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Final Report:

**Reproduction and fish hosts of the Ouachita Rock Pocketbook,
*Arkansia wheeleri***

Project Period: August 4, 2009 – January 31, 2010

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Summary

Fertilization success, suitable host fish, and lab culture methods were examined in the federally endangered Arkansas rock pocketbook mussel, *Arkansia wheeleri*. The proportion of fertilization in the two brooding females recovered was 36.0% and 60.3%. Incomplete fertilization presumably results from low population density limiting availability of sperm. Eight fish species were tested as hosts for the glochidia larvae. The species and the percent successful metamorphosis of attached glochidia were: golden shiner (31%), dusky-stripe shiner (11%), freshwater drum (3%), blue catfish (0%), largemouth bass (0%), hybrid sunfish (0%), orange-spotted sunfish (0%), and walleye (0%). Metamorphosis was complete within 10 days at 23 C. A previous study reported 11 potential hosts for *Arkansia wheeleri*, mainly centrarchids, with metamorphosis success up to 70% (Seagraves 2006). However, an examination of the results of that study suggests that the fish were contaminated with glochidia of another mussel species, *Pyganodon grandis*, and that *Pyganodon* juveniles were recovered and mistaken for juveniles of *Arkansia wheeleri*. Further host tests should focus on cyprinids. Golden shiner appears to be a viable choice for captive propagation. Attempts to lab-culture the *Arkansia* juveniles were unsuccessful. Growth was poor and all juveniles died within 3 months. It appears that the juveniles did not feed well on the microalgae that have been used successfully for other mussel species, and future trials should provide a food source with bacterial components. Future culture efforts should also focus on establishing a captive population of adults to improve fertilization success and allow access to glochidia for culture.

Introduction and objectives

This project is an investigation of the reproductive biology of the federally endangered Ouachita rock pocketbook mussel, *Arkansia wheeleri*, a rare and critically endangered species. The only reported live collections of this species in the past decade are from 3 sites in the Little River in Arkansas and Oklahoma, one site in the Ouachita River, and one site in the Kiamichi River in Oklahoma (Seagraves 2006; Galbraith et al. 2008; Harris et al. in press; W. Posey, AGFC personal communication). Only 1-13 live individuals were found per site in these collections and it is likely that site populations are less than a few hundred individuals (Seagraves 2006). Although potential habitats remain to be surveyed, the limited range, rarity, and the loss or decline of the species at historically known sites of occurrence suggests that *Arkansia wheeleri* may be on the brink of extinction (Harris et al. in press). The objectives of the present study were as follows:

1. Determine proportion of fertilization in brooding females. Male mussels broadcast spermatozeugmata into the water. Female mussels must filter these from the water for the eggs to be fertilized. If population density is too low, there may be insufficient spermatozeugmata available, so that only a fraction or none of the eggs may be fertilized. In this case it might be advisable to sequester animals either in the field or in captive holding facilities in order to enhance fertilization success and access to larvae for propagation.

2. Determine fish hosts that support metamorphosis of glochidia and quantify attachment and metamorphosis. Management of endangered mussels must consider the presence and abundance of the host fish as an essential requirement for natural reproduction and identification of suitable hosts is necessary for captive propagation. Only certain fish species and populations support any particular mussel species, and the quality of hosts varies among species. Glochidia of mussels in the subfamily Unioninae, tribe Anodontini (including *A. wheeleri*) possess specializations that permit attachment to skin and fins of the host as well as gills. Anodontini generally lack adaptations for attracting particular host species, and often a relatively large number of host species are utilized with relatively low metamorphosis success on most of these (Trdan and Hoeh 1982, Barnhart et al. 2008, Crownhart 2009). A previous study of *A. wheeleri* reported that glochidia metamorphosed with highest success on centrarchids (Seagraves 2006).

3. Culture the transformed juvenile mussels for 3-6 months after metamorphosis, to a size suitable for caging in mussel silos, bunkers or pond culture in floating upweller systems.

Mussel reproductive output is high, and captive propagation can potentially be used to augment existing populations or to establish new populations.

These objectives address the following actions recommended in the Recovery Plan for *Arkansia wheeleri* (USFWS 2004):

#3. Determine reproduction, habitat, genetics, and captive propagation requirements.

#6. Develop an enhanced management program.

Methods

Recovering glochidia and estimating fertilization and viability

Glochidia were collected by flushing the marsupial gills with water from a syringe. The glochidia were then passed through a filter (~300 microns) to remove larval threads and debris, and then placed in a beaker with a known volume of water. The glochidia were suspended by gently agitating the water using a large rubber-bulb pipette. While agitating the water, a volumetric pipette was used to collect ten 200- μ L samples from the suspension. These samples were placed as individual drops on a 15 cm diameter polycarbonate Petri plate. The numbers of unfertilized eggs, open glochidia and closed glochidia in each drop were enumerated using a stereo microscope. Fertilization success was calculated as the proportion of developed glochidia to total propagules (i.e. glochidia plus undeveloped eggs). Drops of concentrated saline solution were then added to each of the subsamples to induce the closing response (Lefevre and Curtis 1912). Those glochidia that remained open after the addition of the saline solution were counted. Glochidia that were open initially and that closed in response to the salt solution were considered viable.

