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Ecological relationships between six rare Minnesota mussels and their host fishes

Abstract

Of 297 freshwater mussel species living in North America, 213 are either endangered, threatened, or of special concern. The identification of fish hosts is listed as an urgent research objective in the National Strategy for Freshwater Mussel Conservation. Suitable hosts were determined by artificially infesting various fishes and amphibians with glochidia from one of six mussel species. A fish was considered a suitable host when larval metamorphosis to the juvenile stage was observed. Although twenty-five fish species and mudpuppy were exposed to spectaclecase glochidia, none of the species tested facilitated glochidial metamorphosis. Three-fold shell growth was observed on pistolgrip juveniles collected from yellow and brown bullheads. Transformation of ellipse glochidia was observed in mottled sculpin, four darters, and brook stickleback. Metamorphosis of butterfly glochidia was not observed. Blackside darter and logperch were found to be suitable hosts for snuffbox. Purple wartyback glochidia transformed on four catfishes.

We used microscopy and initiated molecular techniques to identify a subsample of approximately 5000 juvenile mussels collected from freshwater drum naturally infested with glochidia. Light microscopes and a scanning electron microscope were used to study the juvenile mussels and glochidia from mussels whose length is less than 100 μm. Species identification was limited to subfamily using light microscopes. Analysis of shell surface sculpture, shell outline, and shell height from scanning electron micrographs suggest the subsample of juveniles are either *Truncilla truncata* or *T. donaciformis*. We verified the use of published molecular markers for identifying mussel species but specific markers for St. Croix River mussels that release glochidia under 100 μm in length are still under development. Studies were initiated to determine if some mussels produce chemical cues to attract host fishes. Improved understanding of glochidial host requirements and ecological relationships between mussels and their hosts will help managers determine the viability of imperiled mussel populations.

Introduction

The Freshwater Mollusk Conservation Society (formerly known as the National Native Mussel Conservation Committee) has identified the need to increase fundamental knowledge of basic freshwater mussel biology as a top priority in the National Strategy for Freshwater Mussel Conservation (Biggins *et al*. 1995). Identification of fish hosts is the highest priority item listed under the basic biology research goal. The host requirements of six rare mussels living in Minnesota, spectaclecase (*Cumberlandia monodonta*), pistolgrip (*Tritogonia verrucosa*), ellipse (*Venustaconcha ellipsiformis*), creek heelsplitter (*Lasmigona compressa*), snuffbox (*Epioblasma triquetra*), and purple wartyback (*Cyclonaias tuberculata*) are unknown or little understood. The spectaclecase, pistolgrip, and ellipse are three mussel species listed as threatened in Minnesota. Lack of information on mussel host requirements makes it nearly impossible to determine the viability of imperiled mussel populations either in degraded habitats, where they now occur, or in habitats being considered for translocation of mussels. To enable managers to more effectively manage and conserve our mussel fauna, we determined which fish species they need in order to complete their life cycle.

The purpose of this study was to determine the fish hosts for the larvae (glochidia) of six rare freshwater mussels that live in Minnesota. Towards this end, we established two objectives:

1) to identify those fish species which facilitate glochidia metamorphosis, using laboratory application of artificial infestation procedures; and
2) to identify the species of selected juvenile mussels collected from naturally infested fish from the St. Croix River, using morphometric measurements and molecular genetic markers.

Determination of the 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. Transformation studies involve artificially infesting fish with glochidia and observing if transformation occurs (Yokley 1981, Neves *et al*. 1985). 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).

Laboratory observations of gravid pistolgrip and purple wartyback during this project prompted us to initiate a third research objective. Brooding pistolgrip and purple wartyback displayed previously undescribed mantles. Their bulbous mantles do not appear to resemble fish food items (*e.g.* small fish or aquatic insects) as has been described in other mussels. Pistolgrip and purple wartyback glochidia appear to

exclusively use catfishes as hosts and we observed that the mantle display of brooding pistolgrip was more inflated at night; the period of time when catfish most actively feed. These behaviors prompted us to pursue the previously unexplored possibility of mussels using chemical cues to attract hosts. We believe that naiades may have evolved to produce chemical attractants simply because chemical cues are generally the primary means by which fish find food items in turbid freshwaters from a distance. Further, chemicals are readily distributed in the water and are detected with great sensitivity and specificity by fishes.

