Host fish requirements of some upper Mississippi River mussel species

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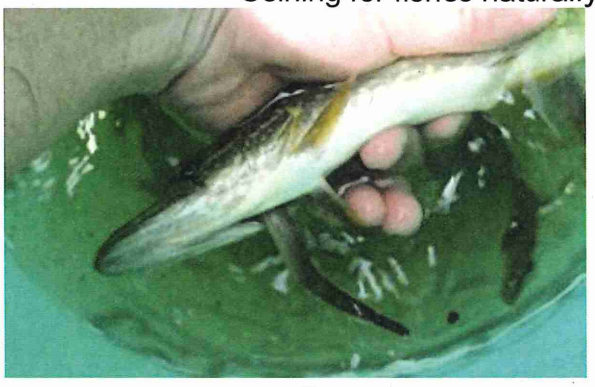
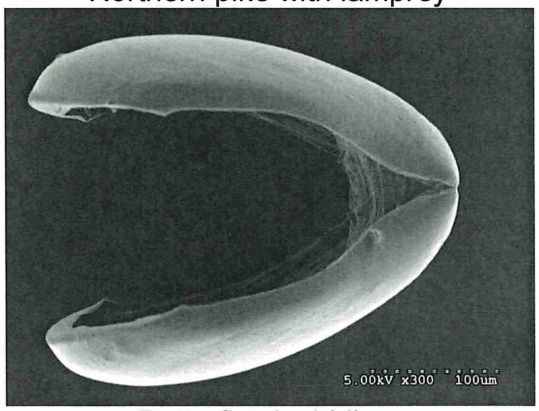
Seining for fishes naturally infested with glochidia

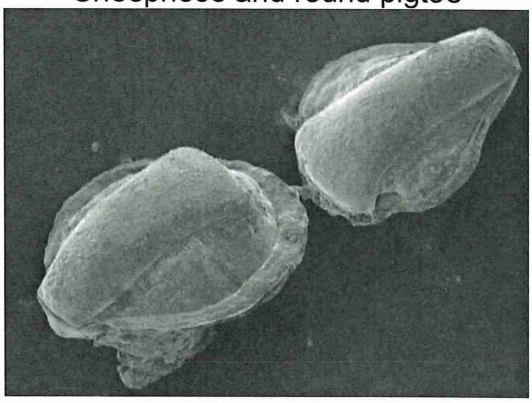


Northern pike with lamprey

Sheepnose and round pigtoe



Butterfly glochidium



Juvenile pink heelsplitters

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Abstract

The National Native Mussel Conservation Committee recommended increasing the understanding of basic knowledge of mussel biology as a top priority. Most freshwater mussel larvae (glochidia) attach to a host in order to metamorphose into juveniles. We conducted a variety of studies to increase knowledge of the life history requirements of some rare and common Mississippi River mussel species. The sheepnose will be listed as Federally Endangered on April 19th, 2012. We studied the brooding period, and suitable and natural hosts for this species. We also conducted laboratory host suitability studies for the following Minnesota mussel species: butterfly, deertoe, hickorynut, fragile papershell, pink heelsplitter, round pigtoe, threehorn wartyback, and wartyback. Sheepnose brood glochidia between late May and mid-August, their glochidia will metamorphose on several minnow species and banded killifish in the laboratory, and naturally infest and transform on mimic shiners. We determined that round pigtoe will metamorphose on several minnow species and mosquito fish, and that lake and shovelnose sturgeons, central mudminnow, goldeye, and mosquito fish are suitable hosts for hickorynut glochidia. We observed five catfish species facilitate metamorphosis of wartyback glochidia in the laboratory but neither of two fish species we exposed to threehorn wartyback glochidia facilitated metamorphosis. Our previous work has shown that butterfly, deertoe, fragile papershell, and pink papershell are likely host specialists utilizing freshwater drum. Although we exposed many fish species to glochidia of these four mussel species the only non-drum species that facilitated glochidia metamorphosis was the brook stickleback for the butterfly. Several previously unknown suitable hosts were identified in this study. This information can be used to improve juvenile mussel propagation programs and help preserve extant mussel populations by ensuring glochidia hosts co-occur with them.

Introduction

According to a study conducted by the Nature Conservancy, freshwater mussels are more imperiled than any other U.S. floral or faunal group (Master *et al.*, 1998). Biologists concerned with conservation of rare freshwater mussels have identified the need to increase fundamental knowledge of basic freshwater mussel biology as a top priority in the national strategy for freshwater mussel conservation (The National Native Mussel Conservation Committee 1998). Most freshwater mussel larvae (glochidia) must attach to a host in order to metamorphose into juveniles. Identification of hosts is the highest priority item listed under the national strategy basic biology research goal.

There have been many glochidia host studies for many mussels living in the upper Mississippi River watershed but most analyses are incomplete (Watters 1994). Some of these species living in Minnesota include: butterfly (*Ellipsaria lineolata*), deertoe (*Truncilla truncata*), fragile papershell (*Leptodea fragilis*), hickorynut (*Obovaria olivaria*), pink heelsplitter (*Potamilus alatus*), round pigtoe (*Pleurobema sintoxia*), sheepnose (*Plethobasus cyphyus*), threehorn wartyback (*Obliquaria reflexa*), and wartyback (*Quadrula nodulata*). Identification of a suitable host species that facilitate glochidia metamorphosis is essential in initiating most juvenile mussel propagation efforts. Limited studies on deertoe, fragile papershell, and pink heelsplitter show that freshwater drum is a suitable glochidial host or are naturally infested with glochidia of these species (Howard 1913, Wilson 1916, Coker *et al.*, 1921, Weiss and Layzer 1995, Brady *et al.*, 2004). The few studies that have been conducted on hickorynut show that shovelnose sturgeon has been naturally infested with this mussel (Howard 1914, Coker *et al.*, 1921) and limited host suitability trials show that lake sturgeon support glochidia metamorphosis (Brady *et al.*, 2004). Earlier studies showed that channel catfish, white crappie, and flathead catfish are naturally infested with wartyback (Surber 1913, Wilson 1916, Coker *et al.*, 1921). Recent studies show that some minnows, topminnows and a non-native livebearer will facilitate metamorphosis of sheepnose glochidia (Watters *et al.*, 2005, Guenther *et al.*, 2009). Various minnow species and bluegill have been shown to be suitable hosts for round pigtoe in the laboratory (Hove 1995, Hove *et al.*, 1997, Watters *et al.*, 2005) and bluegill are naturally infested with this species (Surber 1913, Coker *et al.*, 1921). Silverjaw minnow, common shiner, and longnose dace are potential hosts for threehorn wartyback (Watters *et al.*, 1998a, Watters *et al.*, 1998b). Lack of accurate glochidia host fish information 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 reintroduction of locally extirpated species following an improvement in conditions or translocation of mussels to rescue them from spread of zebra mussels or from other adverse effects. To improve mussel management we had three research objectives: 1) determine the sheepnose brooding period, 2) determine fish species that facilitate glochidia metamorphosis of butterfly, deertoe, hickorynut, fragile papershell, pink heelsplitter, round pigtoe, sheepnose, threehorn wartyback, and wartyback glochidia, and 3) identify juvenile mussels recovered from naturally infested Chippewa River fishes in hopes of identifying natural sheepnose hosts.