Host infection

Host fish were infected by swimming in a glochidia suspension of 2000-4000 glochidia L^{-1} . Fish were placed in the solution for 15 minutes while the glochidia were kept in suspension by aeration and intermittent stirring. In some tests, fins and gills of fish were inoculated separately by pipetting glochidia directly onto gills or by dipping the fins in a concentrated glochidia suspension while holding the opercula closed and the head of the fish out of the water. Following the inoculation, fish were removed using a mesh net, rinsed, and placed individually into tanks in a recirculating recovery system (see below).

Attachment and metamorphosis success

Inoculated fish were housed individually in an AHAB recirculating research aquarium system (Aquatic Habitats, Inc. Apopka, Florida). The system was modified for quantitative recovery of glochidia and metamorphosed juveniles (Barnhart 2003). Water condition was maintained by mechanical, biological, carbon filtration and ultraviolet sterilization. Water temperature was recorded hourly (iButton® model DS1922L Maxim Integrated Products, Inc). Each individual tank received water from a manifold at a rate of approximately 1 L* min⁻¹. After the water exited each tank, it flowed through a 150 µm mesh filter to capture glochidia and juveniles. The first day after inoculation and every other day thereafter (unless noted) the water flow through each tank was increased 2-3X for 15 minutes to flush all particulates from the tank. Afterward the filters were rinsed to recover untransformed glochidia and juveniles.

Juveniles were distinguished from glochidia by foot and valve movements. Untransformed glochidia were characterized as open or closed. Filtrates from each fish were monitored using a Bogorov tray at 2-day intervals until at least 4 days passed with no further recovery of glochidia or juveniles. At that time, host fish were examined for any attached glochidia. If none were found, observations were concluded. The number of glochidia that attached to each fish was approximated as the sum of sloughed glochidia and metamorphosed juveniles that were recovered. The percent metamorphosis (%M) for each fish was calculated by dividing the number of juveniles by the sum of glochidia and juveniles recovered from that fish.

Results and Discussion

Brooding females

Two brooding female *A. wheeleri* were collected by AGFC personnel in December 2008 in the Little River, Hempstead County, Arkansas, along the left descending bank opposite the mouth of Hudson Creek (Lat/Long = 33.6292 /-93.90966) approximately 1.5 miles below only railroad bridge crossing between Millwood Dam and mouth of Little River. These females were transferred to Missouri State University, and used as the source of glochidia for host tests carried out on December 10, 2008 (test #1) and December 31, 2008 (test #2).

Glochidia and fertilization success

The glochidia of *Arkansia wheeleri* had mean height of 311 microns (Table 1), which is a typical size for glochidia of Anodontini (Barnhart et al. 2008). The shape of the glochidium is

distinctive, with the sides in lateral view being straight to slightly concave. The ventral hooks are well-developed and bear conspicuous denticles (Figure 1).

The fertilization success of *Arkansia wheeleri* has not been reported previously (Seagraves 2006). The two females observed in the present study both had large proportions of unfertilized eggs (64% and 40%, Table 1 and Figure 2). Male mussels release sperm into the water in clusters termed spermatzeugmata. Females are fertilized when the spermatzeugmata enter with the feeding current.

The small numbers of *A. wheeleri* recovered in quantitative sampling makes population estimates highly uncertain. Only two quantitative estimates of site population density are apparently available. Density of 0.04 ± 0.08 mussels/m² was estimated from 1995 survey data at a 2600 m² site in the Ouachita River near Frenchport, AR (Posey 1997). A density of 0.2 ± 0.24 mussels/m² was estimated for a 1000 m² site on the Little River in Little River Co AR (Seagrave 2006). These population densities of *A. wheeleri* may be in the range where fertilization can be limited by population density. Of course, males must be up-current of females for fertilization to occur, so population density is important in the sense that it affects the upstream proximity of males to females. The two brooding females observed in this study are not an adequate sample, but low fertilization suggests that population density at the site might be below the level needed for successful fertilization. Population density must limit fertilization at some level when male gametes no longer reach females in sufficient numbers. Moles and Layzer (2008) observed that fertilization rate of mucket (*Actinonaias ligamentina*) varied among 3 sites and was lowest (33%, i.e. 67% unfertilized) at the site with lowest population density (0.2 individuals/m²). However, those authors also noted that *Lampsilis cardium* and *Lampsilis fasciola* were >90% fertilized although density of these species was $\leq 0.07/\text{m}^2$.

