in these studies are similar to the methods we used in our study, conduct relocations during moderate temperatures, hold mussels in water whenever possible, and provide optimal moisture and temperature conditions during transport.

While conducting work on this project during the summer of 1999 we made an important discovery. Winged mapleleaf (*Quadrula fragosa*) shells were collected at the relocation site. This federally endangered species has never been observed in the upper Saint Croix River and presence of the shells could have a substantial impact on whether or not to relocate this species from the lower St. Croix River. The USFWS provided us with support to conduct a survey for live winged mapleleaf in the area. Although no live winged mapleleaf were collected, two additional winged mapleleaf shells were collected.

Result 2 - Monitoring mussel populations in the St. Croix River

We quantitatively assessed mussel communities at seven locations in the St. Croix River. The greatest diversity of native mussel species was observed at Interstate State Park with 26 species and the lowest at Bayport with 13 species (Table 3). All sites had a species diversity index of 1.9 or 2.0 except Bayport that had an index of 1.5. Lakeland had five dominant species, Prescott had three dominant species, Wild River State Park and Franconia had 2 dominant species, and Interstate State Park, Osceola, and Bayport had one dominant species (figures 3-9). The two

federally endangered mussels living in the St. Croix River were collected during the study. Higgins eye (*Lampsilis higginsii*) were observed at Interstate State Park, Osceola, Lakeland, and Prescott, and winged mapleleaf (*Quadrula fragosa*) at Interstate State Park. Non-native bivalves were observed at downstream sites. Zebra mussels and Asian clams were observed at Lakeland and Prescott.

Table 3. Native mussel species diversity and species diversity index observed at each study location between 1999-2000.

Location	Number of Mussel Species	Species Diversity Index
Wild River State Park	18	1.9
Interstate State Park	26	2.0
Franconia, MN	15	2.0
Osceola, WI	21	1.9
Bayport, MN	13	1.5
Lakeland, MN	21	2.0
Prescott, WI	16	2.0

Over the last ten years Dan Hornbach has sampled each of these seven mussel communities at least once: 1993 and 1996 at Wild River State Park, 1992, 1995 and 1998 at Interstate State Park, 1990, 1991, and 1995 at Franconia, 1993 at Osceola, 1992 at Bayport, 1995 at Lakeland, and 1994 at Prescott. Mussel density was greatest at Wild River State Park (average 28-36 mussels/m²), followed by Interstate State Park (average 29-38 mussels/m²), Lakeland (14-19 mussels/m²), Franconia (average 4-10 mussels/m²), Bayport (5-11 mussels/m²), Osceola (7-9 mussels/m²), and Prescott (average 7-8 mussels/m²). At most locations mussel density was lower in 2000 compared to previous years. The most dramatic declines were at Franconia (58%) and at Bayport (51%); other populations had declines of 8-27% (Figure 10). None of the changes were statistically significant. However, juvenile mussel density (mussels < 30mm), at Interstate State Park and Franconia showed a significant decline over the period (Figure 11).

Two possible reasons for the decline in juvenile density could be high juvenile mortality, or lack of recruitment. Shell-length frequency diagrams for dominant species show a general lack of recruitment during

this time period (figures 12-18). Since mussels require approximately 6 years to mature, long periods of low recruitment may be possible.

There was a decrease in the size of average river bottom substrates over time. An increase in fine sediments was observed at each location (Figure 19). Neither increased sedimentation nor lack of recruitment above the dam at St. Croix Falls was observed in previous studies. This suggests that increased sedimentation below the dam may be influencing mussel recruitment or juvenile mussel survival.

Acknowledgements

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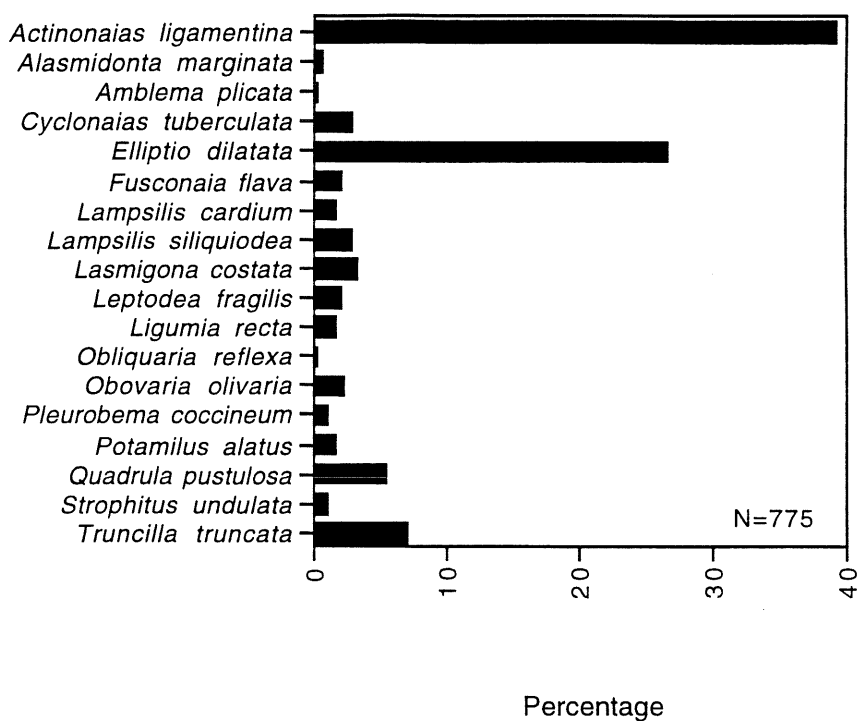


Figure 3. Species assemblage at Wild River State Park in 1999.

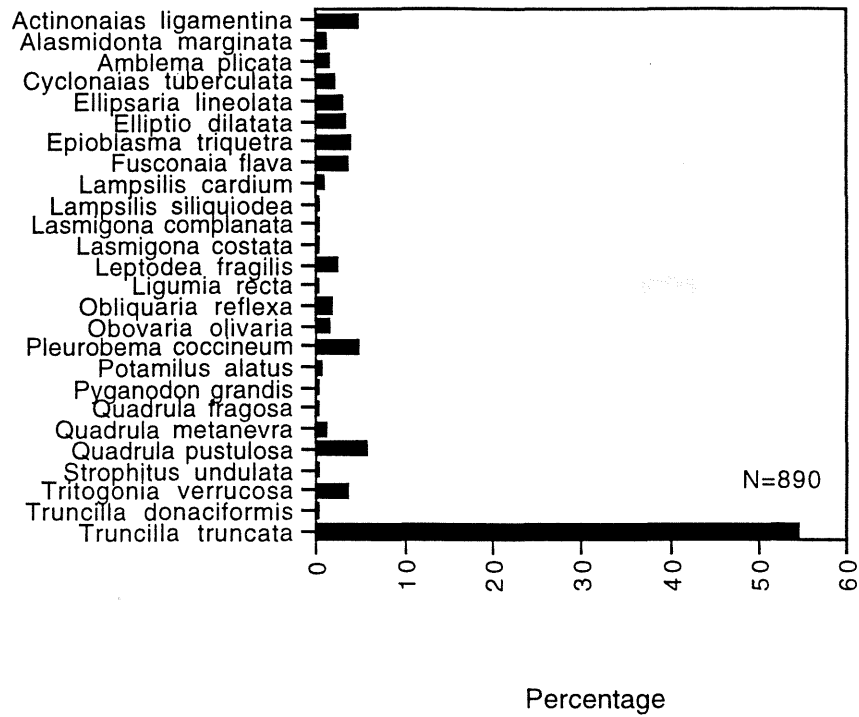


Figure 4. Species assemblage at Interstate State Park in 2000.