Methods

Determine Suitable Hosts

Suitable fish hosts were determined using a standard protocol similar to that described in Neves *et al.* (1985). Fish for artificial infestation trials were collected with a seine, angling gear, and electrofishing equipment from streams and rivers outside of the St. Croix and Zumbro rivers. This precaution was taken to avoid testing fish that may have been previously exposed to the species of glochidia under investigation and subsequently developed an immunity to subsequent exposures (Reuling 1919). Test fish were held in holding tanks (40 L or 400 L) at least 20 d prior to glochidia infestation, at temperatures between 18-23 °C.

Gravid female mussels were collected from two Minnesota rivers, held briefly in the laboratory, and returned to the collection site. Spectaclecase, purple wartyback, butterfly, snuffbox, and pistolgrip were collected from the St. Croix River at Interstate State Park, Minnesota. Gravid female ellipse were collected from the North Fork Zumbro River, near Zumbrota.

Gravid mussels were held in beakers in aquaria until they released glochidia naturally. 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.

Fish were infested with glochidia by placing them in a 1-2 L bath with several hundred to several thousand glochidia under vigorous aeration. Fish were exposed to glochidia for 5 minutes to 3 hours, depending on susceptibility of the species to infestation. The state of infestation was assessed 30, 60, and 180 seconds, and 5, 10, 30, 60, 120, and 180 minutes after initial exposure to glochidia. When a treated fish had at least 10-20 glochidia on its gills, it was transferred to a clean aquarium.

Infested fish were held in aquaria at the University of Minnesota Wet Laboratory. Water temperature was held between 18°C ± 2 °C and most fish were fed frozen brine shrimp (*Artemia* sp.) 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. Glochidia infestation levels were also checked three time a week. Infested fish were 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 were no longer attached to the fish. Each search for juveniles was terminated when glochidia were no longer observed attached to the test subjects' gills. A mussel was considered a juvenile when foot movement or valve closure was 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

Pistolgrip and spectaclecase are short-term brooders, releasing 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 State Park, Minnesota during late June and early July, 1996-98. 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 visualized with light and scanning electron microscopes was based on morphologic characters described in Surber (1915), Waller (1987), and Hoggarth (1988).

We measured morphological characters to preliminarily identify juvenile mussels collected from naturally infested fish. In the early spring many species of freshwater mussels release glochidia into the St. Croix River. However, only five species (*Truncilla donaciformis*, *T. truncata*, *Leptodea fragilis*, *C. monodonta*, and *Tritogonia verrucosa*) release glochidia with length or width dimensions less than 100 microns (Surber 1913, Oesch 1984, Hoggarth 1988). We used dissecting and compound light microscopes, and a scanning electron microscope to visualize the glochidia of the five mussel species and the juvenile mussels. Only juvenile mussels with glochidial valves less than 100 microns were analyzed in this study.

Methods for identification and application of species-specific DNA markers followed those described in White (1994). This technique involved 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, species-specific combinations of DNA-cutting restriction enzymes sites are diagnostic for each mussel species of interest (White *et al*.

1994). Identification of juvenile mussels from naturally infested fish is possible based on the restriction sites (or RFLP patterns) found in their DNA.

To create a diagnostic suite of restriction sites, we conducted preliminary tests using DNA isolated from *C. monodonta* and *Tritogonia verrucosa* glochidia and tissue samples from *Truncilla truncata*, *T. donaciformis*, and *Leptodea fragilis*. 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 is inadvertently amplified by PCR will not be 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 (Figure 1).

