Life history requirements of rare mussels: Plethobasus cyphyus and Cumberlandia monodonta

2008 Annual Report

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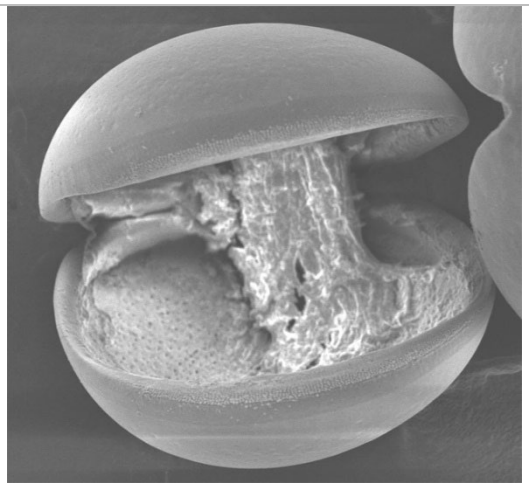

sheepnose glochidium

longnose dace – suitable sheepnose host

creek chub – suitable sheepnose host

central stoneroller – suitable sheepnose host

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Abstract

The National Native Mussel Conservation Committee recommends increasing understanding of basic knowledge of mussel biology as a top conservation priority. We conducted a series of studies to improve our understanding of sheepnose and spectaclecase life history. We visited a sheepnose population in the Chippewa River near Eau Claire, WI monthly during spring and summer 2008 and found brooding animals between late May - early August. Standard artificial glochidia infestation procedures revealed that select minnow species (bleeding shiner, central stoneroller, common shiner, creek chub, golden shiner, longnose dace and spotfin shiner) and blackspotted topminnow serve as suitable hosts for sheepnose glochidia. Glochidia metamorphosis was not observed among any of the twenty fish species exposed to spectaclecase glochidia. Analysis of scanning electron micrographs of sheepnose, spectaclecase, threeridge, deertoe, Wabash pigtoe, spike, round pigtoe and fawnsfoot revealed that spectaclecase produce unusually small and unique glochidia that can easily be distinguished from all species studied. Sheepnose glochidia are similar in size to threeridge and spike but could be distinguished from these species by its asymmetric valve outline. Additionally, sheepnose valve height is less than threeridge, and hinge length is less than spike. Additional work is needed to confirm sheepnose brooding period, expand the number of minnow and other fish species tested for glochifia host suitability, and describe glochidia of other mussel species similar in size to sheepnose. Biologists can use information from these studies to improve protection of rare mussel species by ensuring glochidia hosts co-occur with them, as well as using this information to assist with juvenile mussel propagation efforts.

Introduction

Freshwater mussels are more imperiled than any other U.S. floral or faunal group (Master *et al*., 1998). A national strategy developed for the conservation of rare freshwater mussels has identified the need to increase fundamental knowledge of basic freshwater mussel biology as a top priority (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 basic biology research goal.

Most North American freshwater mussel species release glochidia in the spring and early summer (Parmalee and Bogan 1998). Watters *et al*., (2005) describe sheepnose living in Ohio brooding glochidia between May-June, and further south, sheepnose have been shown to

brood glochidia between May-July (Gordon and Layzer 1989). Sheepnose live at latitudes north of Ohio including into Wisconsin and Minnesota (Cummings and Mayer 1992) and likely brood their young during a period similar to southern populations but observations are needed to confirm this assumption.

The host requirements of most mussels living in the upper Mississippi River watershed are unresolved (Watters 1994). Two of these species living in Minnesota include: sheepnose (*Plethobasus cyphyus*) and spectaclecase (*Cumberlandia monodonta*). Identification of a suitable host species that facilitate glochidia metamorphosis is important for conservation efforts and essential in initiating most juvenile mussel propagation programs. Although several host suitability trials have been run in several laboratories the host(s) for spectaclecase is unknown. A small number of trials using sheepnose glochidia showed that select minnow species are suitable hosts (Watters *et al*., 2005). 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 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) monitor the brooding behavior of a sheepnose population, (2) conduct host suitability trials using sheepnose and spectaclecase glochidia, and (3) use scanning electron microscopy to describe sheepnose, spectaclecase and similarly sized glochidia released by select upper Mississippi River mussel species.