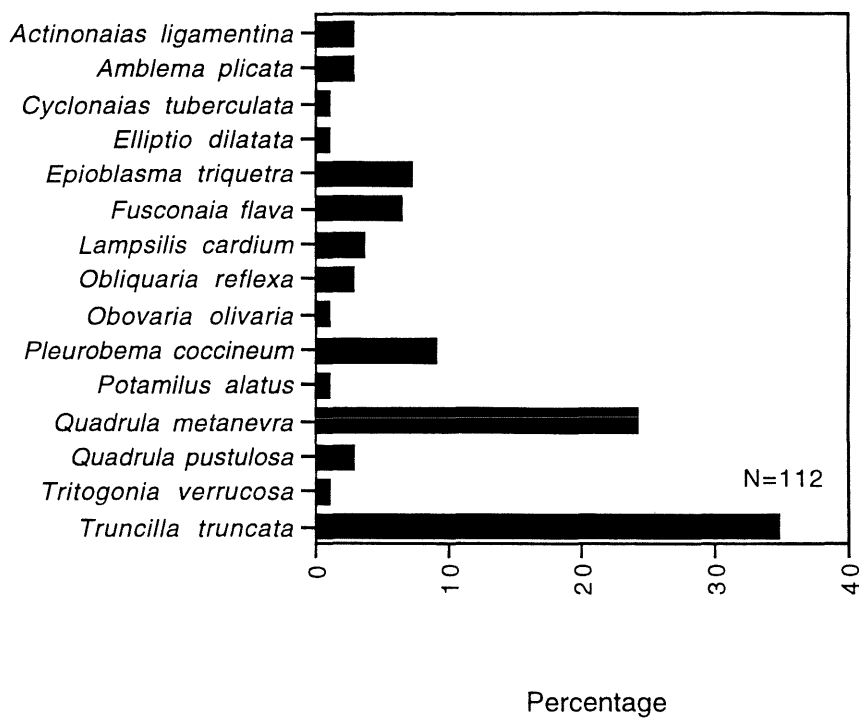


Figure 5. Species assemblage at Franconia in 1999.

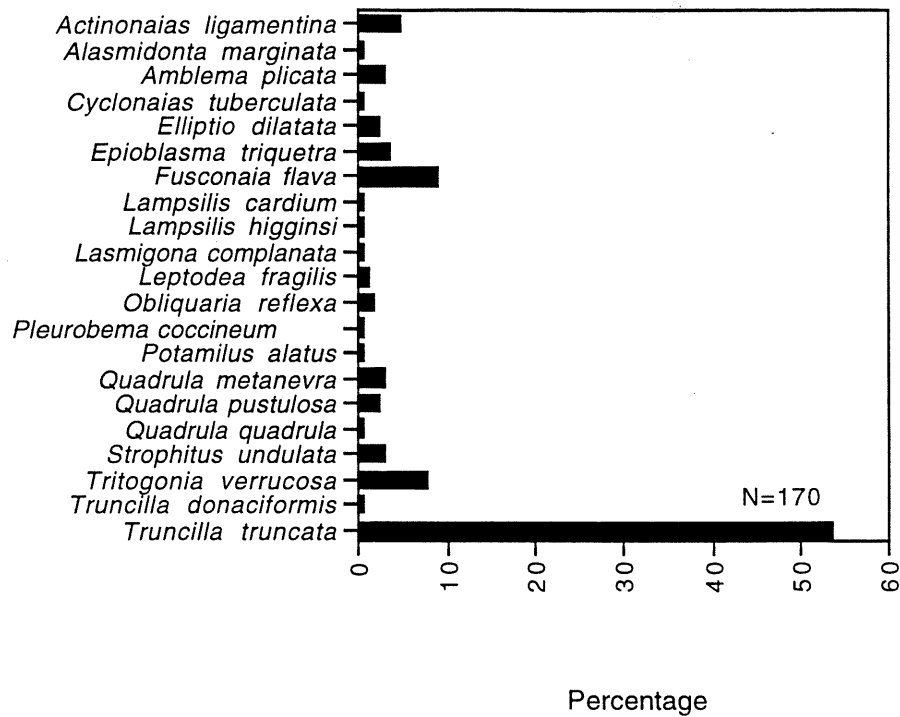


Figure 6. Species assemblage at Osceola in 2000.

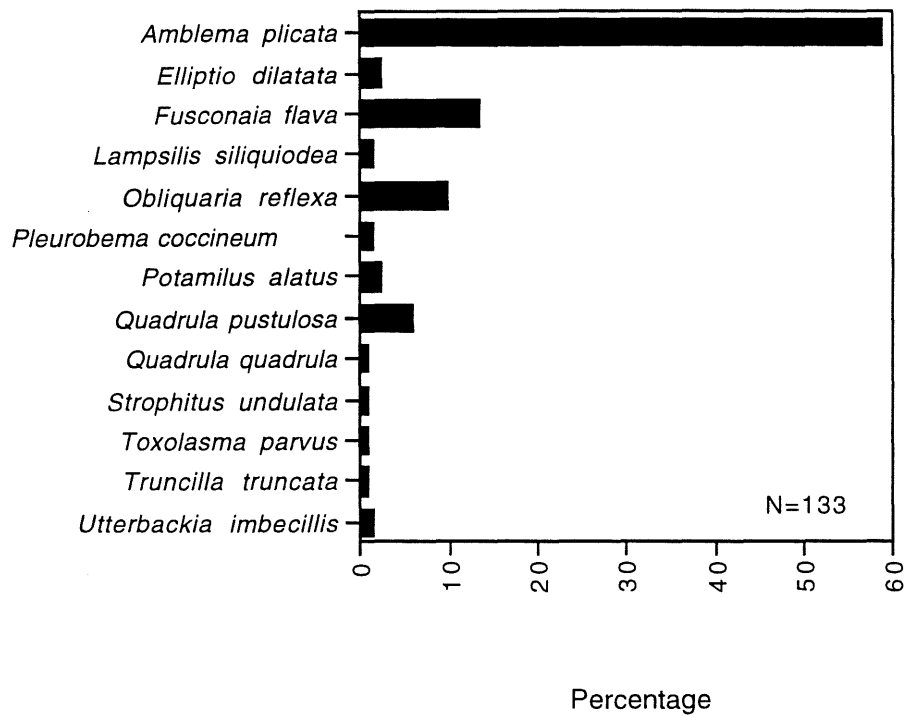


Figure 7. Species assemblage at Bayport in 2000.

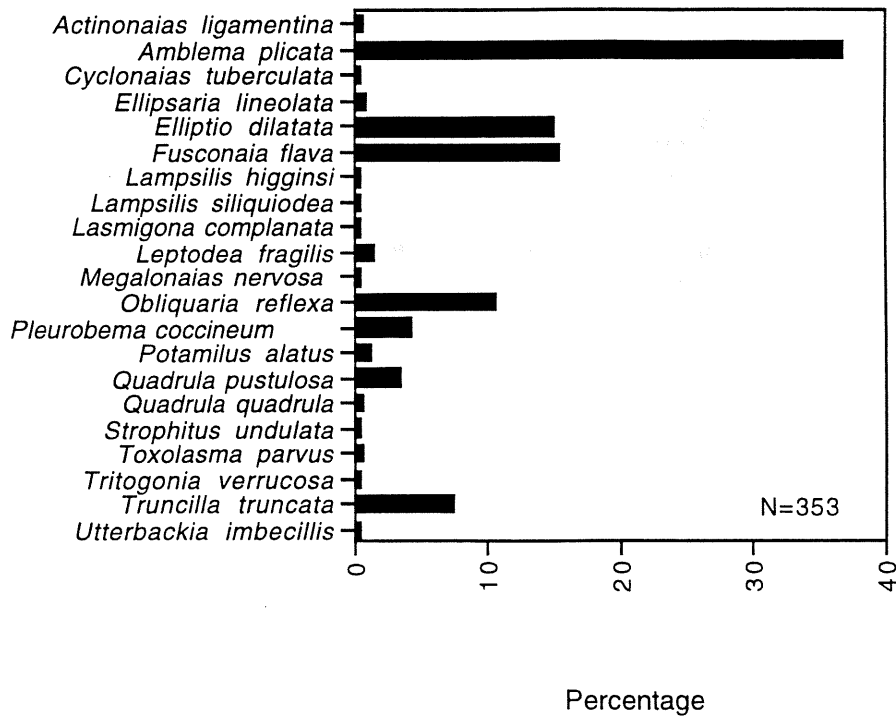


Figure 8. Species assemblage at Lakeland in 2000.