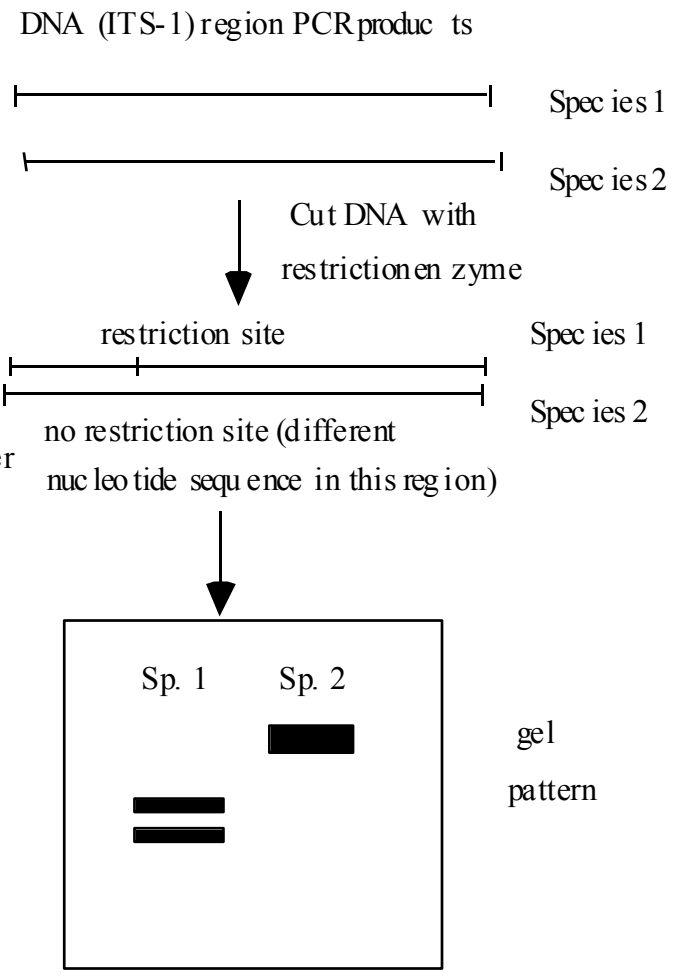

Figure 1. Schematic diagram of the process we used to determine diagnostic restriction enzymes. In this example, the diagnostic enzyme recognized and cut the DNA at its unique cut site in the DNA of Species 1 but did not find a cut site in Species 2. This created unique DNA banding patterns for both species. Another enzyme may cut DNA of both species in the same location therefore creating the same banding pattern. Such an enzyme would not be diagnostic.

Determine if Chemical Cues are Used by Gravid Mussels to Attract Hosts

To determine if catfish are attracted to mussel odor we observed catfish under the following conditions: (1) in the presence of brooding pistolgrip, (2) in the presence of a purple wartyback conglutinate, and (3) while exposed to the water from a container holding brooding purple wartyback or pistolgrip. In the first experiment, year-old channel catfish were placed in a 10-gallon aquarium that contained both brooding and non-brooding pistolgrip. After being permitted to acclimate for 15 min., the fishes' behavior was observed. The design of the second experiment was the same as the first, except that a purple wartyback conglutinate was substituted for the brooding pistolgrip. The third experiment involved six trials. Five different channel catfish and one yellow bullhead were placed in a 75-gallon flow-through tank with painted floor lines to form five quadrants. After a 24 hour acclimation period 200 ml of water from an aquarium holding brooding purple wartyback or pistolgrip was introduced to one end of the tank through a plastic tube. The number of times a fish crossed a quadrant line and its orientation to the added water was recorded.

Results & Discussion

Determination of Suitable Hosts

Host suitability tests were conducted on a variety of candidate species using spectaclecase, pistolgrip, ellipse, butterfly, snuffbox, and purple wartyback glochidia. None of the thirty-three fish species or mudpuppies exposed to spectaclecase glochidia facilitated glochidia metamorphosis (Table 1). Of thirty fish species tested only yellow and brown bullheads facilitated pistolgrip glochidia metamorphosis (Tables 1 and 2). Although no juveniles were collected from black bullheads and creek chubs, growth was observed in pistolgrip glochidia collected from these fishes. Metamophosis of ellipse glochidia was observed on six of sixteen fish species tested (Tables 1 and 2). Butterfly glochidia did not transform on any of the fish species tested (Table 1). Snuffbox glochidia were exposed to four fish species. Blackside darters and logperch served as hosts (Tables 1 and 2). Four of seven Ictalurids were found to be suitable hosts for purple wartyback glochidia (Tables 1 and 2).

Premature deaths of test fish probably lowered the number of suitable hosts that would have otherwise been identified. We believe ellipse glochidia likely transform on slimy sculpins. However, due to the early demise of the test fish, transformation was not observed on slimy sculpin. Early death of test fish may have prevented our observation of metamorphosis *T. verrucosa* glochidia on flathead catfish. Additional host suitability studies should be conducted on these species to clarify host relationships.