Knowledge of glochidia morphology between mussel species can be used to identify juvenile mussels recovered from naturally infested fishes, and improve understanding of taxonomic relationships between species. Species-specific characters of glochidia have been used to identify juvenile mussels recovered from naturally infested fishes (Kennedy and Haag 2005, Allen *et al*., 2007). Additionally, glochidia morphometrics have been used in taxonomic analysis (Rand and Wiles 1982, Kwon *et al*., 1993). Only a few old studies have described the glochidia of spectaclecase (Surber 1915) and sheepnose (Surber 1912) and accurate descriptions are needed in order to construct a morphological key to identify juvenile mussels that have excysted from naturally infested fishes.

Methods

Sheepnose brooding period

We used snorkeling gear and SCUBA to study sheepnose brooding period in the Chippewa River during the spring and summer of 2008. A sheepnose population located near Meridean, WI was visited monthly between May-September, 2008. We attempted to collect 10-20 sheepnose and briefly peer between the valves to observe whether or not gills were inflated with glochidia. Those with gills inflated at least three times normal thickness were considered gravid. Notes about gill color and the number of gills used as marsupia were recorded. We also studied and photographed sheepnose siphoning in the river and the laboratory to see if brooding animals were behaving in a way to attract hosts (*e.g.*, mantle fish lure).

Suitable Glochidia Host(s)

We conducted glochidia host suitability trials using standard methods (Zale and Neves 1982, Hove *et al.,* 2000). Gravid female sheepnose and spectaclecase were collected from the Chippewa River and St. Croix River, respectively. Fish for artificial infestation trials were collected using a seine, trap net, angling or electrofishing equipment primarily from streams and rivers outside of the St. Croix River and Chippewa River drainages. This precaution was taken to avoid testing fish that may have been previously exposed to the species of glochidia under investigation and subsequently developing immunity to subsequent exposures (Reuling 1919). Host suitability trials were conducted at the University of Minnesota Wet Laboratory. Test subjects were held in holding tanks (40 L or 400 L) at least 30 d prior to glochidia infestation, at temperatures adjusted near that of the St. Croix River. Gravid female mussels were held in beakers in aquaria until they release glochidia naturally. 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 spring and summer of 2008. Fish were infested with glochidia by placing fish in a 1-20 L bath with several hundred to several thousand glochidia under vigorous aeration for a few minutes to hours depending on species' susceptibility to infestation. 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 ± 2 °C and fed daily. Fathead minnows (*Pimephales promelas*) were given to piscivorous fish 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, etc.) and benthic feeding fishes (*e.g*., catostomids) 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.

Glochidia descriptions

We studied morphological characters of glochidia of select upper Mississippi River mussel species to develop a key for the identification of juvenile mussels recovered from naturally infested fishes. We used scanning electron microscopy to photograph, measure and describe

characteristics of glochidia of sheepnose (Chippewa River) and other mussel species (St. Croix River). Glochidia were preserved in 95% ethanol and rinsed in 100% ethanol prior to mounting onto scanning electron microscope specimen stubs. Glochidia were adhered to specimen stubs using conductive, double-faced adhesive tape. Mounted specimens were covered with gold using a Fullam Sputter Coater, and viewed with a Hitachi S3500N variable pressure scanning electron microscope with Windows NT operating system and Quartz PCI digital imaging software. Glochidia morphology nomenclature follows Hoggarth (1999).

Results

Sheepnose brooding period

We observed sheepnose brooding glochidia between May-August 2008. A visual cue useful in finding non-/brooding sheepnose was the presence of their branched papillae around the incurrent aperture (Figure 1). Brooding sheepnose appeared to behave and orient themselves much like non-brooding sheepnose. We did not observe brooding sheepnose exhibit a specialized mantle structure for attracting host fishes (*e.g.*, mantle flaps or magazine) although brooding sheepnose may have raised themselves out of the substrate somewhat more than nonbrooding animals. The relative abundance of sheepnose brooding glochidia peaked in late June and was over by mid-September (Figure 2). Sheepnose were observed brooding glochidia in outer gills and gill color of various individuals varied from red to white throughout most of the brooding period. Individuals with swollen white gills tended to bear mature glochidia.