Methods

Sheepnose brooding behavior

We used snorkeling gear and SCUBA to study sheepnose brooding behavior in the Chippewa River, WI during the spring and summer of 2011. We visited a sheepnose population in Rusk County bimonthly between April and August 2011. A visual cue we used to find sheepnose was the branched papillae around the incurrent aperture. We measured water temperatures with a iBCod datalogger. We attempted to collect 10-20 sheepnose during each visit, briefly peered between the valves to determine gill inflatedness and color, and returned mussels to the collection site. If mussels had gills inflated at least three times normal thickness we considered them gravid. Glochidia with fully formed valves and the soft tissue between the valves comprised <1/3 the volume between the valves they were considered mature. Age was estimated by counting rings on the periostracum. We conducted statistical analyses using JMP v. 3.2 statistical software (SAS Institute, Cary, NC).

Suitable Glochidia Host(s)

We conducted glochidia host suitability trials using standard methods (Zale and Neves 1982, Hove *et al.,* 2000). Most gravid mussels were collected from the St. Croix River, sheepnose were collected from the Chippewa River at Meridean, WI, and wartyback from the Mississippi River near Hastings, MN. Fish for most artificial infestation trials were collected using a seine, trap net, angling or electrofishing equipment from streams and rivers outside of the St. Croix River drainage. This precaution was taken to avoid testing fish that may have been previously exposed to the species of glochidia under investigation and thereby developing immunity to subsequent exposures (Reuling 1919). Several host suitability trials were conducted at the University of Minnesota Wet Laboratory, and at the MN DNR Wet Laboratory in the MPCA building in St. Paul. Test subjects were held in holding tanks (40 L or 400 L) at least 14 d prior to glochidia infestation, at temperatures between 14-23 °C. Gravid female mussels were held in beakers in aquaria until they released glochidia naturally or glochidia were extracted by puncturing and flushing the marsupia with a water filled syringe. To determine glochidia health we exposed a subsample to a 0.1-1% NaCl solution. If ≥ 70% of the glochidia closed their valves upon exposure to salt, the rest of the glochidia were used for host tests. After completion of experiments, we returned female mussels to where they were collected. Fish and adult mussel identifications were based on Becker (1983) and Sietman (2003), respectively. Fish and mussel nomenclature follows Turgeon *et al.*, (1998) and Robins *et al.*, (1991), respectively.

Host suitability trials were conducted during 2011. Fishes were infested with glochidia by placing fishes in a 1-7 L bath with several hundred to several thousand glochidia under vigorous aeration for a few minutes to hours depending on species' susceptibility to infestation. We inoculated fish with a combination of up to 3 mussel species whose glochidia were easy to distinguish using a dissecting microscope. We did not combine *Leptodea fragilis* or *Truncilla truncata* glochidia on any fish species because they cannot be readily distinguished with a dissecting microscope. The state of infestation was monitored regularly to ensure over-infestation did not occur. Once treated fish were infested with at least 10-20 glochidia on their gills they were transferred to clean aquaria. Infested fish were held in aquaria at 20°C ± 3 °C and fed at least three times a week. Fathead minnows (*Pimephales*

promelas) were given to piscivorous fish at least 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 generally siphoned and siphonate checked for presence of glochidia and juveniles three times a week. A given search for juveniles was terminated after three consecutive searches failed to reveal a glochidium or juvenile mussel. At this termination point, each fish was anesthetized and searched for attached glochidia using a dissecting microscope. If we found a glochidium, the fish was revived and the experiment continued until we no longer observed glochidia attached to the fish. A mussel was considered a juvenile when we observe foot movement or valve closure among those individuals collected ≥ 10 d after infestation. A fish was considered a suitable host if we observed glochidia encystment and metamorphosis to the juvenile stage.

Natural glochidia infestations

We used glochidia from Chippewa River mussel species that were morphologically similar to sheepnose to identify juvenile mussels recovered from naturally infested fishes collected from the Chippewa River near Meridean, WI. Between 2009-2011 we collected fishes from the study site, identified individuals naturally infested with glochidia, and held them at the Grantsburg High School biology laboratory. We searched holding aquaria for juvenile mussels using procedures described above. With scanning electron microscopy we used standard methods to photograph, measure, and describe characteristics of glochidia from known mussel species and juvenile mussels recovered from naturally infested fishes (Hove *et al.* 2011). We reviewed Balding (2002) to determine mussel species inhabiting the Chippewa River and used Hoggarth (1999) to describe glochidia morphology.