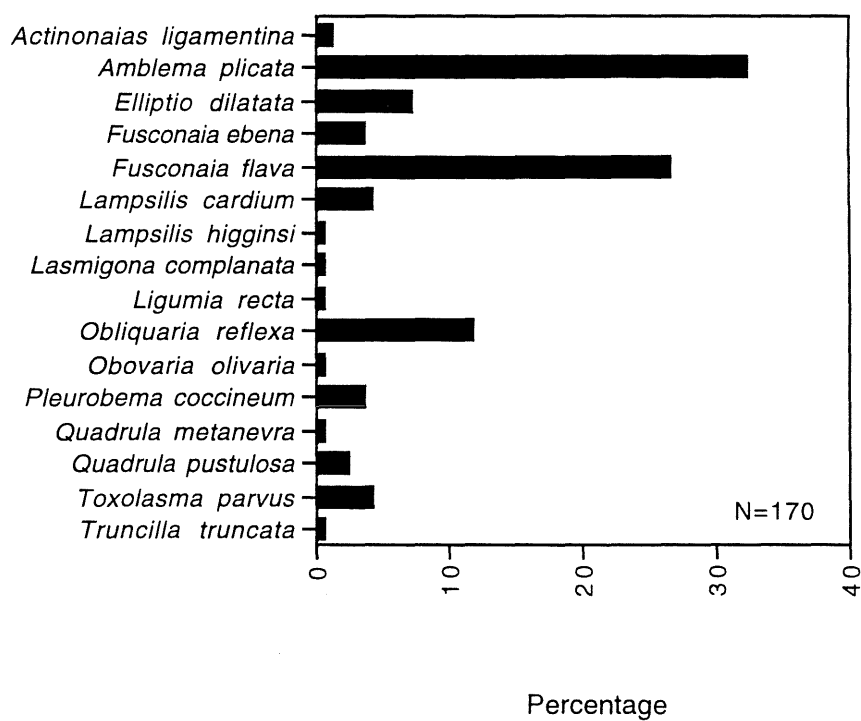


Figure 9. Species assemblage at Prescott in 1999.

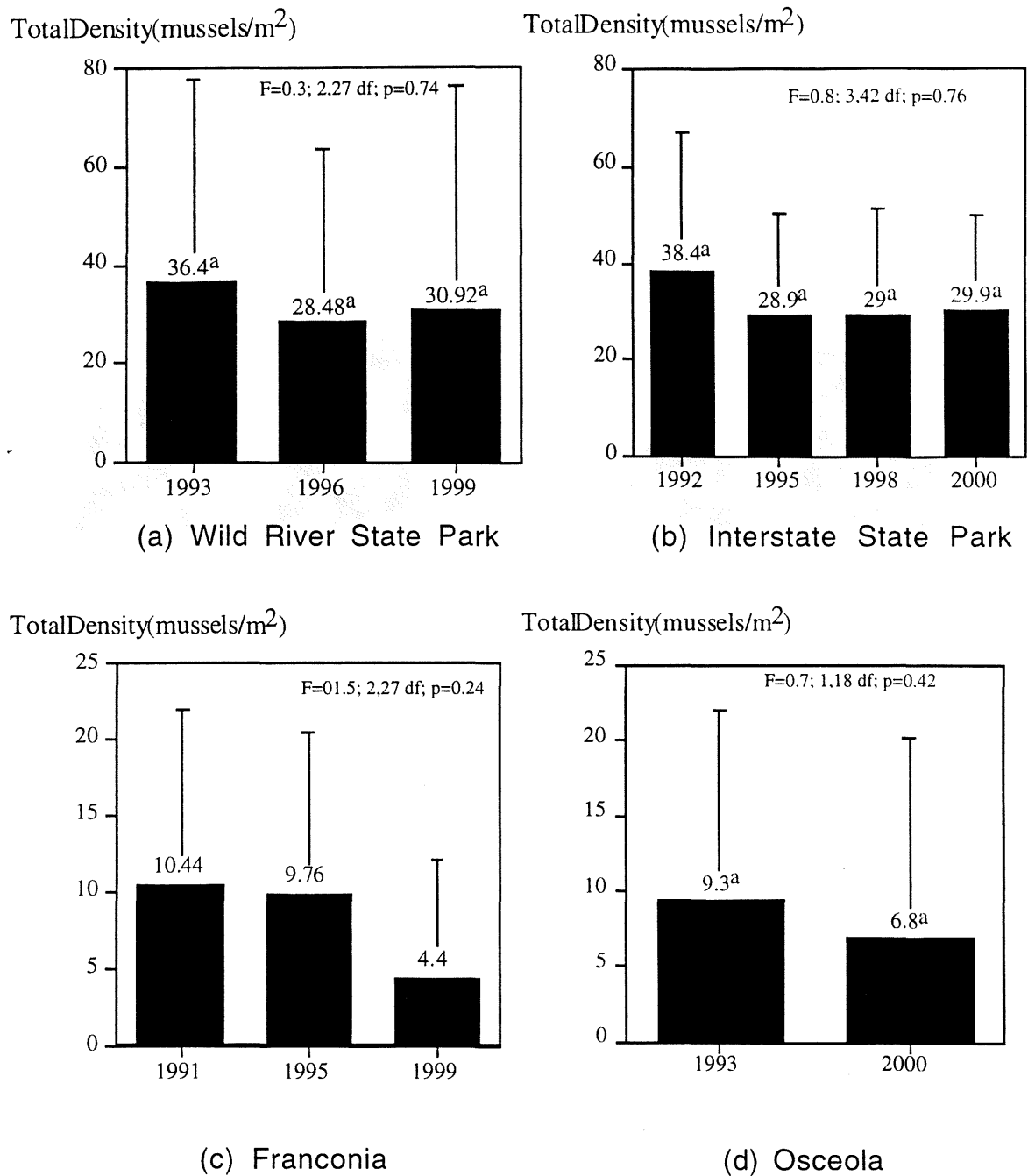


Figure 10. Mussel density at: (a) Wild River State Park, (b) Interstate State Park, (c) Franconia, and (d) Osceola.

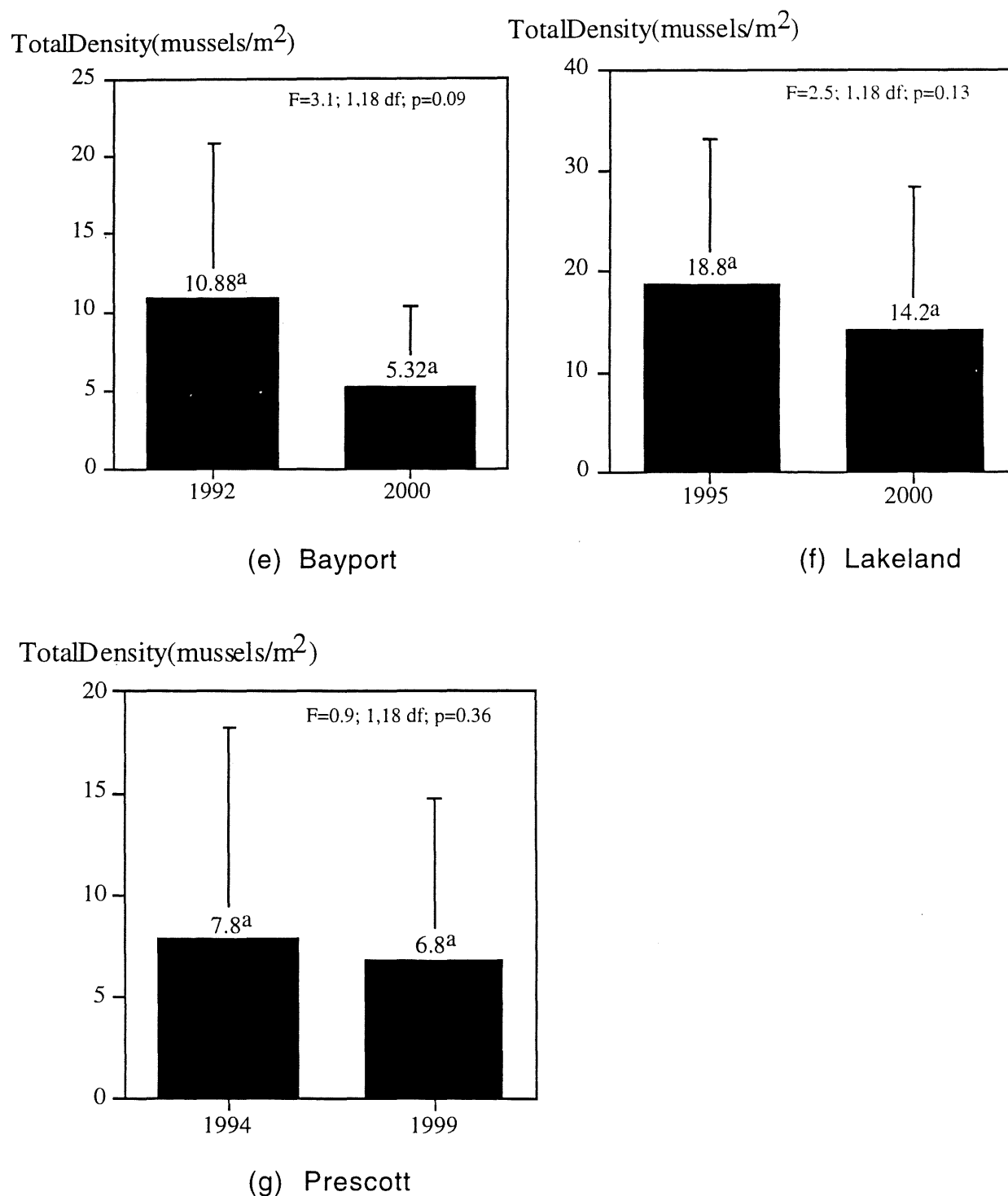
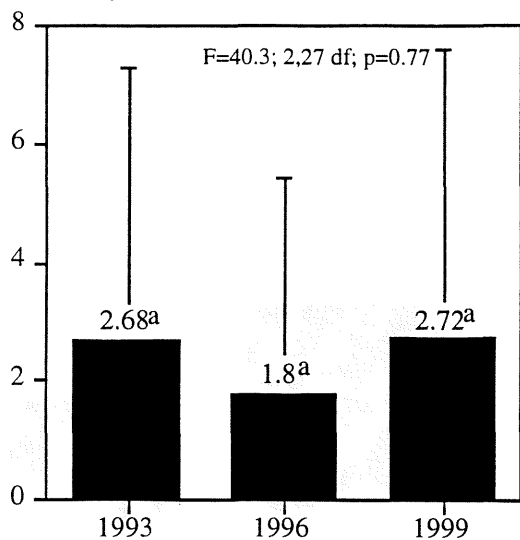