Suitable Glochidia Host(s)

We observed sheepnose glochida metamorphose on select minnow species and blackspotted topminnow; none of the spectaclecase glochidia metamorphosed into juveniles. Sheepnose glochidia metamorphosed on eight of forty fish species (nine families) exposed in the laboratory. Metamorphosis was observed on bleeding shiner, central stoneroller, common shiner, creek chub, golden shiner, longnose dace, spotfin shiner and blackspotted topminnow (Table 1). No glochidia growth was observed on freshly excysted juvenile mussels or among sloughed glochidia (Table 2). We did not observe spectaclecase glochidia metamorphose on any of the twenty species tested (Table 2).

Glochidia descriptions

Conchological characteristics of glochidia varied between mussel species. Sheepnose were dissimilar to all species except threeridge and spike, but could be distinguished from these species by its asymmetric valve outline, and sheepnose valve height is less than threeridge and hinge length is less than spike (Table 3). Round pigtoe and Wabash pigtoe glochidia were very similar in size and shape. Spectaclecase are similar in size to deertoe and fawnsfoot but is easily distinguished from the two *Truncilla* species by its round outline.

Discussion

Sheepnose brooding period

As is commonly to many species in Ambleminae sheepnose were bearing glochidia in spring and summer. Most upper Mississippi River Ambleminae brood glochidia in the spring or early summer: Amblema (May-Aug), Cyclonaias (Apr-July), Elliptio (Apr-Aug), Fusconaia (May-Aug), Pleurobema (May-Aug), select Quadrula (Apr-Aug), Tritogonia (Apr-June) (Howard 1914, Coker *et al*. 1921, Heath *et al*., 2001). We observed sheepnose brooding glochidia between May-August during 2008, which is similar to other sheepnose populations, May-June in Ohio (Watters *et al*., 2005) and May-July further south (Gordon and Layzer 1989). The first time we went out in spring (May 24) we observed brooding sheepnose so we likely missed the beginning of the brooding period. Next year we will confirm the brooding period and plan to begin searching for brooding sheepnose earlier in order to record the beginning of the period.

Suitable Glochidia Host(s)

We observed sheepnose glochidia metamorphose on species that have been reported as suitable hosts, as well as other species. Central stonerollers, fathead minnows and creek chubs are known to be suitable hosts (Watters *et al*., 2005) but to our knowledge the other minnow species and blackspotted topminnow have not been shown to facilitate metamorphosis of sheepnose glochidia. Several other minnow species were tested for host suitability in this study but juvenile sheepnose were not recovered. Next field season we plan retest these minnow species in higher numbers as well as additional minnow and non-minnow species to better understand potential host species for sheepnose. Spectaclecase glochidia did not metamorphose on any of the fishes we tested. Next season we will attempt to run host suitability trials on fish families or genera that co-occur with spectaclecase and have not yet been tested.

Glochidia descriptions

Conchological characters of glochidia described in this study can be used to distinguish most of the mussel species studied. Our measurements of glochidia were are similar to most measurements made in other studies (Table 3) (Surber 1912, Surber 1915, Kennedy and Haag 2005, Hoggarth 1999). Sheepnose glochidia are larger and easily distinguishable from Wabash and round pigtoes but similar in size to threeridge and spike. Our initial measurements suggest we can use the smaller hinge line of sheepnose to distinguish it from spike and the smaller sheepnose valve height to separate it from threeridge. Additional measurements are needed of threeridge glochidia to better understand the variability in this species. Additionally, measurements are needed of glochidia from other Chippewa River mussel species that are glochidia similar in size to sheepnose (*e.g*., *Obliquaria reflexa*, possibly *Toxolasma parvus*) in order to complete an identification key for use in identifying possible juvenile sheepnose recovered from naturally infested Chippewa River fishes. The glochidia of spectaclecase are uniquely small and round in outline and are easy to distinguish from glochidia of other mussel species (Hoggarth 1999).