Host tests

Eight potential host species were tested (Table 2). Water temperature averaged 23 C during experiments. The highest metamorphosis success (30.5%) was obtained with golden shiner in a January trial, although a subsequent trial in February gave less than 10%. The lower success in the February trial could reflect declining condition of the glochidia, although viability (measured by closing response to saline) remained high at 98%. Juveniles were also recovered from dusky stripe shiner (10.1%) and freshwater drum (2.8%). Three species of centrarchids gave less than 1% metamorphosis (Table 2). Mean time to metamorphose and leave the host was

about 8 days on golden shiner at 23 C (Figure 3). Over 90% of the glochidia that failed to transform dropped off within 5 days in most trials. Golden shiner is a potentially useful host for artificial propagation, because it is commonly cultured as bait and forage and is readily available from hatcheries. Future host tests should explore other possible cyprinid hosts that occur sympatrically with *Arkansia*.

The hooked glochidia of mussels in the subfamily Unioninae as well as certain other taxa are able to attach to both skin and gills of host fish (Barnhart et al. 2008). Crownhart (2009) tested two mussel-host species-pairs, by placing glochidia separately on fins or gills. The mean %M values of both *Lasmigona complanata* (Anodontini) on golden shiner and *Megaloniaias nervosa* (Quadrulini) on blue catfish were two times higher when attached to fins than when attached to gills. Glochidia that failed to transform on fins tended to be lost earlier than those that were lost from gills. In the present study, glochidia of *Arkansia* were placed on both skin and fins of largemouth bass. A much smaller number of glochidia attached to fins than to gills (Figure 4), but this result reflects the conditions of the inoculation and is not necessarily representative of attachment in the field. Largemouth bass was not a suitable host and no juveniles were recovered, but it is interesting that the time course of sloughing was similar for both fins and gills (Figure 5) in contrast to Crownhart's results with other species. Fins present a smaller surface area than gills and are more difficult to inoculate effectively, so that gill infection is more practical for captive propagation.

Previous host studies

Previous host fish tests with *A. wheeleri* were reported by Seagrave (2006). Twenty-nine species were tested, with at least a few juveniles recovered from 11 of these species. Species with the highest percent metamorphosis were centrarchids (23-70%). However, these results are suspect. Two of the most successful species (*L. cyanellus*, 70% and *L. macrochirus*, 40%) were retested and yielded negligible results in the second trial (0.7% and 0.2%). An examination of the photographs provided by Seagrave (2006) raises a question regarding identity of juveniles recovered in that study. Only two juveniles were figured, but both of these are significantly different in shape from glochidia of *A. wheeleri* (Figure 6). No scale was provided, so it is not possible to compare size. However, the juveniles illustrated have a broader hinge line, relative to length and height, than *Arkansia* glochidia, and they have a greater length relative to height. The

mean hinge/height ratio of the two juveniles was 0.74 ± 0.004 95% CI, and the length/height ratio was 0.99 ± 0.005 . These ratios differ significantly from *Arkansia* glochidia (Table 2).

At least three explanations can be suggested for the disparate shape of the juveniles. First, growth might have altered shell shape. Early growth generally increases length more than height. However, there is no visible marginal growth of either individual. A second possible explanation is that the individuals were not in full lateral view. Rotation around an axis parallel to the hinge could make them look less tall. However, the entire margin of the shell is in good focus, both individuals appear similar in shape, and slight rotation would not alter the apparent ratio of hinge/length.

The third possibility is that these two juveniles were not *Arkansia*, but rather another mussel species that was present on the host fish when they were collected. Likely suspects are *Pyganodon grandis* and *Utterbackia imbecillis*, both of which are closer in shape to these two juveniles than is *Arkansia* (Figure 7) (Hoggarth and Gaunt 1988, Hoggarth 1999). The sources of host fish were not reported (Seagraves 2006) but *Pyganodon* and *Utterbackia* are widespread and often present in ponds. Although all fish were reportedly inspected for gill parasites including glochidial infestation, it is very difficult to inspect live fish thoroughly for glochidia. The dates of fish collection were not reported, but the first test was apparently begun in mid-late December and the second in February. It was stated that the fish were held for 5 days before the tests. Therefore, the fish were presumably collected in mid-winter. Glochidia of *Pyganodon* were present on hosts from January through May at sites in Canada (Jansen 1991). Although local observations are lacking, it is not implausible that *Pyganodon* are present on fish in December-February in Arkansas.

The timing of recovery of juveniles also argues for contamination of fish in the Seagraves study. In the first trial, which yielded large numbers of juveniles, the juveniles were recovered from day 8 through day 57 following inoculation. In contrast, in the second trial juveniles were recovered only from day 44 to 57 (Figure 5 of Seagraves 2006). Water temperature during both tests was 15 C. There is no apparent reason that the timing of *Arkansia* metamorphosis should have been so variable and generally shorter in the first trial than in the second. However, if other glochidia were already present on the fish collected for the first test (but perhaps not the second) they might have attached at any time prior to collection, and therefore could have completed

metamorphosis at varying times, altering the apparent timing as well as inflating the apparent success of metamorphosis.

The Seagraves study is the most substantial body of work on this critically endangered species, and it is obviously important to investigate whether other information supports the evidence it presents regarding fish hosts. One likely answerable question is where the fish used in trial 1 were collected, and whether glochidia of *Pyganodon* are present on centrarchids from that source in winter. Other measurements, photos or preserved specimens of juveniles, if available, would also be informative.