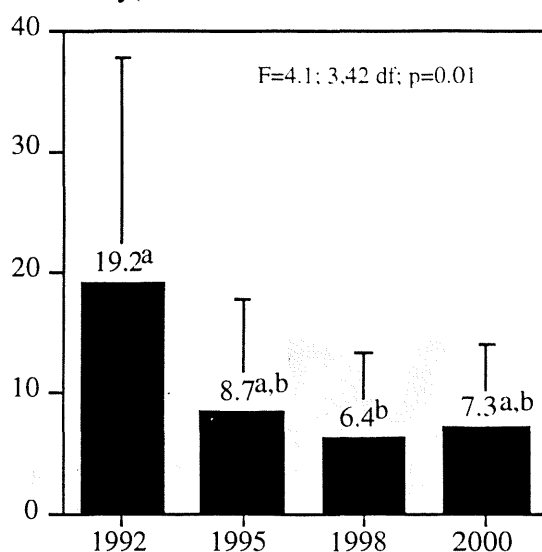
Figure 10. Mussel density at: (e) Bayport, (f) Lakeland, and (g) Prescott.

JuvenileDensity(mussels/m²)



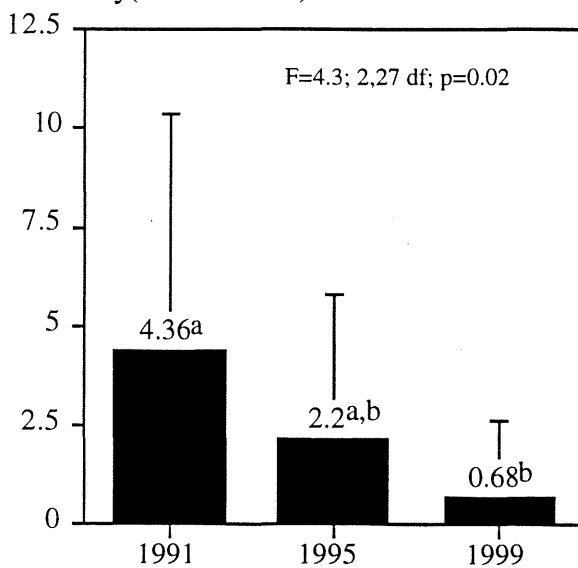
(a) Wild River State Park

JuvenileDensity(mussels/m²)



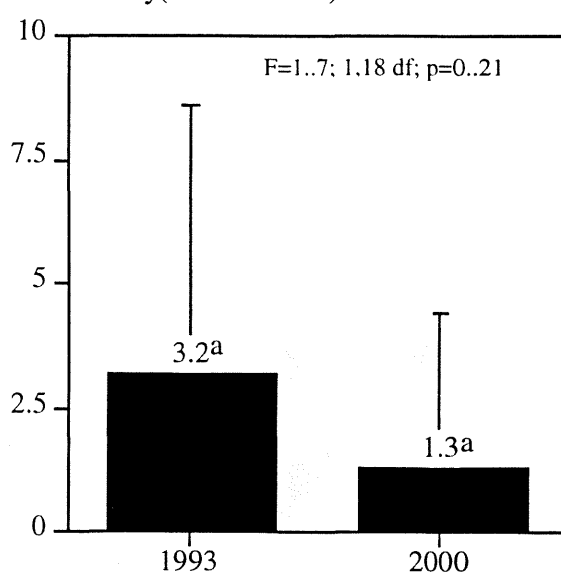
(b) Interstate State Park

JuvenileDensity(mussels/m²)



(c) Franconia

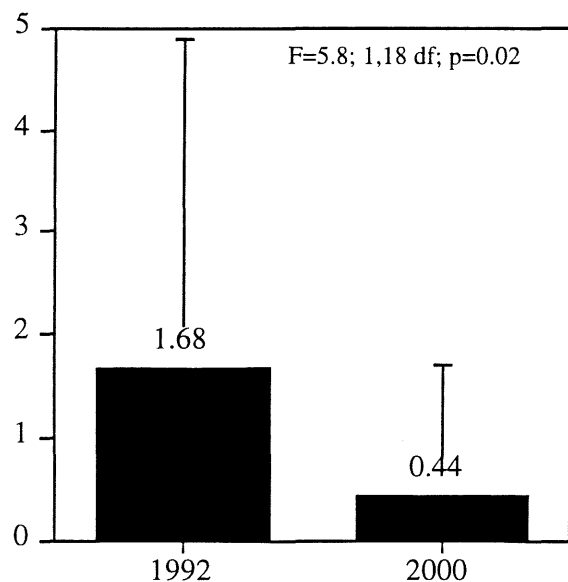
JuvenileDensity(mussels/m²)



(d) Osceola

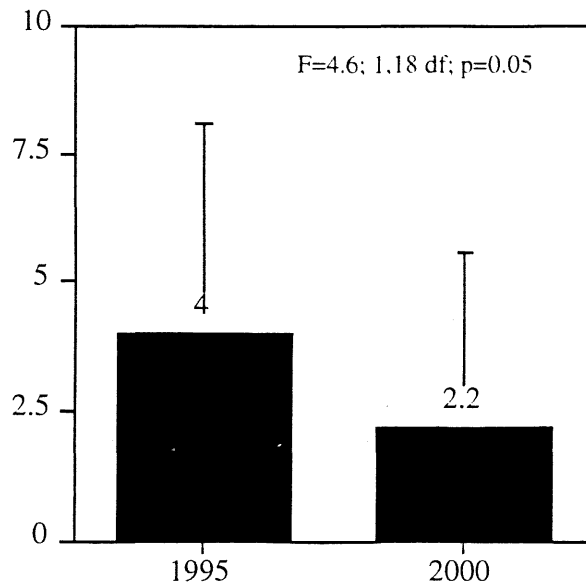
Figure 11. Juvenile mussel density at: (a) Wild River State Park, (b) Interstate State Park, (c) Franconia, and (d) Osceola. Means with the same letter are not significantly different.