We identified several previously unknown suitable hosts for four mussel species. Prior to this study, the hosts for *Tritogonia verrucosa* were unknown (Watters 1994). We found yellow and brown bullheads serve as suitable hosts, and recently flathead catfish were found to be suitable hosts (Howells 1996). Hosts for the ellipse were also unknown (Watters 1994). We have identified six stream-dwelling fishes that serve as suitable hosts for ellipse glochidia, although additional host suitability studies are needed to test other

fishes not included in this study. Extensive tests suggested that logperch were the sole host for snuffbox glochidia (Sherman 1995). Tests conducted in our laboratory have verified the host status of logperch and revealed that blackside darter is a marginal host for this species. Additional studies are needed to determine if other darters serve as marginal hosts for the snuffbox. We have shown that purple wartybacks use yellow bullheads and channel catfish as hosts (Hove 1992) and in this study we learned that flathead catfish and black bullheads are also suitable hosts.

Determination of Natural Glochidial Infestation of Fish

Nearly four hundred fish (fourteen species) were collected from Interstate State Park, Minnesota and transported to the University of Minnesota. Juvenile mussels were collected from eleven fish species (Table 3). No juvenile mussels were collected from smallmouth bass, white suckers, Johnny darters, or a mudpuppy (Table 4). Some of the juvenile mussels collected in 1997 were identified to subfamily using dissecting and compound light microscopes. Anodontine juveniles were collected from mimic and emerald shiners and Moxostomids. Amblemine juveniles were recovered from logperch. Juvenile mussels collected from freshwater drum include: pink heelsplitter (*Potamilus alatus*), Lampsilines, and Amblemines. On several occasions all three kinds of juveniles were collected from individual drum. Some of these juveniles had very small glochidial valves. Observations made in 1997 using a compound microscope suggested the juveniles were spectaclecase. However, scanning electron micrographs taken of juveniles from freshwater drum in 1998 and the five mussel species that produce glochidia less than 100 µm long show the juveniles are not spectaclecase. Glochidial valves are evident on the umbo of freshly excysted juvenile mussels (Figure 2). Examination of scanning electron micrographs of small glochidia show that the juveniles are probably either deertoe (*Truncilla truncata*) or fawnsfoot (*T. donaciformis*) (Figure 3). The outline of spectaclecase glochidia is more round than the glochidial shell of the juvenile, and the surface sculpture of fragile papershell (*Leptodea fragilis*) and pistolgrip (*Tritogonia verrucosa*) is much rougher than either the deertoe or fawnsfoot. Also, pistolgrip and fragile papershell glochidial shells are 10-20 µm larger than the glochidial shells of the juveniles.

Freshwater drum may be a good species for changing peoples opinion of non-sport fishes. Freshwater drum are hosts for numerous mussel species (Watters 1994). However, we observed for the first time that individual fish facilitate metamorphosis of multiple mussel species at one time. In fact, hundreds of juvenile mussels excysted from some drum. *Truncilla truncata*, *Leptodea fragilis*, and *Potamilus alatus* are common mussels in the St. Croix River at Interstate State Park and their glochidia metamorphose on freshwater drum. Although freshwater drum are considered by some to be unimportant trash fish, they probably help maintain healthy mussel populations, and may influence species composition of mussel communities. Educating people about the role drum play in aquatic ecosystems may bring about greater respect for this species. Perhaps it is time to discard the idea of “rough fish” and re-emphasize the importance each species has in our ecosystem.

Table 1. 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 1. 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 1. Trials where glochidial metamorphosis was not observed. (Continued.)

Common name	Number of individuals inoculated	Number of survivors	Glochidia attachment period (days)
ellipse			
bluntnose minnow I	8	8	2-5
bluntnose minnow II	8	8	1-4
emerald shiner	6	5	1-4
goldfish	7	7	1-4
channel catfish	6	0	*
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	1	0	*
green sunfish	8	8	17-20
pumpkinseed	8	6	13-15
logperch I	3	2	8-11
logperch II	8	8	8-18
river darter	1	1	23-25
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.
** - Only barbels were infested with glochidia.

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
ellipse			
brook stickleback	8/3	18-35	74
Iowa darter	8/8	18-30	41
fantail darter	8/6	18-35	56
blackside darter I	5/3	51-53	1
blackside darter II	4/4	30-34	7
blackside darter III	7/7	18-21	3
logperch	6/0	25-32	3*
mottled sculpin	4/0	19-36	64*
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

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

Table 3. St. Croix River fishes that facilitated glochidia metamorphosis.