Results

Sheepnose brooding behavior

We observed several aspects of sheepnose brooding behavior. Sheepnose brooded glochidia between mid May-early August between water temperatures 15-29 °C (Figure 1). When we checked glochidia maturity in 2011 we observed developing glochidia between May 20-Aug 17 and mature glochidia (fully formed valves) between June 3-Aug 17. We observed sheepnose brooding glochidia in outer gills, where gill thickness varied 2-5 mm, and gill color varied from that of the surrounding tissue in nongravid individuals to red or pink, to light pink or light orange (73%) to cream or white (27%) in brooding animals. Gill thickness was not significantly different between females bearing mature versus immature glochidia (t = 0.74, df = 55, P=0.46). The swollen gills of gravid animals bearing mature glochidia exhibited a range of color from white to red where color did not change regularly within the brooding period. We did not observe any sheepnose with mantle displays (Barnhart *et al.*, 2008). Of 16 mussels collected in 2009 and recaptured in 2011 15 were gravid both years. Sheepnose collected in 2011 ranged in age between 4-30 yr and gravid individuals ranged between 5-26 yr. Sheepnose sometimes released immature glochidia in full conglutinates, although mature glochidia were released individually or in broken conglutinates in a clear gelatinous mass (Figure 2). Examining mussels for gravidity did not cause glochidia abortion for at least some mussels as we observed several mussels gravid twice during 2011.

Suitable Glochidia Host(s)

We conducted host suitability trials using butterfly, deertoe, hickorynut, fragile papershell, pink heelsplitter, round pigtoe, sheepnose, threehorn wartyback, and wartyback glochidia. We observed glochidial metamorphosis among most mussel species tested. Of 55 species exposed to butterfly glochidia only freshwater drum and brook stickleback facilitated metamorphosis (Tables 1 and 2). Similarly, freshwater drum was the only fish species that facilitated metamorphosis of fragile papershell, deertoe, and pink heelsplitter glochidia of 37, 18, and 37 fish species tested, respectively. Fifty-six fish species were exposed to hickorynut glochidia, only 5 species were found to be suitable hosts: shovelnose and lake sturgeons, central mudminnow, goldeye, and mosquito fish. Of 36 fish species exposed to round pigtoe 12 of 15 minnow species and mosquito fish facilitated metamorphosis. Sheepnose also metamorphosed on several minnow species as well as banded killifish. We exposed threehorn wartyback glochidia to lake sturgeon and freshwater drum but neither fish species facilitated metamorphosis. Wartyback glochidia were exposed to 16 fish species and all catfishes tested, black and yellow bullheads, and blue, channel and flathead catfishes, were suitable hosts

Natural infestations

We used conchological characteristics of glochidia from Chippewa River mussel species at Meridean, WI similar in size and shape to sheepnose to identify juvenile mussels recovered from naturally infested Chippewa River fishes. Glochidia morphometrics varied between mussel species. Sheepnose valve height was significantly different from other mussel species (Table 3) and sheepnose glochidia had a uniquely round, asymmetric valve outline (Figure 3). Additionally, sheepnose length and hinge length were significantly different than most other species, although not all. Threeridge and spike, and round pigtoe and Wabash pigtoe were similar in size and outline although Wabash pigtoe hinge line length was significantly greater than round pigtoe. Scanning electron micrographs showed that the glochidial valve outline and dimensions of glochidial valves of juvenile mussels from mimic shiners were sheepnose (n=7), Wabash pigtoe (n=8), and round pigtoe (n=1), and spotfin shiners released two Wabash pigtoe.

Discussion

Sheepnose brooding behavior

Like many other members of the Tribe Pleurobemini (Campbell *et al.*, 2005) living in the upper Mississippi River we found sheepnose brood glochidia during spring and summer. Upper Mississippi River Pleurobemini that brood glochidia in the spring or early summer include: *Elliptio* (Apr-Aug), *Fusconaia* (May-Aug), and *Pleurobema* (May-Aug) (Howard 1914, Coker *et al.* 1921, Heath *et al.*, 2001). Our observations of sheepnose brooding period (mid-May-early Aug) were similar to what has been reported by others, May-June in Ohio (Watters *et al.*, 2005) and May-July south of the upper Midwest (Gordon and Layzer 1989) and falls within the range of many Ambleminae genera. Knowledge of when sheepnose brood mature glochidia will assist biologists searching for glochidia for juvenile mussel propagation programs or for research.

Suitable Glochidia Host(s)

Our results broaden our understanding of suitable hosts for butterfly, deertoe, hickorynut, fragile papershell, pink heelsplitter, round pigtoe, sheepnose, threehorn wartyback, and wartyback glochidia. This confirms the likelihood that freshwater drum is the sole host for butterfly, deertoe, fragile papershell, and pink heelsplitter glochidia (Cummings and Watters, accessed February 2012). We did show that brook stickleback will serve as a suitable host for butterfly but this fish species lives more often in streams, ponds, and lakes rather than medium to large rivers where most of these mussel species occur (Becker 1983) and the number of transformed glochidia from brook stickleback was low. Our work confirmed that lake and shovelnose sturgeon is a suitable host for hickorynut (Coker *et al.*, 1921, Brady *et al.*, 2004), and showed for the first time that central mudminnow, goldeye, and mosquito fish also facilitate metamorphosis, however transformation was low for the non-sturgeon species, and their normal habitats do not overlap with hickorynut (central mudminnow and mosquitofish) or they are rare where they co-occur (goldeye) (Becker 1983). Round pigtoe are known to metamorphose on various minnow species and bluegill in the laboratory (Hove 1995, Hove *et al.*, 1997, Watters *et al.*, 2005), and bluegill are known to be naturally infested with this species (Surber 1913, Coker *et al.*, 1921). This study added several minnows (bigmouth shiner, blacktail shiner, bleeding shiner, common shiner, creek chub, eastern blacknose dace, golden shiner, Ozark minnow, spottail shiner, striped shiner, and whitetail shiner) and mosquito fish to the list of suitable round pigtoe hosts, but we did not confirm bluegill as a suitable host. Sheepnose glochidia will metamorphose on several minnow species, blackspotted topminnow, and mosquito fish (Watters *et al.*, 2005, Guenther *et al.*, 2009). Blackspotted topminnow and mosquito fish will metamorphose on several distantly related mussel species and have been regarded as universal hosts (Haag and Warren 1997). In this study we expanded the number of suitable minnow hosts and added banded killifish to the list of potential sheepnose hosts. Threehorn wartyback are known to metamorphose on various minnow species in the laboratory (Watters *et al.*, 1998a, Watters *et al.*, 1998b) but this species did not metamorphose on the two non-minnow fish species we tested. Finally, we observed wartyback metamorphose on black and yellow bullheads, blue, channel, and flathead catfishes, which is consistent with the observation that wartyback infest catfishes under natural conditions (Surber 1913, Wilson 1916, Coker *et al.*, 1921), and that members of the *Q. pustulosa* clade of quadrulines specialize on catfish hosts (Howard 1914, Haag and Warren 2003). These observations show that there is still much to learn about suitable and natural glochidia host relationships as there are many fish species that co-occur with these mussels but were not part of our study.