JuvenileDensity(mussels/m²)



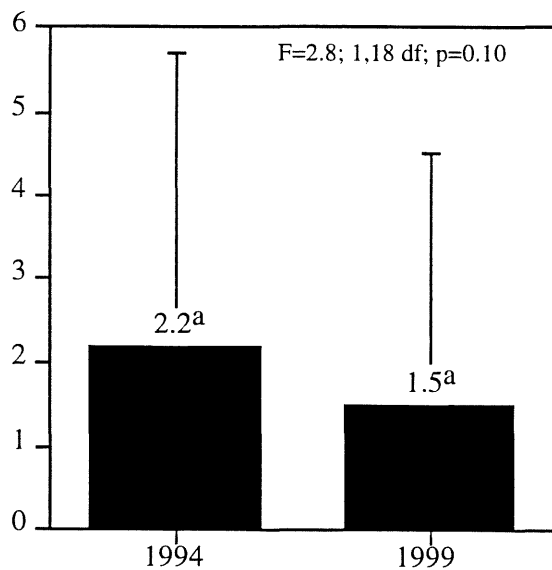
(e) Bayport

JuvenileDensity(mussels/m²)



(f) Lakeland

JuvenileDensity(mussels/m²)

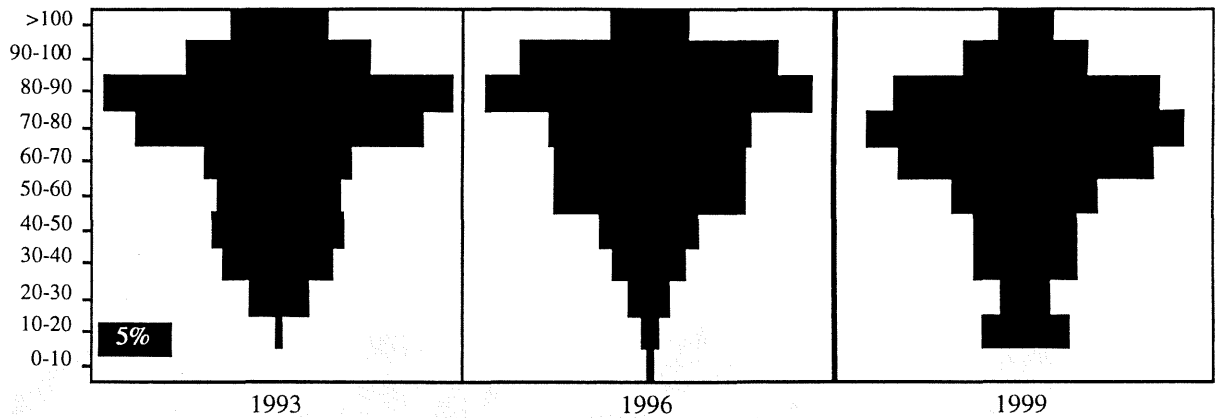


(g) Prescott

Figure 11. Juvenile mussel density at: (e) Bayport, (f) Lakeland, and (g) Prescott. Means with the same letter are not significantly different.

Shell Length (mm)

Actinonaias ligamentina



Shell Length (mm)

Elliptio dilatata

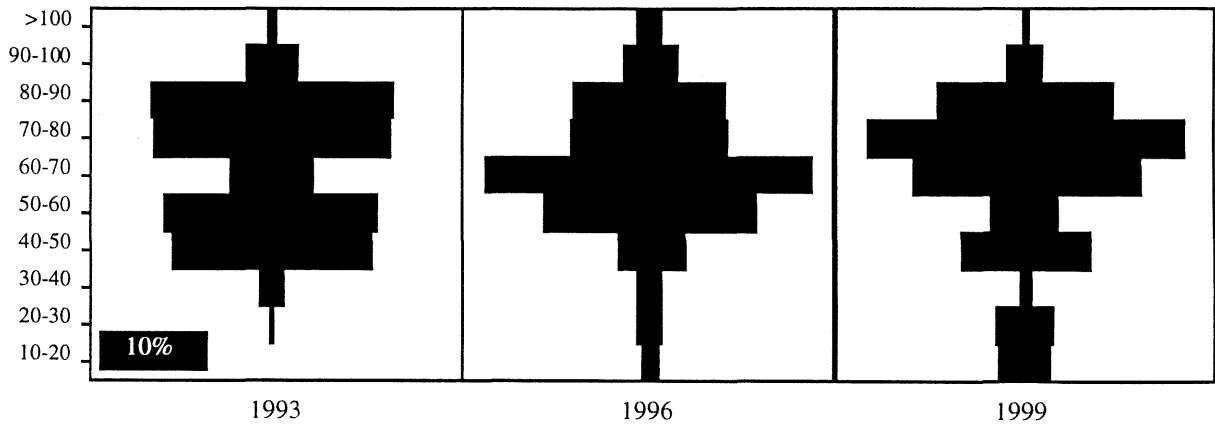


Figure 12. Shell-frequency diagram for dominant species at Wild River State Park.

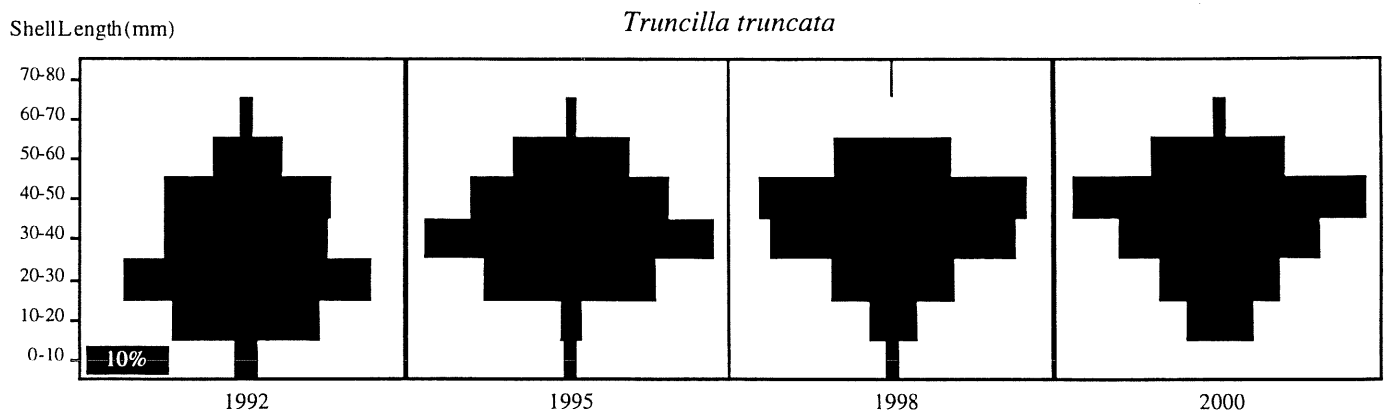


Figure 13. Shell-frequency diagram for dominant species at Interstate State Park.