Culture of juveniles

Attempts to lab-culture the *Arkansia* juveniles were unsuccessful. Survivorship was less than 10% after one month, then stable for two months, but growth was poor and all juveniles died within the third month. It appears that the juveniles did not feed well on the microalgae that have been used successfully for other mussel species, and future trials should provide a food source with bacterial components.

Management actions

If population density is low enough to inhibit fertilization, one management strategy might be to sequester animals to improve fertilization success. This could be done at field sites, but might be logistically difficult and could increase the chances that spates, predation, or other impacts would destroy a large fraction of available animals. A second possible strategy would be to collect mussels and take them into captive culture. Facilities are available where adult mussels could be held in captive culture with reasonable assurance of survival and reproduction, for example at the Virginia Aquatic Wildlife Conservation Center in Marion, VA, and the Alabama Aquatic Biodiversity Center in Marion, AL. Both facilities have held adult mussels of other species through complete reproductive cycles (Mike Pinder, AWCC, and Paul Johnson, AABC, personal communications). This action could greatly facilitate obtaining glochidia for further propagation efforts. This action should be considered seriously and promptly, given the difficulty in obtaining glochidia from the remaining wild populations.

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Table 1. Brood condition of *Arkansia wheeleri* tested Dec 10, 2008. Numbers are in thousands. Figures are estimated from counts of 10 volumetric subsamples from the total sample. Both females had relatively low percent fertilization.

<i>A. Numbers (thousands)</i>	Female ID#	
	2008-A	2008-B
1. Total sample	22,922	42,488
2. Undeveloped eggs	14,672	16,838
3. Glochidia	8,250	25,650
4. Live, open glochidia	7,500	23,850
5. Live, closed glochidia	250	1350
6. Dead glochidia	250	450
<i>B. Proportions</i>		
1. % brood fertile	36.0%	60.3%
2. % brood infertile	64.0%	39.6%
3. % glochidia live	93.9%	98.2%
4. % glochidia dead	6.1%	1.8%
5. % live glochidia open	93.8%	94.6%
6. % live glochidia closed	6.2%	5.4%
7. % total glochidia viable	93.9%	93.0

Table 2. Measurements (mm) and measurement ratios of ten glochidia of *Arkansia wheeleri*. A-P = anterior-posterior. D-V = dorsal-ventral (figure 6).

Specimen	Hinge (A-P)	Length (A-P)	Height (D-V)	hinge/height	length/height
1	0.175	0.259	0.317	0.552	0.817
2	0.1755	0.264	0.309	0.568	0.854
3	0.178	0.254	0.295	0.603	0.861
4	0.181	0.2615	0.318	0.569	0.822
5	0.184	0.2685	0.3125	0.589	0.859
6	0.185	0.2575	0.303	0.611	0.85
7	0.1855	0.2625	0.317	0.585	0.828
8	0.19	0.266	0.3125	0.608	0.851
9	0.193	0.2565	0.3125	0.618	0.821
10	0.1935	0.272	0.312	0.62	0.872
mean	0.184	0.262	0.311	0.592	0.844
stdev	0.0065	0.0055	0.007	0.023	0.02
95% CI	0.0042	0.0035	0.0044	0.0145	0.0122

Table 2. Species and sources of fish tested as potential hosts.

Species	Source
Largemouth bass (<i>Micropterus salmoides</i>)	Missouri Dept. of Conservation, Chesapeake Hatchery, Chesapeake, MO
Hybrid sunfish (<i>Lepomis macrochirus</i> X <i>L. cyanellus</i>):	Missouri Dept. of Conservation, Chesapeake Hatchery, Chesapeake, MO
Orangespot sunfish (<i>Lepomis humilis</i>)	Missouri Dept. of Conservation, Lost Valley Hatchery, Warsaw MO
Walleye (<i>Sander vitreum</i>)	Missouri Dept. of Conservation, Lost Valley Hatchery, Warsaw MO
Golden shiner (<i>Notemigonus crysoleucas</i>)	Missouri Dept. of Conservation, Lost Valley Hatchery, Warsaw MO
Duskystripe shiner (<i>Luxilis pilsbryi</i>)	Collected from the James River, Greene Co MO
Freshwater drum (<i>Aplodinotus grunniens</i>)	Langston University, Langston OK
Blue catfish (<i>Ictalurus furcatus</i>)	AGFC Joe Hogan Hatchery, Lonoke AR

Table 3. Results of host tests.