Natural infestations

We showed for the first time that mimic shiners facilitate metamorphosis of natural infestations of sheepnose and Wabash pigtoe and that spotfin shiners are natural hosts for Wabash pigtoe. A few studies support our identifications mimic shiners as natural sheepnose hosts, and mimic and spotfin shiners as natural Wabash pigtoe hosts. In previous years we have twice observed mimic shiners support sheepnose metamorphosis in the laboratory. Our collection of Wabash pigtoe from a mimic shiner (*Notropis volucellus*) is consistent with the observation of another *Notropis* species, silver shiners (*N. photogenis*), serving as suitable hosts for this mussel (Watters and O'Dee 2000). Additional research is needed to determine if suitable hosts identified in this study are also hosts under natural conditions.

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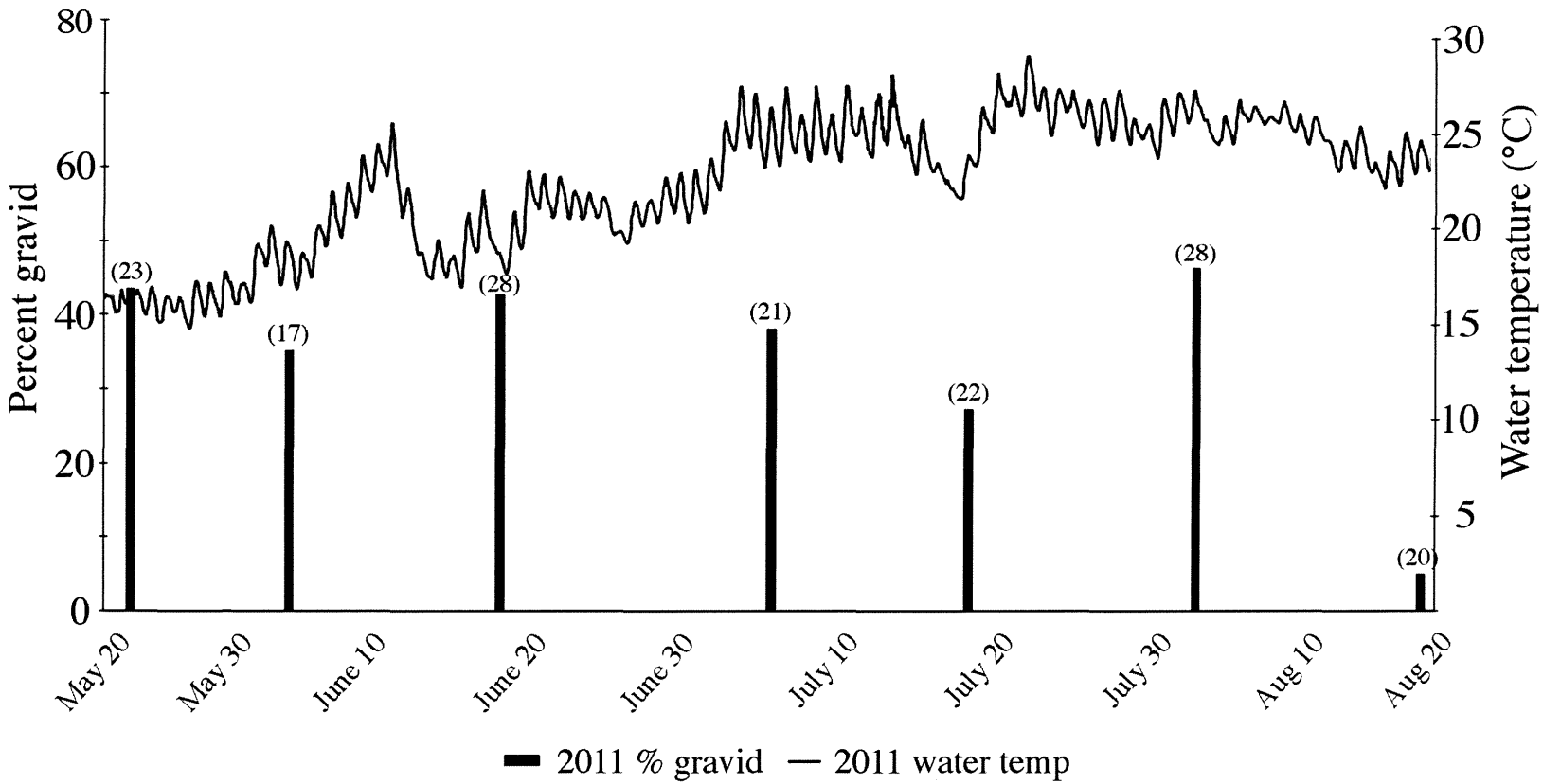

Figure 1. 2011 sheepnose gravidity period. Numbers in parentheses describe the number of sheepnose checked for gravidity.