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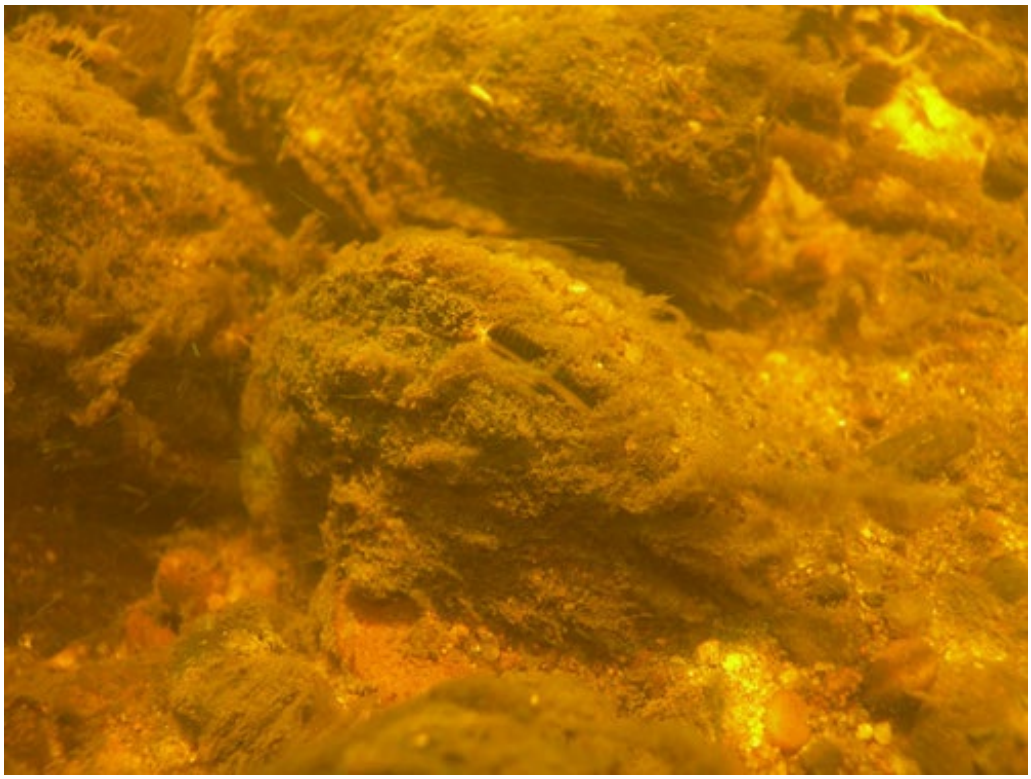
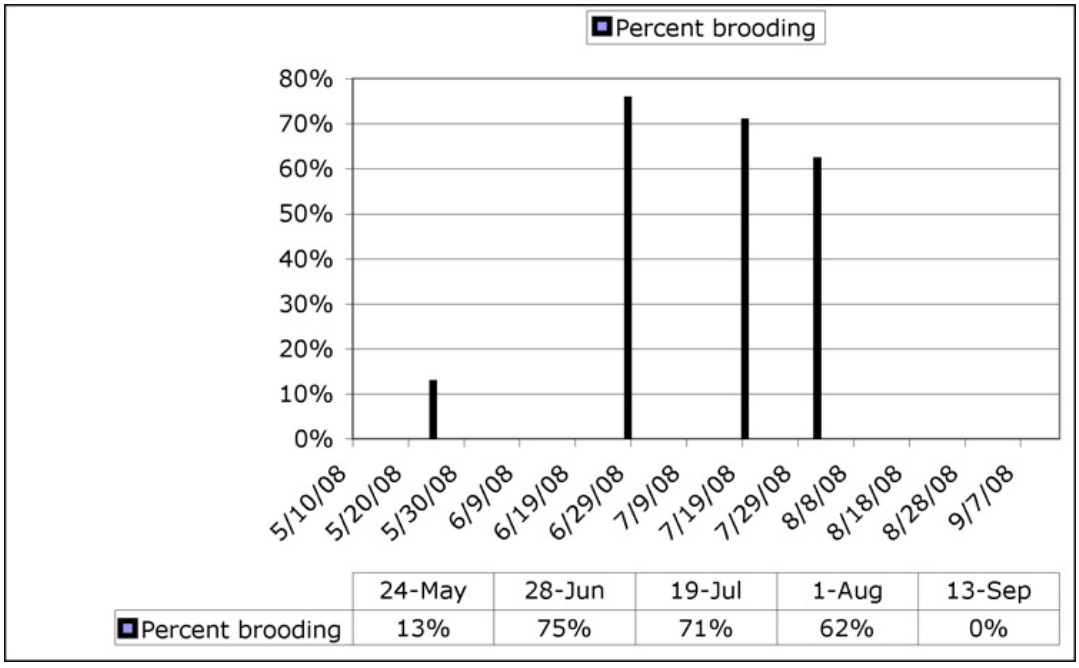