Species	Trial dates (n tested)	% Metamorphosis success (juveniles/total attached)
Largemouth bass	12/10/08 (20) (on gills)	Zero (0/926)
	12/10/08 (20) (on fins)	Zero (0/44)
Hybrid sunfish	12/31/08 (7)	<1% (1/2631)
Orangespot sunfish	1/13/09 (5)	<1% (2/1198)
Walleye	1/13/09 (2)	Zero (0/262)
Golden shiner	1/13/09 (4)	30.5% (164/537)
	2/19/09 (17)	9.1% (40/438)
Duskystripe shiner	12/10/08 (4)	10.7% (16/149)
Freshwater drum	12/10/08 (4)	2.8% (8/281)
Blue catfish	12/10/08 (4)	Zero (0/899)

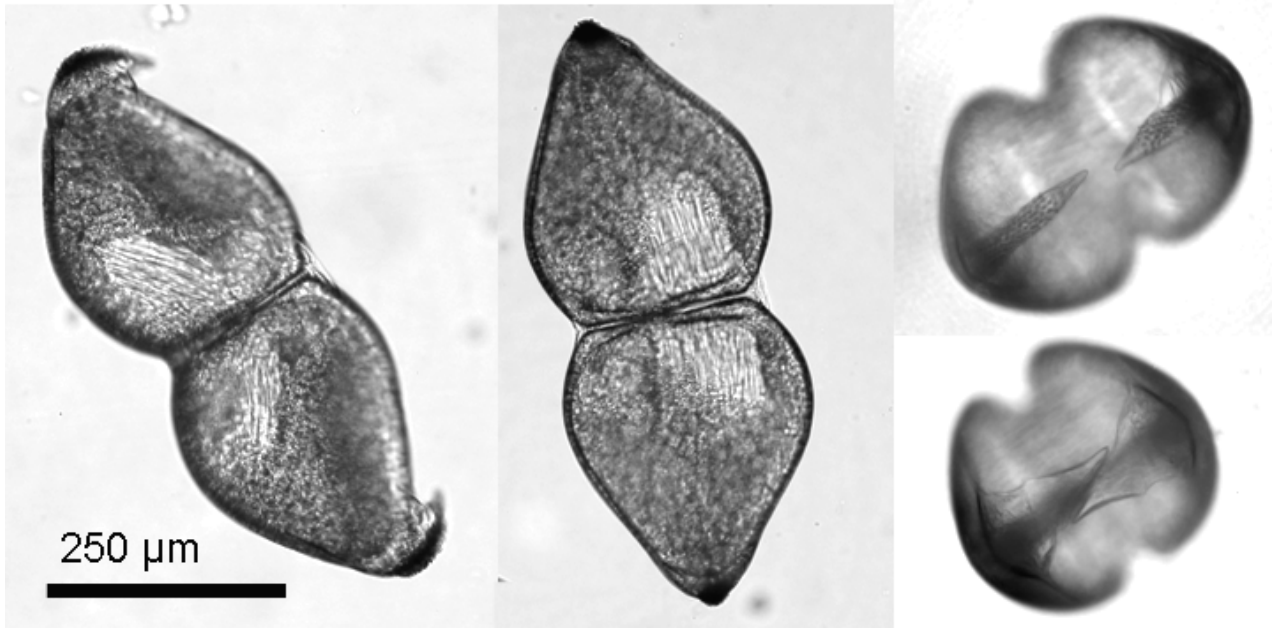


Figure 1. Glochidia of *Arkansia wheeleri*.