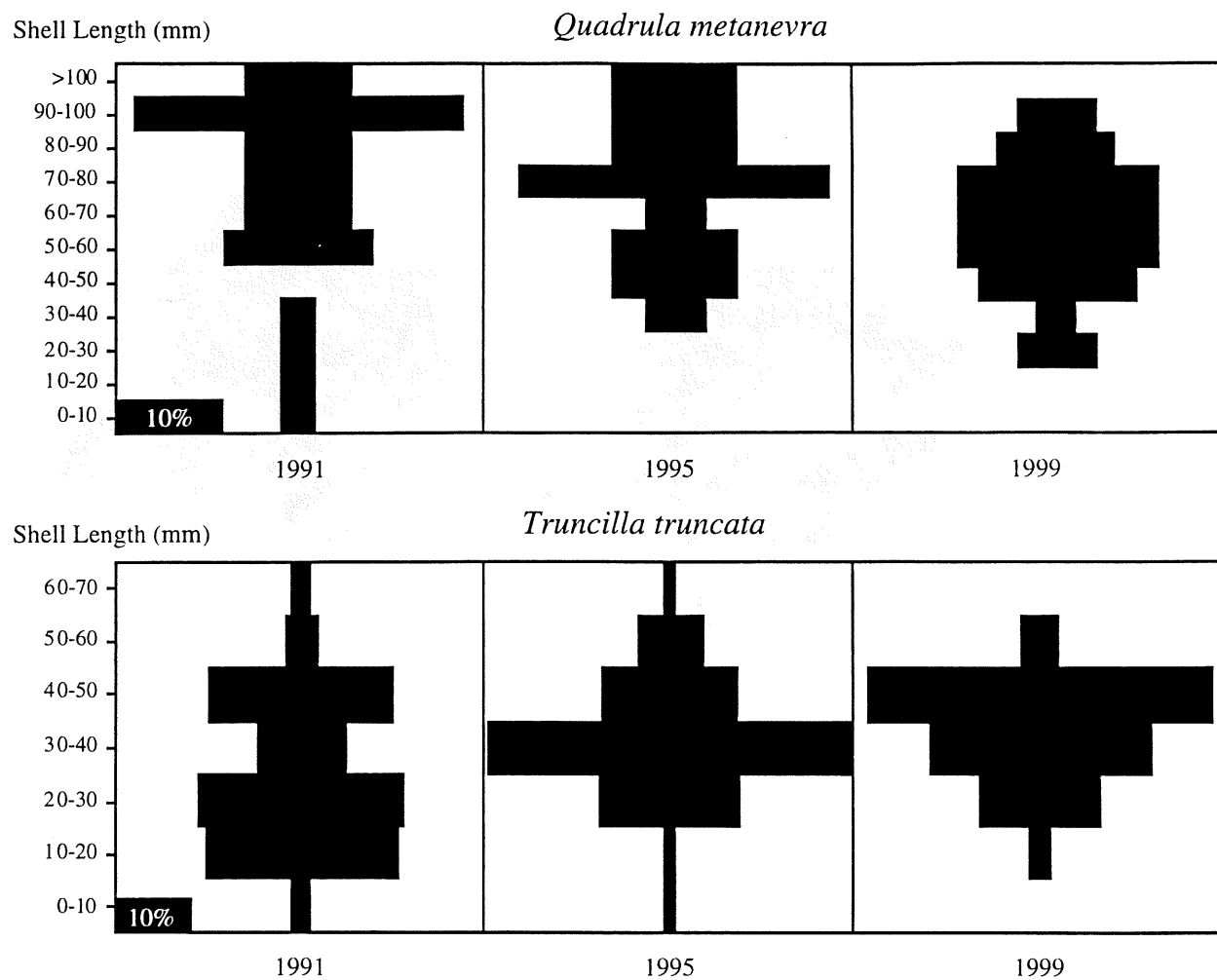


Figure 14. Shell-frequency diagram for dominant species at Franconia.

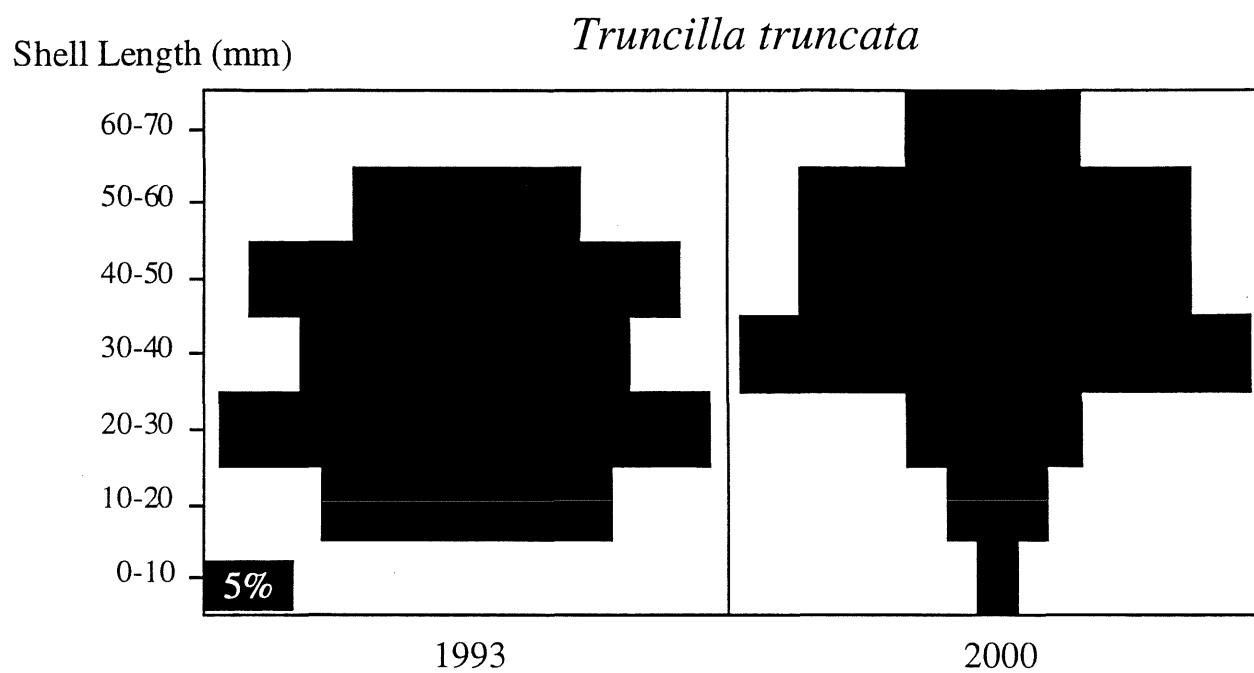


Figure 15. Shell-frequency diagram for dominant species at Osceola.

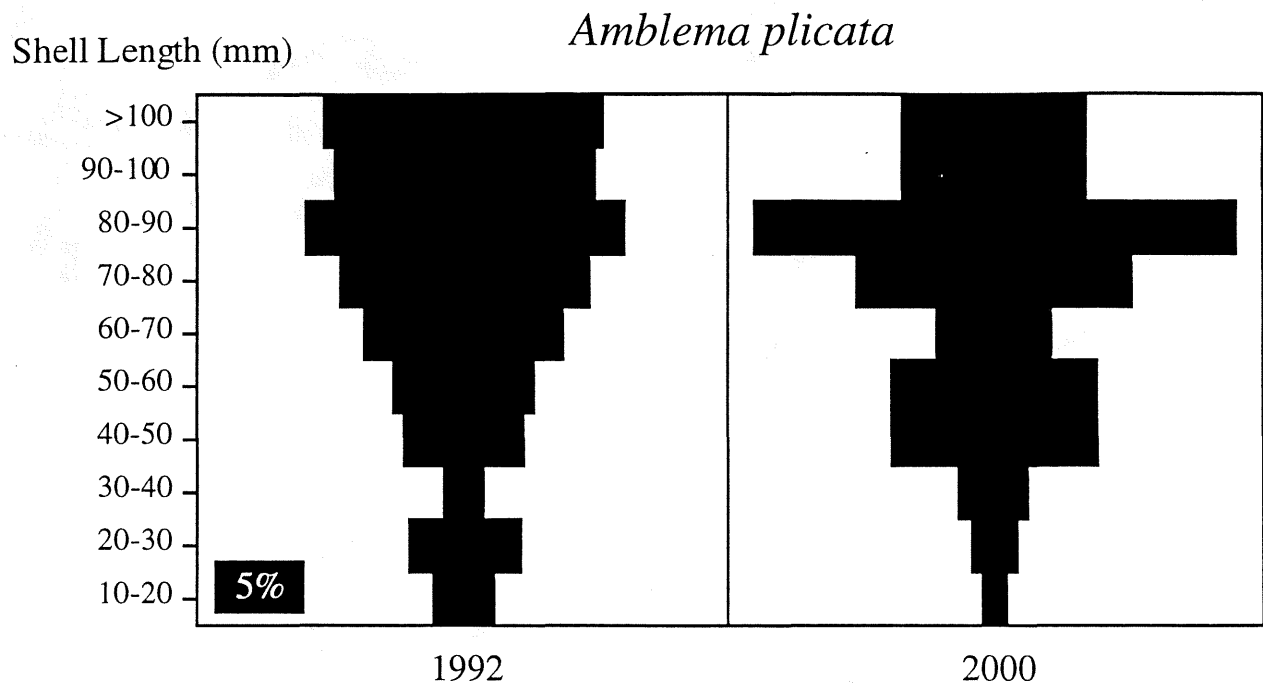


Figure 16. Shell-frequency diagram for dominant species at Bayport.