Figure 1. Sheepnose siphoning in the Chippewa River near Meridean, WI.



	24-May	28-Jun	19-Jul	1-Aug	13-Sep
Percent brooding	13%	75%	71%	62%	0%

Figure 2. Sheepnose brooding period observed during 2008.

Table 1. Fishes that facilitated sheepnose glochidia metamorphosis.

Common name (Scientific name)	No. of individuals inoculated	No. of survivors	Juvenile recovery period (days)	No. of juveniles recovered
Cyprinidae				
central stoneroller (*Campostoma anomalum*)	4	3	12-33	90
spotfin shiner (*Cyprinella spiloptera*)	3	3	15-25	20
common shiner (*Luxilus cornutus*)	2	2	15-23	7
bleeding shiner (*Luxilus zonatus*)	2	1	12-27	8
golden shiner (*Notemigonus crysoleucas*)	6	3	12-22	53
longnose dace (*Rhinichthys cataractae*)	1	1	12-25	9
creek chub (*Semotilus atromaculatus*)	3	3	22-25	4
Fundulidae				
blackspotted topminnow (*Fundulus olivaceus*)	1	1	13-25	5

Table 2. Trials where glochidia metamorphosis was not observed.

Common name	Trial	No. of individuals inoculated	No. of survivors	Glochidia attachment period (days)
sheepnose (*Plethobasus cyphyus*)				
Anguillidae				
American eel (*Anguilla rostrata*)		1	1	6-10
Cyprinidae				
goldfish (*Carassius auratus*)		2	2	1-5
common carp (*Cyprinus carpio*)		2	2	1-4
common shiner (*Luxilus cornutus*)	1	3	3	5-17
	2	2	2	6-9
redfin shiner (*Lythrurus umbratilis*)		2	0	*
emerald shiner (*Notropis atherinoides*)		5	4	6-9
bigmouth shiner (*Notropis dorsalis*)		3	2	9-15
mimic shiner (*Notropis volucellus*)		3	0	*
eastern blacknose dace (*Rhinichthys atratulus*)		2	0	*
creek chub (*Semotilus atromaculatus*)		3	3	5-17
Catostomidae				
white sucker (*Catostomus commersonii*)		2	2	1-4
Ictaluridae				
black bullhead (*Ameiurus melas*)		4	4	1-5
yellow bullhead (*Ameiurus natalis*)		1	1	1-3
blue catfish (*Ictalurus furcatus*)		4	4	1-4