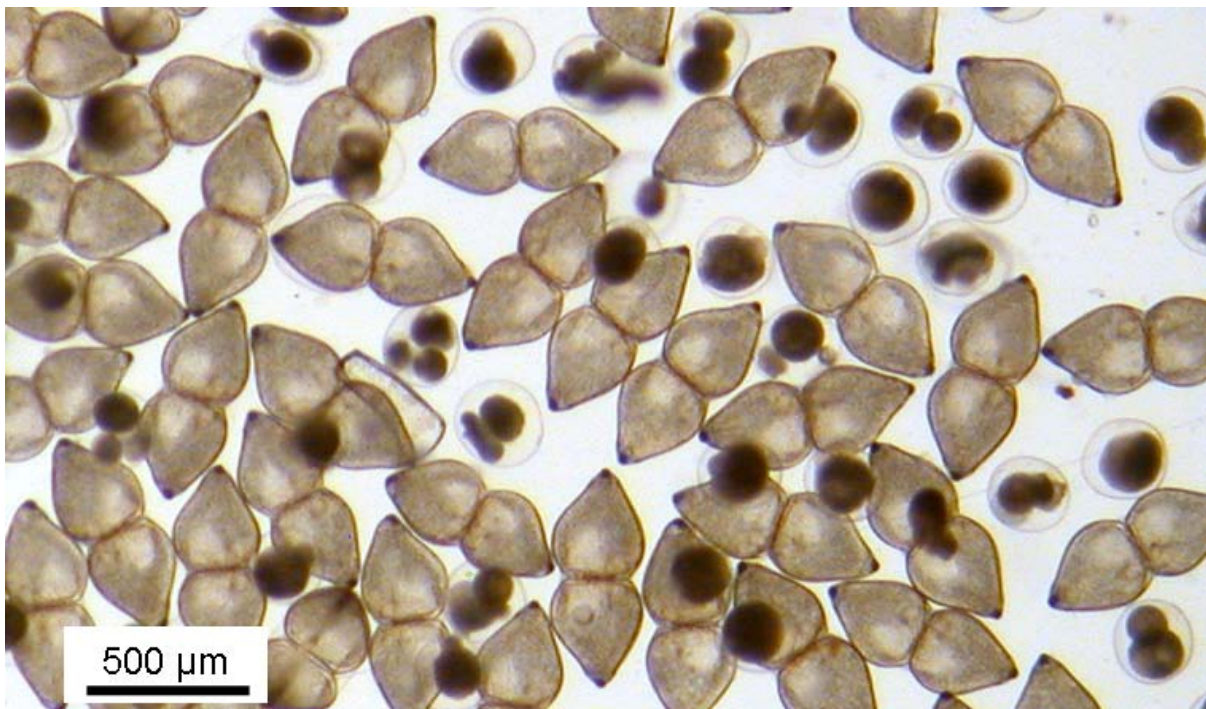


Figure 2. Glochidia and unfertilized eggs of *Arkansia wheeleri*. Many of the undeveloped ova have divided once or even twice, which is not unusual for unfertilized bivalve eggs.

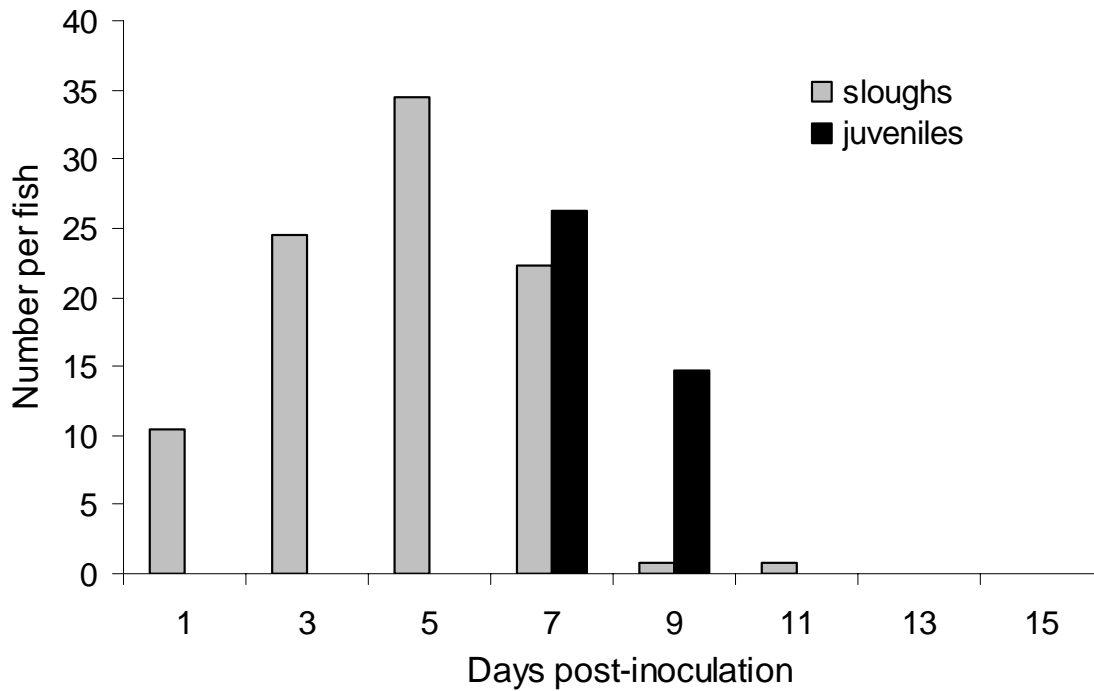


Figure 3. Host test results for *Arkansia wheeleri* with golden shiner (*Notemigonus crysoleucas*) from Lost Valley Hatchery. “Sloughs” were untransformed or dead glochidia. Bars show the mean number of sloughs and juveniles recovered per individual fish. Overall metamorphosis success in this trial was 30.5%.