Common name	Number of fish collected	Number of juveniles recovered	Mussel subfamily or species
1996			
spotfin shiner	40	4	Unknown
yellow perch	6	1	Unknown
logperch	3	16	Unknown
1997			
emerald shiner	25	*	Anodontinae
mimic shiner	70	*	Anodontinae
redhorse	5	*	Anodontinae
logperch	5	*	Ambleminae
freshwater drum	6	*	*Potamilus alatus* , & 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	*Potamilus alatus* , & other species

* - Not recorded.

Table 4. St. Croix River fishes and amphibians that did not facilitate glochidia metamorphosis.

Common name	Approximate Number collected	Number of individuals less than 1 yr old
1996		
emerald shiner	10	0
mimic shiner	150	0
smallmouth bass	6	6
western sand darter	3	0
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

Methods for molecular marker identification were customized for use in our laboratory. Methods in White *et al*. (1994) were optimized for our laboratory and results published in White *et al*. (1994) were reproduced. However, none of the species-specific molecular markers identified in White *et al*. (1994) include species under investigation in this study. Species-specific molecular markers for mussels that release glochidia less than 100 µm high are still under development. Once markers have been identified we plan to use them to identify juvenile mussels collected during this project.

Determine if Chemical Cues are Used by Gravid Mussels to Attract Hosts

In the first experiment, year-old channel catfish were placed in a 10-gallon aquarium that contained both brooding and non-brooding pistolgrip. On two occasions a fish was seen to brush against the mantle of a displaying pistolgrip but this did not elicit feeding behavior (vigorous snapping and nosing against object). Although the fish did not appear attracted to the inflated pistolgrip mantle, one fish did nose and nudge the excurrent siphon of a gravid, non-displaying pistolgrip. Although inconclusive, these results are suggestive of a role of chemical cues, leading us to the second experiment.

The second experiment using a purple wartyback conglutinate also produced suggestive but as yet inconclusive results. Feeding behavior was not observed during two trials where young, hatchery-reared channel catfish were placed in a 10-gallon aquarium with a petri dish holding the conglutinate. During a third trial however, a conglutinate was placed in a 40-gallon aquarium with three 3-5 year old, wild-caught channel catfish. One of the fish briefly inhaled and expelled the conglutinate.

Tests are currently underway to address our initial suggestive findings that mussel odor might be important to host attraction. Six trials with different channel catfish and one yellow bullhead were conducted to determine their response to water from aquaria holding brooding mussels. During the first four trials fish moved very little. However, in the last two trials, we reduced test chamber light level and a yellow bullhead began exhibiting searching behavior approximately five minutes after water was added to the tank. The fish repeatedly approached the water inlet tube, rubbed against it, and bit it. After 1-3 minutes, it returned to searching behavior, eventually investigating the tube again. These behaviors lasted 5-10 minutes, after which time the bullhead resumed resting.

We plan to continue testing mussel odor to see if we can repeat the last finding. In addition, we hope to expand this study to observe the reaction of different Ictalurids to gravid purple wartyback and pistolgrip, and compare the behavior of wild-caught and hatchery-reared fishes.

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Authorization for and Scope of Work Performed

This work was performed under authorization from the Natural Heritage and Nongame Research Program, Minnesota Department of Natural Resources.

Accomplishments are summarized below:

1. We conducted host suitability studies on six species of rare freshwater mussels including: spectaclecase, pistolgrip, ellipse, snuffbox, butterfly, and purple wartyback. Previously unknown hosts were identified for each species except spectaclecase and butterfly.

2. We collected approximately 5000 juvenile mussels from naturally infested fish from the St. Croix River and identified a subsample of them to subfamily, genus, and

species. Methods for identification of published markers were customized for our use and confirmed in our laboratory.

3. We coordinated efforts between the University of Minnesota, Virginia Polytechnic Institute and State University, and Southwest Missouri State University in 1997 to determine suitable host requirements of spectaclecase.

4. We presented results from this project at several meetings, in four media interviews, and in nine articles. Study results were presented at the following meetings: (1) 1996 Annual Meeting of the American Malacological Union, Chicago, Illinois, (2) 1996 Annual St. Croix River Research Rendezvous, Marine on St. Croix, Minnesota, (3) three undergraduate courses at the University of Minnesota, (4) 1997 Annual Meeting of the Mississippi River Research Consortium, La Crosse, WI, 1997, (5) two laboratory tours for upper midwest threatened and endangered species managers, (6) 1998 Annual Meeting of the Minnesota Chapter of the American Fisheries Society, Camp Ripley, Minnesota, (7) Freshwater Mussel Symposium: Conservation, Captive Care, and Propagation, Columbus, Ohio, and (8) 1998 World Congress of Malacology, Washington, D.C.