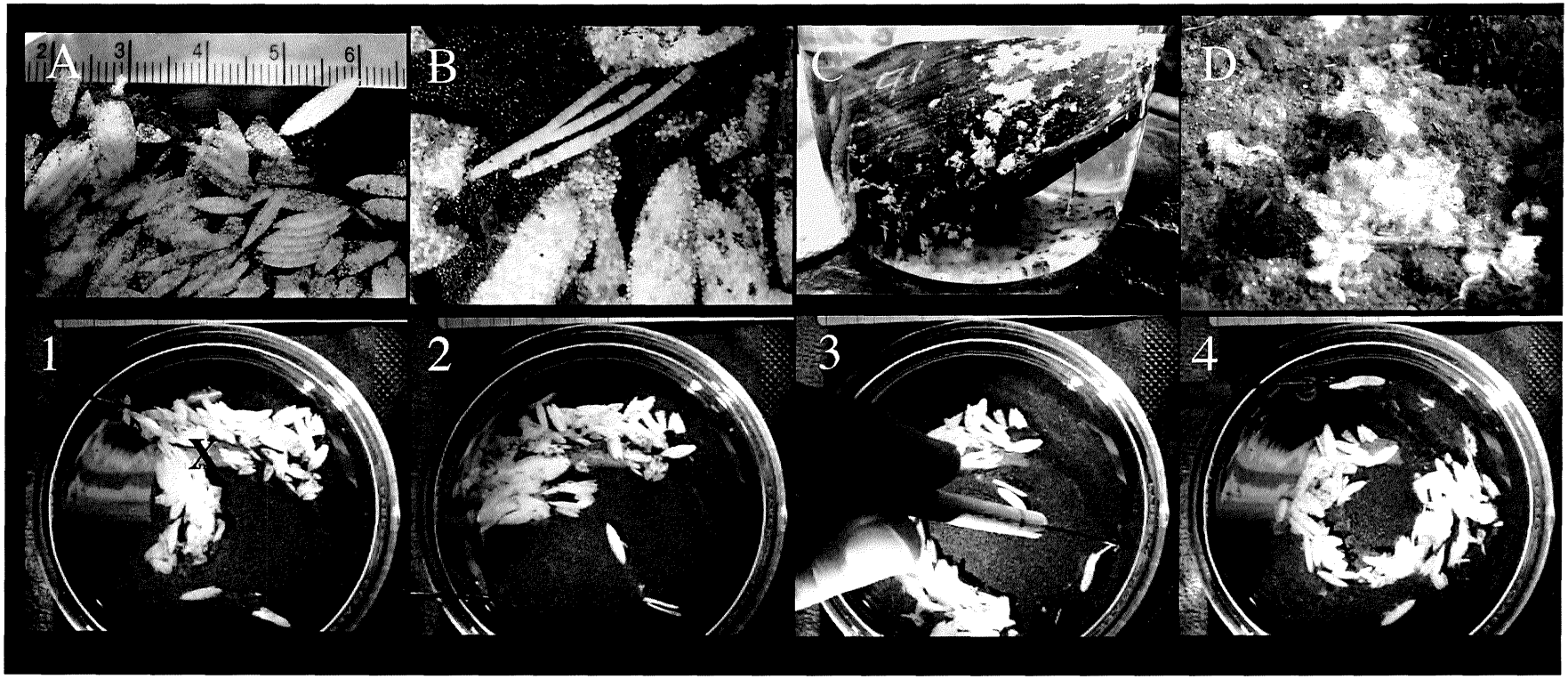

Figure 2. Sheepnose released glochidia in various states of maturity: (A) immature glochidia in conglutinates (scale bar-1 cm numbered increments), (B) conglutinates with immature glochidia were solid or bifurcate, (C) mature glochidia released in the laboratory were released individually (covering beaker floor) or in broken conglutinates in a clear matrix, (D) conglutinate matrix on bed of Chippewa River, and (1-4) adhesive properties of clear conglutinate matrix illustrated with a photo sequence showing dissecting needle starting at "X" moving counter-clockwise around perimeter of Petri dish.

Table 1. Fishes that facilitated glochidia metamorphosis.

Common name (Scientific name)	Trial	No. of individuals inoculated	No. of survivors	Juvenile recovery period (days)	No. of juveniles recovered
butterfly (*Ellipsaria lineolata*)					
freshwater drum	1	1	1	17-49	55
	2	3	0	15-[a]	166
	3	6	4	15-29	1072
brook stickleback	1	10	5[b]	24-27	3
	2	10	6[b]	20	2
fragile papershell (*Leptodea fragilis*)					
freshwater drum	3	5	2[b]	22-31	3218
	4	6	2	15-50	9130[c]
hickorynut (*Obovaria olivaria*)					
shovelnose sturgeon		3	3	17-31	5955
lake sturgeon		3	3	24-41	596
central mudminnow	1	6	1	13-16	10
	2	2	2	13-16	3
goldeye		2	2	14-22	4
mosquito fish		6	3	16	1
sheepnose (*Plethobasus cyphyus*)					
banded killifish	1	4	4	13-16	5
	2	18	2	16	1
bleeding shiner	1	2	2	14-21	9
	2	2	0		
bluntnose minnow	1	3	0	12	1
	2	4	2	12	1
	3	6	6	7	0
	4	7	6	12	0
common shiner	1	1	1	10-17	5
	2	20	6	9-16	3
creek chub	1	1	1	15-19	18
	2	6	3	9-14	3
fathead minnow	1	5	2	16-18	6
	2	17	4	16-27	5

golden shiner		10	4	13-32	67
hornyhead chub	1	9	6	12-23	36
	2	10	9	14-18	43
Ozark minnow	1	9	8	22-24	6
	2	10	0		
spotfin shiner	1	6	0	12-23	17
	2	42	0	11-20	46
spottail shiner	1	3	0	10	1
	2	6	0	16-18	8
	3	18	0	11-20	22
steelcolor shiner		5	0	12	1
round pigtoe (*Pleurobema sintoxia*)					
bigmouth shiner		7	0	17-20	7
blacktail shiner		6	5	11-14	89
bleeding shiner	1	3	0	18-23	61
	2	5	0		0
bluntnose minnow		4	4	8-12	26
common shiner		3	3	20-25	7
creek chub	1	4	4	17-23	94
	2	4	4	11-18	111
eastern blacknose dace		2	0	11	250
golden shiner		10	10	7-14	2059
mosquito fish		10	7	11	54
Ozark minnow		9	1	17	2
spottail shiner	1	4	3	7	3
	2	7	0	10	0
striped shiner		1	0	11	22
whitetail shiner	1	5	5	18	194
	2	3	0	7	0
pink heelsplitter (*Potamilus alatus)*					
freshwater drum	4	5	2[b]	22-31	915
	5	6	4	15-26	75
wartyback (*Quadrula nodulata*)					
black bullhead		3	3	9-14	250
blue catfish		5	5	9-22	1947
channel catfish		6	4	9-14	3198
flathead catfish	1	3	0	16	20

	2	2	2	9	0
yellow bullhead		2	2	9-14	313
deertoe (*T. truncata)*					
freshwater drum		1	1	14-23	7730[c]

[a] Trial incomplete
[b] Fish died before any juveniles were recovered
[c] Estimate based on subsamples of siphonate

Table 2. Trials where glochidia metamorphosis was not observed.