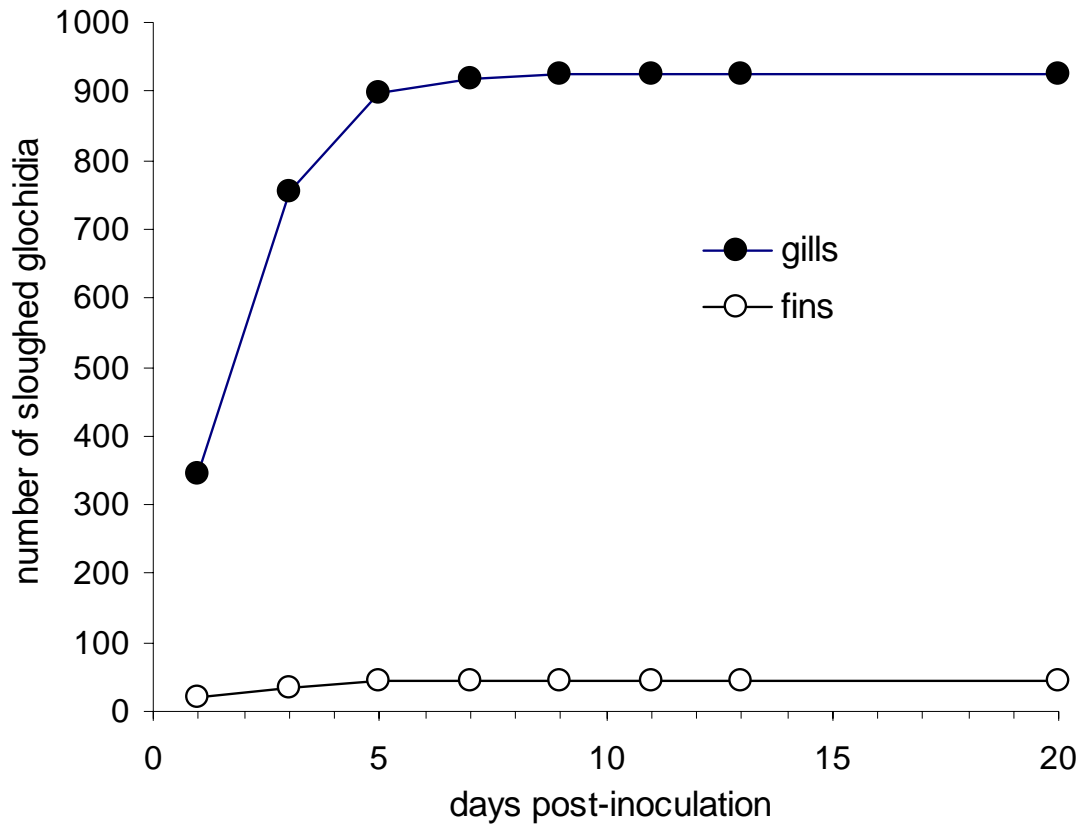


Figure 4. Cumulative recovery of sloughed (unmetamorphosed) glochidia from two groups of largemouth bass inoculated with *Arkansia wheeleri* glochidia on gills or fins. Each point is the total recovered from 20 fish. No metamorphosed juveniles were recovered. Attachment to fins was much less than to gills, but the time course of sloughing was similar from gills and fins and was >90% complete within 5 days (23 C). (See figure 5).