Mark Hove was interviewed by the following organizations: (1) Venture North, public television, (2) twice by University of Minnesota's newspaper, The Daily, (3) University of Minnesota, College of Natural Resources newsletter, and (4) Media Rare, a radio and television studio that produces radio and cable television programming highlighting natural resource activities in Minnesota.

Publications from this study include: (1) an article entitled "Suitable fish hosts for glochidia of four freshwater mussels" published in the Proceedings of the UMRRC Symposium on Conservation and Management of Freshwater Mussels.', (2) nine articles in the USFWS's Triannual Unionid Report, and (3) an article entitled "Considerations for conducting host suitability studies" to be published in the American Malacological Bulletin from a meeting entitled Freshwater Mussel Symposium: Conservation, Captive Care, and Propagation. Copies of abstracts and published articles accompany this report.

5. We expanded this study through additional support from the following institutions: (1) the University of Minnesota, Undergraduate Research Opportunities Program provided $8,500 for seven university undergraduate students to assist with field and laboratory studies, (2) the Mentor Connection program provided us with an intelligent and highly motivated high school student for the 1996-97 academic year, (3) the University of Minnesota, College of Biological Sciences, Summer Science Research Program provided $2500 to support an undergraduate student during the summer of 1998, (4) the Legislative Commission on Minnesota Resources provided $39,523 for continued investigation of rare mussel host requirements, and (5) federal aid under Section 6 of the Endangered Species Act of 1973 with matching funds from the Wisconsin Department of Natural Resources (WI DNR) for WI DNR were used to support collection of gravid mussels.

Appendix 1. Scientific names of fishes and amphibians used in this study. Nomenclature follows Robins *et al*. (1981) and Oldfield and Moriarty (1994) for fish and amphibians, respectively.

Common name	Scientific name
banded killifish	*Fundulus diaphanus*
black bullhead	*Ameiurus melas*
black crappie	*Pomoxis nigromaculatus*
blackside darter	*Percina maculata*
bluegill	*Lepomis macrochirus*
bluntnose minnow	*Pimephales notatus*
bowfin	*Amia calva*
brook stickleback	*Culaea inconstans*
brown bullhead	*Ameiurus nebulosus*
burbot	*Lota lota*
carp	*Cyprinus carpio*
central mudminnow	*Umbra limi*
channel catfish	*Ictalurus punctatus*
chestnut lamprey	*Ichthyomyzon casteneus*
common shiner	*Luxilus cornutus*
creek chub	*Semotilus atromaculatus*
emerald shiner	*Notropis atherinoides*
fantail darter	*Etheostoma flabellare*
fathead minnow	*Pimephales promelas*
flathead catfish	*Pylodictus olivaris*
freshwater drum	*Aplodinotus grunniens*
goldfish	*Carassius auratus*
green sunfish	*Lepomis cyanellus*
Iowa darter	*Etheostoma exile*
Johnny darter	*Etheostoma nigrum*
largemouth bass	*Micropterus salmoides*
logperch	*Percina caprodes*
longnose dace	*Rhinichthys cataractae*
mimic shiner	*Notropis volucellus*
mottled sculpin	*Cottus bairdi*
northern pike	*Esox lucius*
northern redbelly dace	*Phoxinus eox*
pumpkinseed	*Lepomis gibbosus*
quillback	*Carpiodes cyprinus*
redhorse	*Moxostoma* sp.
rock bass	*Ambloplites rupestris*
river darter	*Percina shumardi*
slimy sculpin	*Cottus cognatus*
smallmouth bass	*Micropterus dolomieui*
spotfin shiner	*Cyprinella spiloptera*
stonecat	*Noturus flavus*
tadpole madtom	*Noturus gyrinus*
trout-perch	*Percopsis omiscomaycus*
walleye	*Stizostedion vitreum*
white sucker	*Catostomus commersoni*
yellow bullhead	*Amieurus natalis*
yellow perch	*Perca flavescens*
western sand darter	*Ammocrypta clara*
mudpuppy	*Necturus maculosus*
tiger salamander	*Ambystoma tigrinum*