Common name	Trial	No. of individuals inoculated	No. of survivors	Glochidia attachment period (days)
butterfly (*Ellipsaria lineolata*)				
bigmouth shiner	1	---	2	3
	2	---	2	3
black bullhead		---	8	3
black crappie		---	9	6
blackside darter		---	5	3
blackspotted topminnow		---	5	7
blue catfish		---	8	3
bluegill		---	8	3
bluntnose minnow		6	6	4
bowfin	1	---	1	3
	2	2	2	4
brook trout		---	1	3
bullhead minnow	1	---	5	3
	2	---	3	3
central mudminnow		---	9	7
central stoneroller		---	5	3
chain pickerel		---	1	3
common carp		---	3	3
creek chub		---	4	3
eelpout		1	1	4
emerald shiner		---	1	3
fantail darter		1	1	4
fathead minnow		---	7	3
flathead catfish		---	2	3
gizzard shad		3	3	6
golden redhorse		---	2	3
golden shiner		---	11	3
green sunfish		---	3	13
largemouth bass		---	9	7
logperch		---	10	3
longear sunfish		---	5	7
longnose dace		---	3	3

longnose gar	1	---	2	3
	2	1	1	4
mosquitofish		---	5	17
mottled sculpin		---	6	13
northern pike	1	---	1	7
	2	5	5	4
northern studfish		---	2	11
pumpkinseed		---	2	3
quillback		1	1	4
redear sunfish		---	2	6
river shiner	1	---	4	3
	2	---	1	3
rock bass		---	3	3
shorthead redhorse		---	3	3
slender madtom		---	3	3
slenderhead darter	1	---	1	3
	2	4	4	4
smallmouth bass		---	3	7
smallmouth buffalo		---	1	3
spotfin shiner	1	---	6	3
	2	---	1	3
stonecat		3	3	4
striped shiner		---	2	3
tadpole madtom	1	---	2	3
	2	2	2	4
walleye(HR)		---	11	3
white sucker		---	4	3
whitetail shiner	1	---	4	3
	2	---	2	3
yellow bullhead		---	3	3
yellow perch		---	4	3
fragile papershell (*Leptodea fragilis*)				
bigmouth shiner		---	2	3
black crappie		---	5	3
blackside darter		---	5	3
blacktail shiner		---	3	3
blue catfish		---	8	7
bluntnose minnow		6	6	4

bowfin	1	---	1	3
	2	2	2	4
brook stickleback	1	---	6	10
	2	---	5	16
bullhead minnow		---	5	3
central mudminnow		---	9	7
central stoneroller		---	5	3
channel catfish		---	6	7
creek chubsucker		---	3	3
eelpout		1	1	4
fantail darter		1	1	4
fathead minnow		---	7	3
flathead catfish		---	2	3
gizzard shad		3	3	6
golden redhorse		---	2	3
goldeye		---	2	17
largescale stoneroller		---	7	3
longnose gar	1	---	2	3
	2	1	1	4
mosquitofish		---	8	14
northern hog sucker		---	3	3
northern pike	1	---	1	7
	2	5	5	4
pumpkinseed		---	5	3
quillback		1	1	4
shovelnose sturgeon		---	3	7
slenderhead darter		4	4	4
smallmouth buffalo		---	1	3
spotfin shiner		---	6	3
stonecat		3	3	4
striped shiner		---	1	3
tadpole madtom	1	---	2	3
	2	2	2	4
whitetail shiner		---	2	3
yellow bullhead	1	---	3	3
	2	---	2	7
threehorn wartyback (*Obliquaria reflexa*)				
freshwater drum		1	1	3

lake sturgeon	1	2	2	5
	2	4	4	4
hickorynut (*Obovaria olivaria*)				
banded killifish		6	0	12
bigmouth shiner		8	7	5
black bullhead	1	2	1	4
	2	2	2	4
	3	8	8	3
black crappie	1	2	2	4
	2	6	6	6
blackspotted top minnow		6	6	10
blue catfish		8	8	3
bluegill	1	4	4	4
	2	5	5	3
brook trout		1	1	3
brown bullhead		1	1	4
burbot		1	1	4
chain pickerel		3	3	9
channel catfish	1	6	6	4
	2	1	1	3
	3	6	6	3
common carp		1	1	4
common shiner		1	1	4
creek chub		4	4	3
fantail darter		2	2	5
fathead minnow		5	5	5
flathead catfish		1	1	4
golden redhorse		2	2	3
golden shiner		11	11	9
goldfish	1	1	1	4
	2	2	2	5
	3	25	5	3
green sunfish	1	6	6	15
	2	4	4	6
Iowa darter		2	2	5
largemouth bass	1	5	5	4
	2	11	11	9
largescale stoneroller		6	6	3

logperch		6	6	3
longear sunfish	1	5	5	4
	2	4	4	3
longnose dace		3	3	3
longnose gar	1	2	2	4
	2	1	1	3
Mexican molly		7	6	16
mottled sculpin		2	1	21
norther hog sucker		1	1	3
pumpkinseed	1	1	1	4
	2	3	3	6
redear sunfish		1	1	3
rock bass	1	2	2	4
	2	5	5	4
	3	2	2	3
sauger		2	2	4
shorthead redhorse		3	3	3
shortnose gar		4	4	4
shovelnose sturgeon		3	3	17-31
slender madtom		2	2	3
slenderhead darter		3	3	5
smallmouth bass	1	5	5	4
	2	1	1	3
southern redbelly dace		1	1	5
spotfin shiner	1	8	8	5
	2	9	9	5
stonecat	1	4	4	4
	2	1	1	3
tadpole madtom		2	2	4
walleye	1	3	3	4
	2	1	1	4
	3	6	6	6
warmouth		1	1	4
white bass		2	2	7
white sucker		4	4	3
whitetail shiner		8	7	3
yellow perch	1	4	4	6
	2	5	5	7