Lakeland

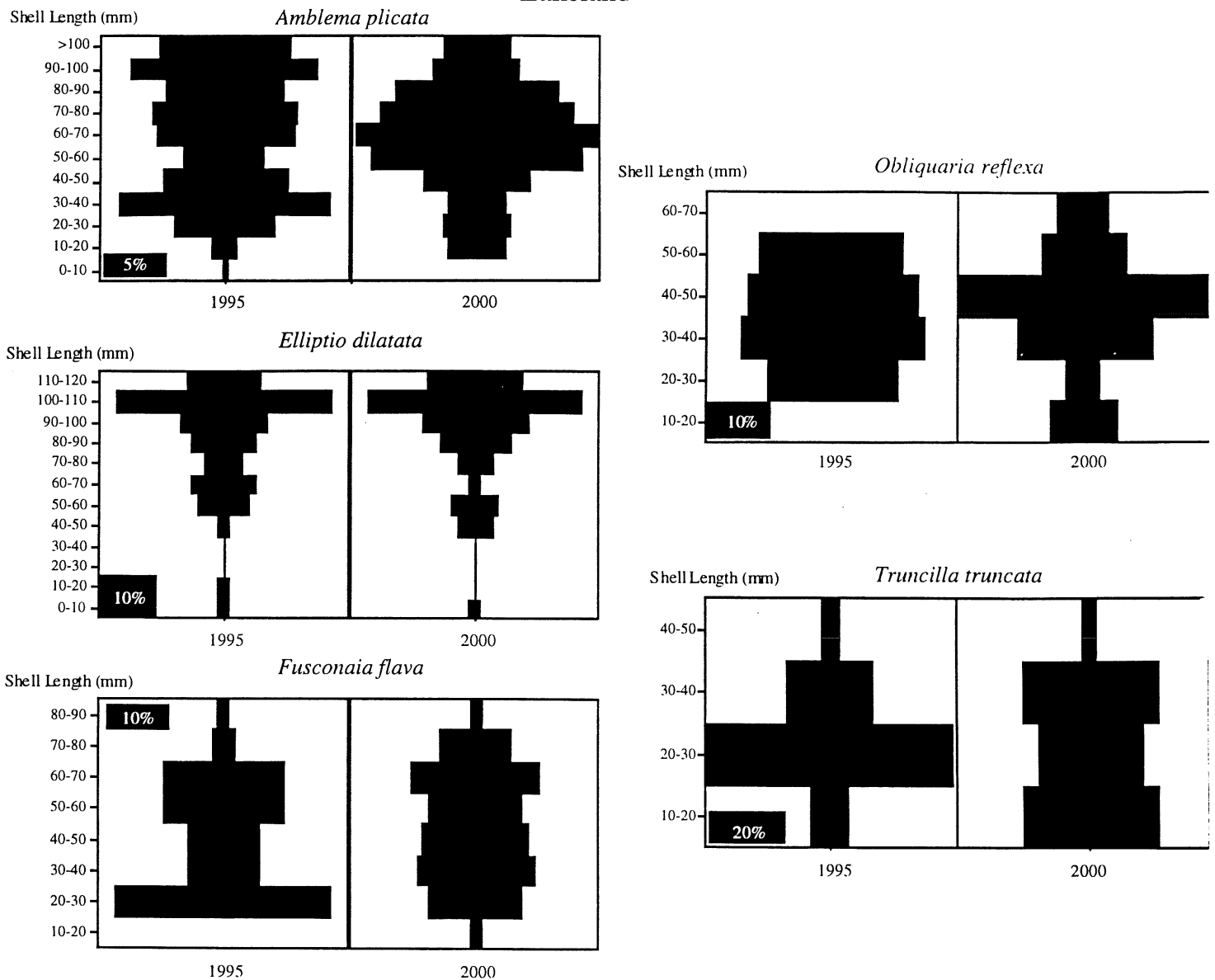


Figure 17. Shell-frequency diagram for dominant species at Lakeland.

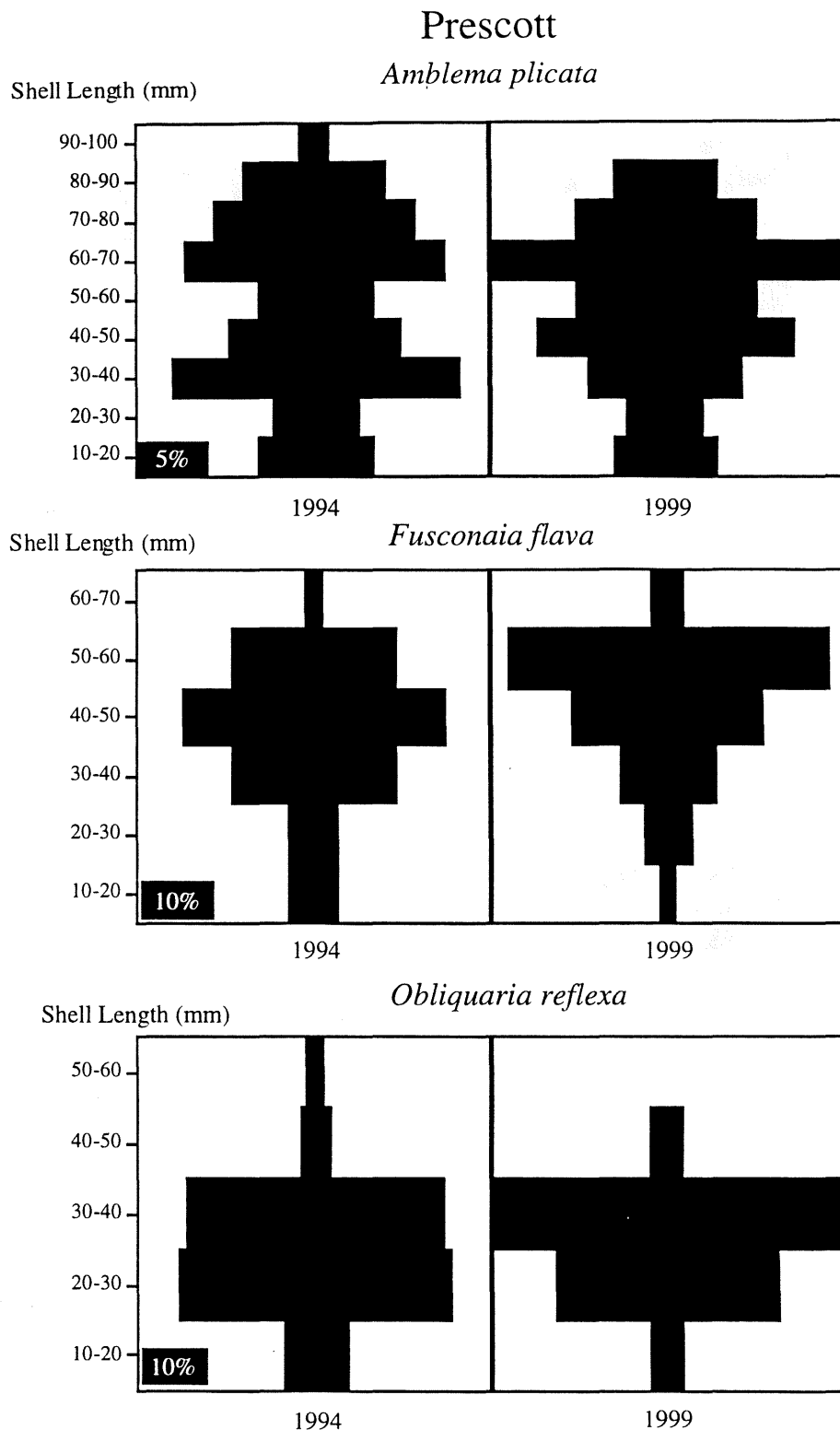
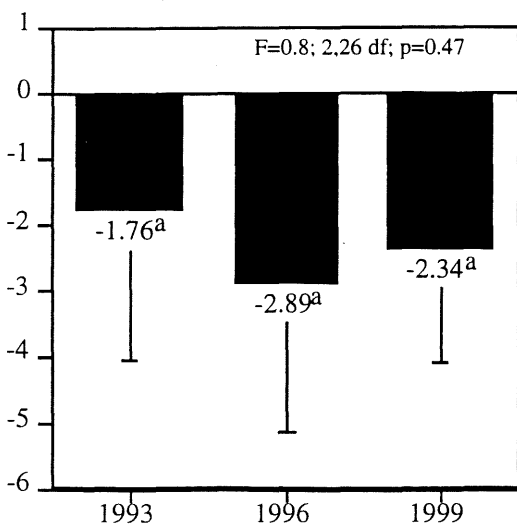