channel catfish (*Ictalurus punctatus*)	1	1	1	1-5
	2	4	4	1-4
stonecat (*Noturus flavus*)	1	1	1	1-5
	2	1	1	1-4
Aphredoderidae				
pirate perch (*Aphredoderus sayanus*)		3	3	1-4
Cottidae				
banded sculpin (*Cottus carolinae*)		1	1	4-6
Centrarchidae				
rock bass (*Ambloplites rupestris*)	1	2	2	1-5
	2	2	2	1-4
green sunfish (*Lepomis cyanellus*)		5	5	3-6
pumpkinseed (*Lepomis gibbosus*)		3	3	1-3
orange-spotted sunfish (*Lepomis humilis*)		4	4	3-6
bluegill (*Lepomis macrochirus*)		6	6	1-5
smallmouth bass (*Micropterus dolomieu*)	1	5	5	5-17
	2	4	4	3-6
largemouth bass (*Micropterus salmoides*)	1	3	3	5-17
	2	1	1	1-3
black crappie (*Pomoxis nigromaculatus*)		3	3	3-6
Percidae				
rainbow darter (*Etheostoma caeruleum*)		9	9	4-6
Johnny darter (*Etheostoma nigrum*)		11	11	1-4
banded darter (*Etheostoma zonale*)		3	3	1-4
yellow perch (*Perca flavescens*)		4	4	9-11
logperch (*Percina caprodes*)	1	4	4	1-5
	2	3	3	1-3
	3	1	1	1-4
blackside darter (*Percina maculata*)		3	3	1-4
slenderhead darter (*Percina phoxocephala*)		4	4	1-4
sauger (*Sander canadensis*)		5	5	1-4
spectaclecase (*Cumberlandia monodonta*)				
Fish				
Petromyzontidae		1	0	*
chestnut lamprey (*Ichthyomyzon castaneus*)				
Anguillidae				
American eel (*Anguilla rostrata*)		1	1	**
Cyprinidae				
central stoneroller (*Campostoma anomalum*)		3	3	1-3
common carp (*Cyprinus carpio*)		2	2	1-4
southern redbelly dace (*Phoxinus erythrogaster*)		9	9	1-3
longnose dace (*Rhinichthys cataractae*)		7	7	1-3

creek chub (*Semotilus atromaculatus*)		7	7	1-3
Aphredoderidae				
pirate perch (*Aphredoderus sayanus*)		5	5	1-3
Cottidae				
mottled sculpin (*Cottus bairdi*)		1	1	1-3
banded sculpin (*Cottus carolinae*)		1	1	1-3
Centrarchidae				
green sunfish (*Lepomis cyanellus*)		1	1	1-3
warmouth (*Lepomis gulosus*)		1	1	1-3
orange-spotted sunfish (*Lepomis humilis*)		1	1	1-3
bluegill (*Lepomis macrochirus*)		3	3	1-3
longear sunfish (*Lepomis megalotis*)		6	6	1-3
largemouth bass (*Micropterus salmoides*)		3	3	1-3
Percidae				
rainbow darter (*Etheostoma caeruleum*)		1	1	1-3
fantail darter (*Etheostoma flabellare*)		5	5	1-3
yellow perch (*Perca flavescens*)		4	4	9-11
Amphibians				
Proteidae				
common mudpuppy (*Necturus maculosus*)		4	0	*

* - Incomplete trial, test subjects died before study completion

** - No glochidia recovered so glochidia attachment period unknown

Table 2. Glochidial valve dimensions (± 1 SD (µm)) of select upper Mississippi River mussel species. Glochidia images are provided to illustrate differences in valve outline and are **not** the same scale. The number of females providing glochidia and sample sizes are in parentheses