sheepnose (*Plethobasus cyphyus)*

bigmouth shiner	1	1	1	7
	2	9	0	
	3	8	0	
black bullhead		6	3	5
black crappie		1	1	5
blacknose dace	1	5	0	
	2	1	0	
	3	1	1	5
blacktail shiner		1	0	
bluegill		4	4	5
bowfin		2	2	5
brassy minnow		1	0	
brook stickleback		7	2	9
central mudminnow		4	4	5
central stoneroller		1	0	
channel catfish		8	8	5
creek chubsucker		1	1	5
fantail darter	1	4	4	8
	2	2	2	5
flathead catfish		2	2	5
freshwater drum		1	1	5
goldfish	1	1	1	8
	2	9	9	5
green sunfish		7	7	5
greenside darter		1	1	5
Iowa darter		6	5	5
Johnny darter	1	3	3	7
	2	4	3	8
	3	3	3	6
	4	10	8	5
lake sturgeon		6	6	5
logperch		3	3	5
longear sunfish		8	8	5
longnose gar		1	1	5
orange-spotted sunfish		1	1	5
orangethroat darter		2	1	5
pearl dace		1	0	

pirate perch		9	9	7
pumpkinseed		4	4	5
quillback		1	1	5
rainbow darter	1	8	8	8
	2	3	3	8
river darter		1	1	5
rock bass		2	2	5
sauger		3	3	5
shorthead redhorse		4	4	5
silver redhorse		2	2	5
slenderhead darter	1	3	3	8
	2	14	14	5
southern redbelly dace	1	1	0	
	2	3	2	7
stonecat		3	3	5
striped shiner		3	3	7
tadpole madtom	1	1	1	7
	2	3	3	5
walleye		4	4	5
warmouth		1	1	5
white bass	1	2	2	8
	2	1	1	5
white sucker		2	2	5
whitetail shiner		2	2	7
yellow bullhead		2	2	5
yellow perch		3	2	5
round pigtoe (*Pleurobema sintoxia*)				
black crappie		3	3	4
blackspotted topminnow		1	0	10
bleeding shiner		5	0	
blue catfish		8	8	4
bluegill		8	8	5
brook trout		1	0	
chain pickerel		3	3	10
emerald shiner		11	8	5
freshwater drum		1	1	7
golden redhorse		2	2	10
goldfish		5	5	5

logperch		10	10	5
longear sunfish		5	5	5
longnose gar		1	1	4
Mexican molly		11	8	7
mottled sculpin		1	0	
pumkinseed		3	3	5
redear sunfish		1	0	
rockbass		3	3	4
sauger		1	1	5
shorthead redhorse		3	3	15
smallmouth buffalo		1	1	4
southern platy		3	1	6
steelcolor shiner	1	3	3	7
	2	3	1	7
pink heelsplitter (*Potamilus alatus)*				
bigmouth shiner		---	2	3
black crappie		---	5	3
blackside darter		---	5	3
blacktail shiner		---	3	3
blue catfish		---	8	7
bluntnose minnow		6	6	4
bowfin	1	---	1	3
	2	2	2	4
brook stickleback	1	4	0[b]	14
	2	10	6	20
	3	---	6	21
bullhead minnow		---	5	3
central mudminnow		---	9	7
central stoneroller		---	5	3
channel catfish		---	6	7
creek chubsucker		---	3	3
eelpout		1	1	4
fantail darter		1	1	4
fathead minnow		---	7	3
flathead catfish		---	2	3
gizzard shad		3	3	6
goldeye		---	2	25
goldren redhorse		---	2	3

Johnny darter		---	5	3
largescale stoneroller		---	7	3
longnose gar	1	---	2	3
	2	1	1	4
	3	---	8	14
northern hog sucker		---	3	3
northern pike	1	---	1	7
	2	5	5	4
pumpkinseed		---	5	3
quillback		1	1	4
rock bass		---	4	3
shovelnose sturgeon		---	3	7
slenderhead darter		4	4	4
stonecat		3	3	4
striped shiner		---	1	3
tadpole madtom	1	---	2	3
	2	2	2	4
whitetail shiner		---	2	3
yellow bullhead	1	---	3	3
	2	---	2	7
wartyback (*Quadrula nodulata*)				
blackspotted top minnow		3	3	12
bowfin		3	3	6
brook stickleback		2	1	9
chain pickerel		3	3	6
creek chub		3	3	3
emerald shiner		2	2	3
golden redhorse		2	2	3
goldfish		4	4	3
mosquito fish		7	6	9
walleye		3	3	3
whitetail shiner		5	5	3
deertoe (*T. truncata)*				
black crappie		---	3	11
blackspotted topminnow		---	6	14
blue catfish		---	8	3
brook stickleback		---	3	14
central mudminnow		---	2	4

chain pickerel		---	2	4
channel catfish		---	6	3
creek chub		---	3	4
golden redhorse		---	2	4
largescale stoneroller		---	6	3
longnose gar		---	1	4
mosquitofish		---	8	14
northern hog sucker		---	1	3
sauger	1	4	4	4
	2	---	1	4
spottail shiner		---	2	4
walleye(HR)		---	15	4
whitetail shiner		---	6	4

[a] Data not available
[b] Trial incomplete

Table 3. Glochidial valve dimensions (mean ± 1 SD (µm)) of mussels similar in size to sheepnose and glochidial valve dimensions of juvenile mussels released from naturally infested Chippewa River shiners. Number of females providing glochidia and number of glochidia are in parentheses. Measurements not connected by same letter are significantly different.