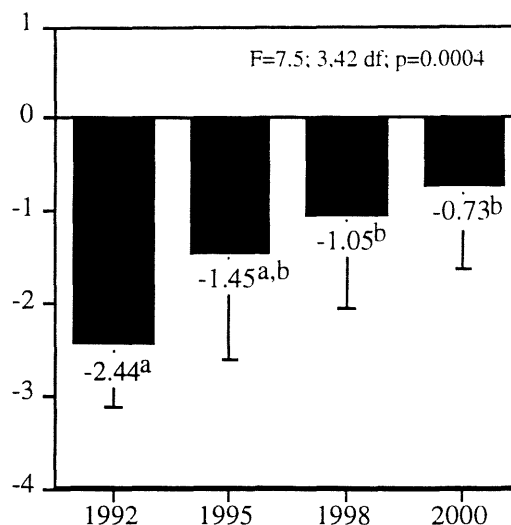
Figure 18. Shell-frequency diagram for dominant species at Prescott.

SedimentSize(ϕ)



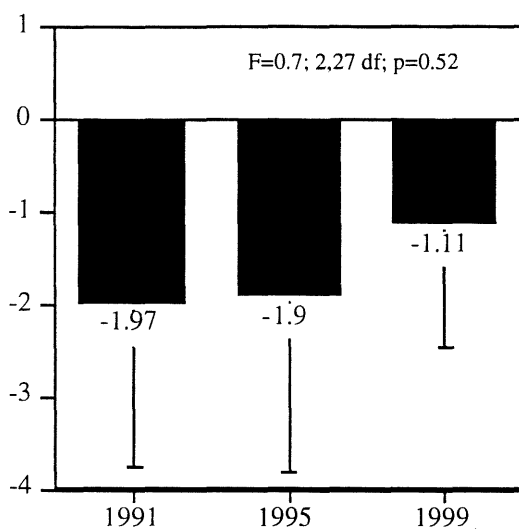
(a) Wild River State Park

SedimentSize(ϕ)



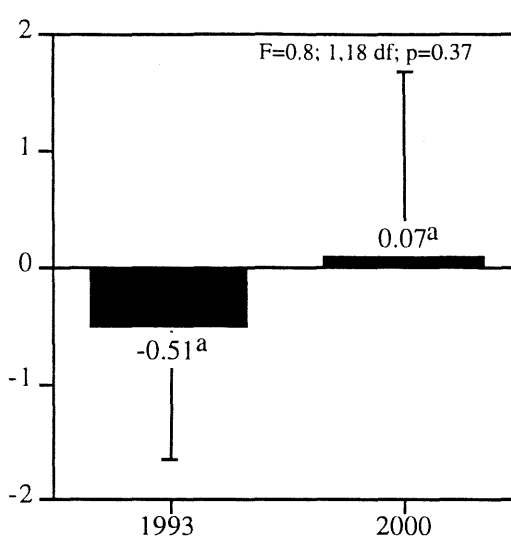
(b) Interstate State Park

SedimentSize(ϕ)



(c) Franconia

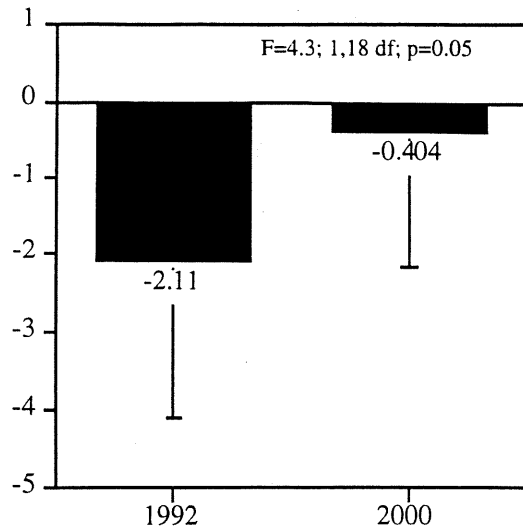
SedimentSize(ϕ)



(d) Osceola

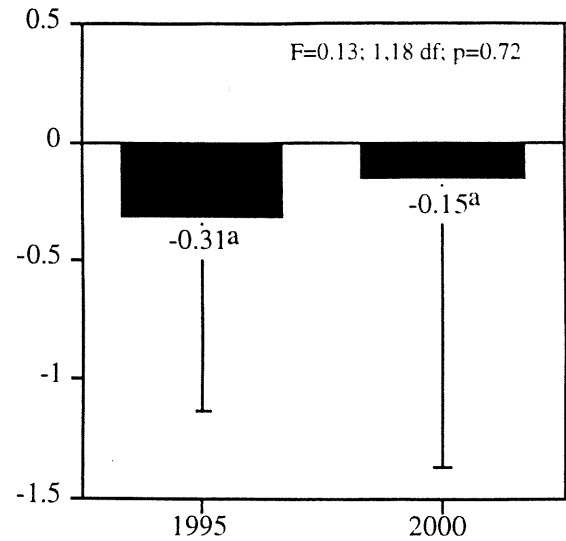
Figure 19. Substrate size at: (a) Wild River State Park, (b) Interstate State Park, (c) Franconia, and (d) Osceola.

SedimentSize(ϕ)



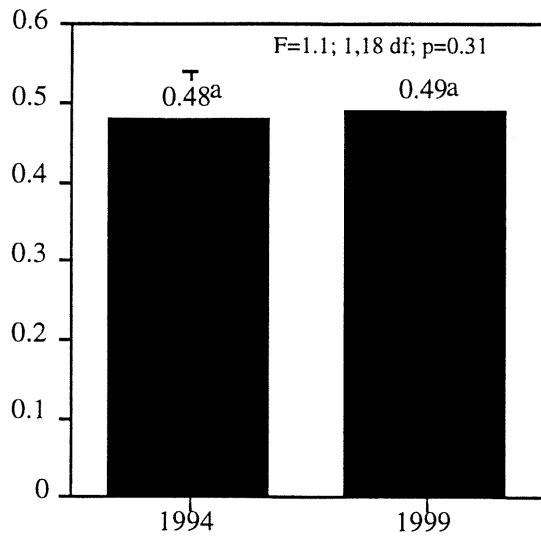
(e) Bayport

SedimentSize(ϕ)



(f) Lakeland

Sediment Size (ϕ)



(g) Prescott

Figure 19. Substrate size at: (e) Bayport, (f) Lakeland, and (g) Prescott.

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V. Timetable

- July 1999-Sept. 1999 - Sample Relocation Site, sample three population monitoring locations (Wild River State Park, Franconia, Prescott)
- Sept. 1999-Dec. 1999 - Analyze data, prepare interim report, present results at St. Croix River Rendezvous
- July 2000-Sept. 2000 - Sample Relocation Site, sample four population monitoring locations (Interstate Park, Osceola, Lakeland, Bayport)
- Sept. 2000-Dec. 2000 - Analyze data, prepare interim report, present results at St. Croix River Rendezvous
- August 2001 - Final report to LCMR

VI. Budget

	Total	Relocation activity	Population monitoring
Personnel			
Project Manager	\$20,400	\$5,100	\$15,300
Summer Students	\$18,100	\$3,960	\$14,140
Web page development	\$3,600	\$1,800	\$1,800
Equipment			
SCUBA equipment	\$3,400	\$1,000	\$2,400
Field equipment	\$3,000		\$3,000
Other			
Travel	\$3,500	\$750	\$2,750
Supplies	\$6,000	\$1,000	\$5,000
Acquisition			
Development			
Total	\$58,000	\$13,610	\$44,390

In addition Macalester College provided summer housing for students, and Dan Hornbach oversaw the project spending approximately 5% of his time on this grant. This support was provided by Macalester College.