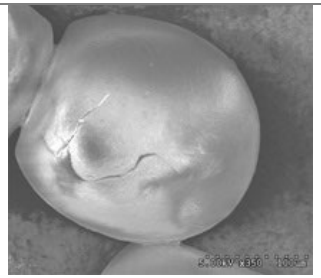
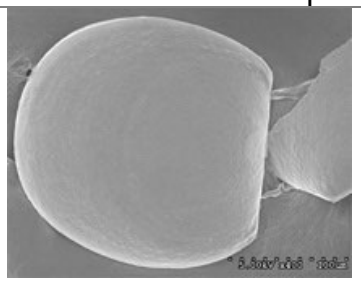
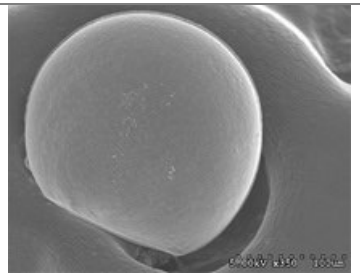
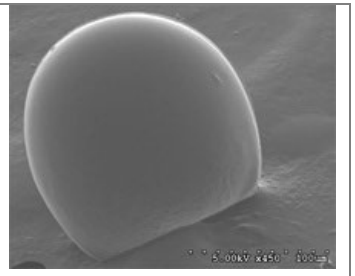
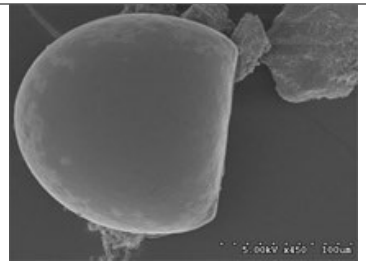
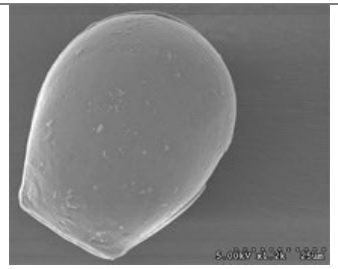
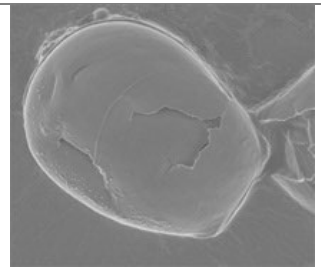
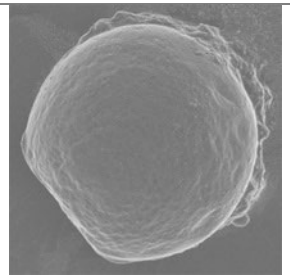
	Species			
Valve character	 threeridge (*Amblema plicata*)	 spike (*Elliptio dilatata*)	 sheepnose (*Plethobasus cyphyus*)	 round pigtoe (*Pleurobema sintoxia*)
Height	237 ± 8 (1, 6)	231 ± 12 (5, 29)	212 ± 16[a] (5, 30)	171 ± 7 (1, 6)
Length	221 ± 9 (1, 6)	220 ± 8 (5, 30)	223 ± 10 (5, 30)	173 ± 3 (1, 6)
Hinge L	140 ± 4 (1, 6)	148 ± 5 (5, 30)	134 ± 9 (5, 30)	125 ± 2 (1, 6)
	 Wabash pigtoe (*Fusconaia flava*)	 deertoe (*Truncilla truncata*)	 fawnsfoot (*Truncilla donaciformis*)	 spectaclecase (*Cumberlandia monodonta*)
Height	166 ± 2 (2, 12)	72 ± 3 (5, 30)	68 ± 1 (1, 6)	65 ± 3 (5, 30)
Length	170 ± 7 (2, 12)	57 ± 2 (5, 30)	52 ± 2 (1, 6)	65 ± 2 (5, 30)
Hinge L	134 ± 8 (2, 12)	32 ± 2 (5, 29)	28 ± 1 (1, 6)	36 ± 4 (5, 25)

Table 3. Glochidial valve dimensions (± 1 SD (µm)) of of select upper Mississippi River mussel species measured in this and other studies.

Species	Height	Length	Hinge length	Reference
threeridge	237 ± 8	221 ± 9	140 ± 4	This study
	218 ± 2	203 ± 2	131 ± 2	Kennedy & Haag 2005
	200	200		Surber 1912
spike	231 ± 12	220 ± 8	148 ± 5	This study
	220 ± 3	216 ± 4	143 ± 3	Hoggarth 1999
	215	200		Surber 1912
sheepnose	212 ± 16	223 ± 10	134 ± 9	This study
	200	220		Surber 1912
round pigtoe	171 ± 7	173 ± 3	125 ± 2	This study
Wabash pigtoe	166 ± 2	170 ± 7	134 ± 8	This study
deertoe	72 ± 3	57 ± 2	32 ± 2	This study
	70	60		Surber 1912
fawnsfoot	68 ± 1	52 ± 2	28 ± 1	This study
	63	60		Surber 1912
spectaclecase	65 ± 3	65 ± 2	36 ± 4	This study
	52	50		Surber 1915