	Species			
Valve character	threehorn wartyback (*Obliquaria reflexa*)	threeridge (*Amblema plicata*)	spike (*Elliptio dilatata*)	sheepnose (*Plethobasus cyphyus*)
Height	256 ± 6^{a} (5, 25)	234 ± 8^{b} (5, 26)	231 ± 12^{b} (5, 29)	212 ± 16^{c} (5, 30)
Length	250 ± 7^{a} (5, 25)	216 ± 6^{c} (5, 26)	220 ± 8$^{b, c}$ (5, 30)	223 ± 10^{b} (5, 30)
Hinge L	146 ± 6$^{a, b}$ (5, 23)	141 ± 5$^{b, c}$ (5, 26)	148 ± 5^{a} (5, 30)	134 ± 9^{d} (5, 30)
Collection location	St. Croix River, MN	St. Croix River, Mississippi River, MN	St. Croix River, MN	Chippewa River, WI
	lilliput (*Toxolasma parvus*)	round pigtoe (*Pleurobema sintoxia*)	Wabash pigtoe (*Fusconaia flava*)	
Height	200 ± 11^{d} (5, 24)	166 ± 6^{e} (5, 26)	167 ± 5^{e} (5, 27)	
Length	181 ± 10^{d} (5, 25)	167 ± 4^{e} (5, 26)	171 ± 6^{e} (5, 27)	
Hinge L	104 ± 6^{f} (5, 25)	122 ± 3^{e} (5, 26)	137 ± 8$^{c, d}$ (5, 27)	
Collection location	Unnamed Creek, Maplewood, MN	St. Croix River, MN	St. Croix River, MN	
	Glochidial valve dimensions from juveniles released from naturally infested shiners			
	sheepnose juveniles (n=7)	Wabash pigtoe juveniles (n=10)	round pigtoe juvenile (n=1)	
Height	210 ± 5	164 ± 6	153	
Length	223 ± 6	169 ± 9	152	
Hinge L	135 ± 7	142 ± 8	118	
Collection location	Chippewa River, WI	Chippewa River, WI	Chippewa River, WI	

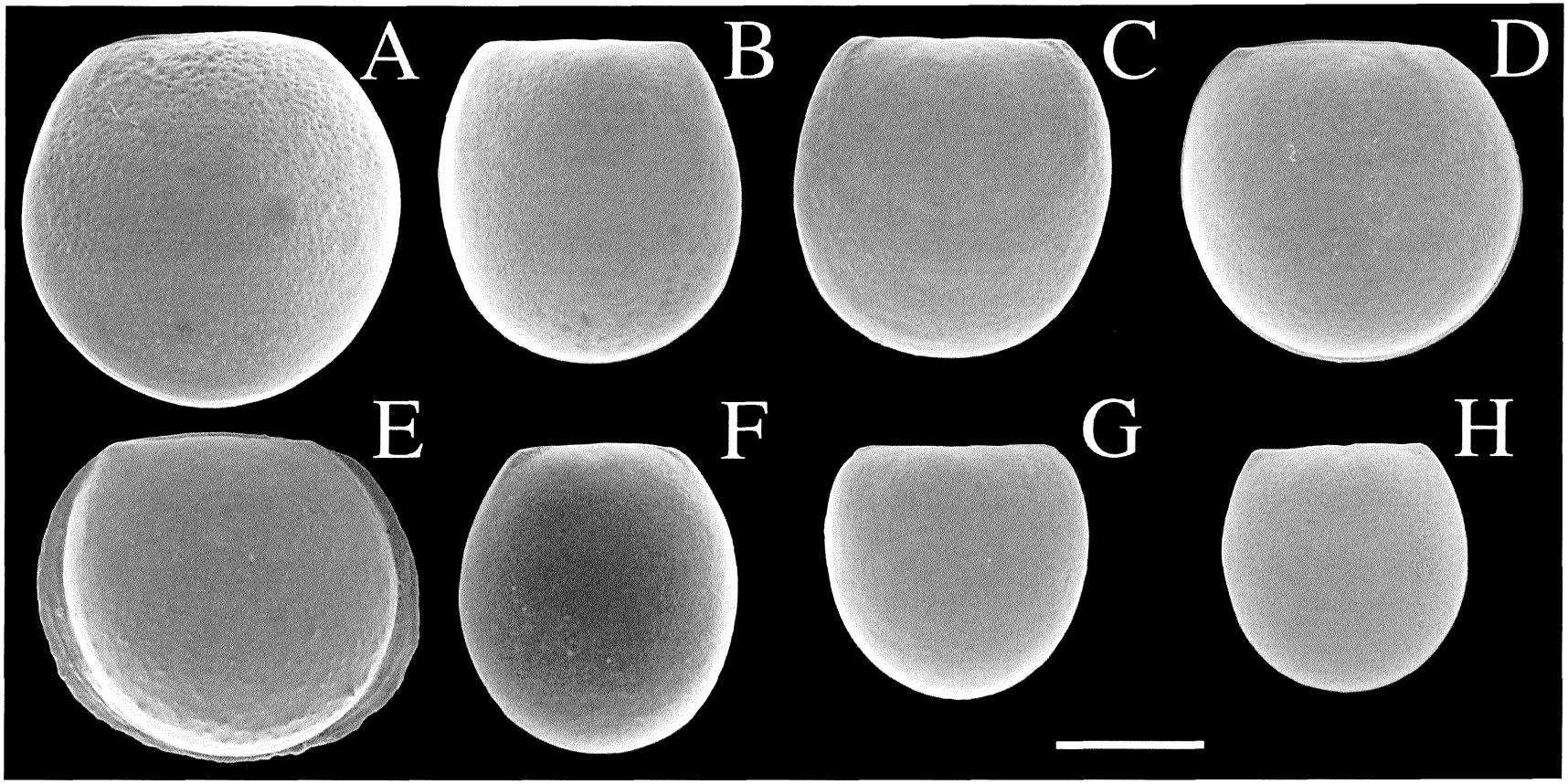

Figure 3. Scanning electron micrographs of glochidia from Chippewa River mussel species that release glochidia similar to sheepnose glochdidia (scale bar = 100 µm). Species are (A) threehorn wartyback (*Obliquaria reflexa*), (B) threeridge (*Amblema plicata*), (C) spike (*Elliptio dilatata*), (D) sheepnose (*Plethobasus cyphyus*), (E) juvenile mussel recovered from a naturally infested mimic shiner (glochidial valve height=217 µm), (F) lilliput (*Toxolasma parvus*), (G) Wabash pigtoed (*Fusconaia flava*), and (H) round pigtoe (*Pleurobema sintoxia*).