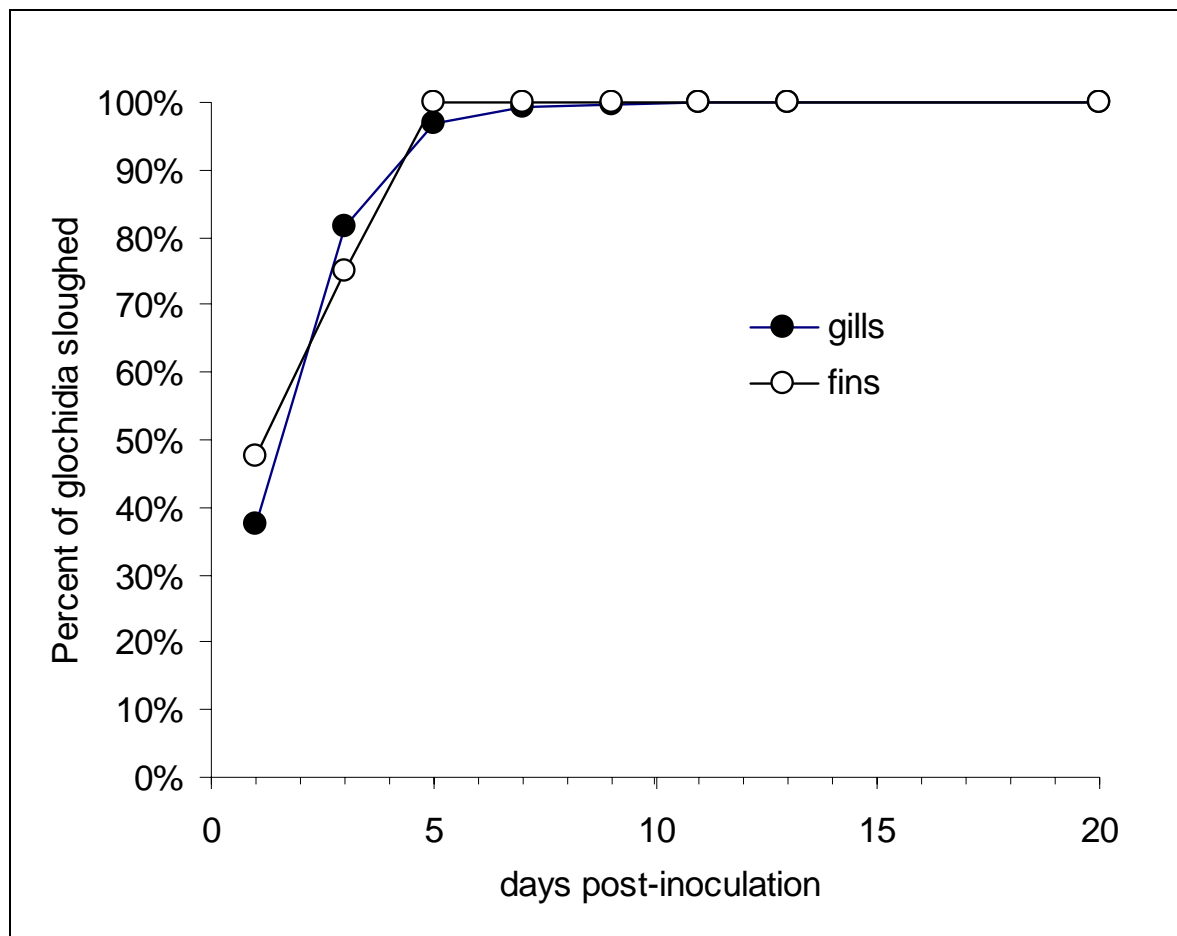


Figure 5. Cumulative recovery of sloughed (unmetamorphosed) glochidia from two groups of largemouth bass inoculated with *Arkansia wheeleri* glochidia on gills or fins. No metamorphosed juveniles were recovered. Time course of sloughing was similar from gills and fins and was >90% complete within 5 days (23C).

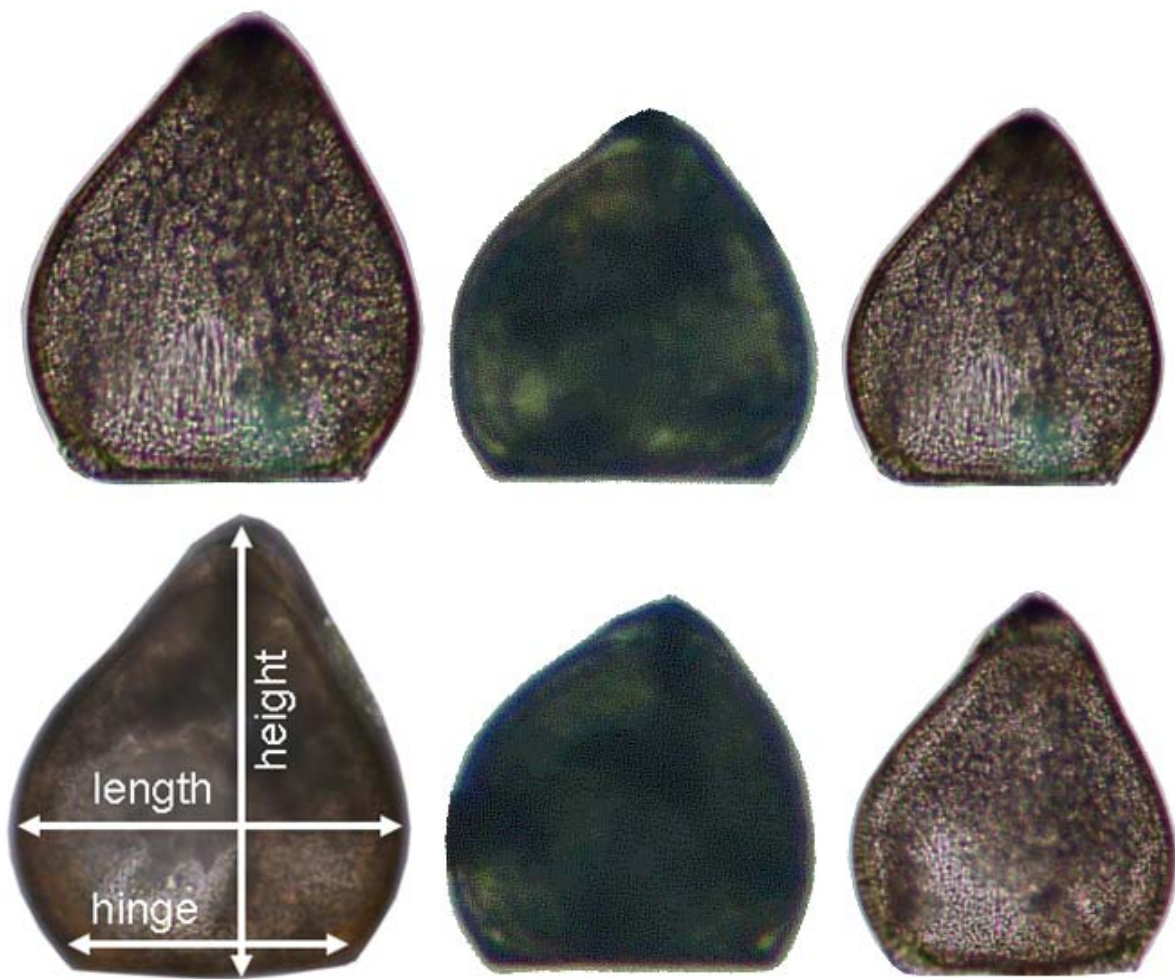


Figure 6. Comparison of *Arkansia wheeleri* glochidia with the two juvenile mussels illustrated by Seagraves (2006, Figure 6D). The Seagraves juveniles are at the center of each row. Each juvenile is flanked by an *A. wheeleri* glochidium (present study) that is scaled to match either the hinge length (left image) or the height (right image) of the juveniles. No absolute scale for size was provided for the juveniles.

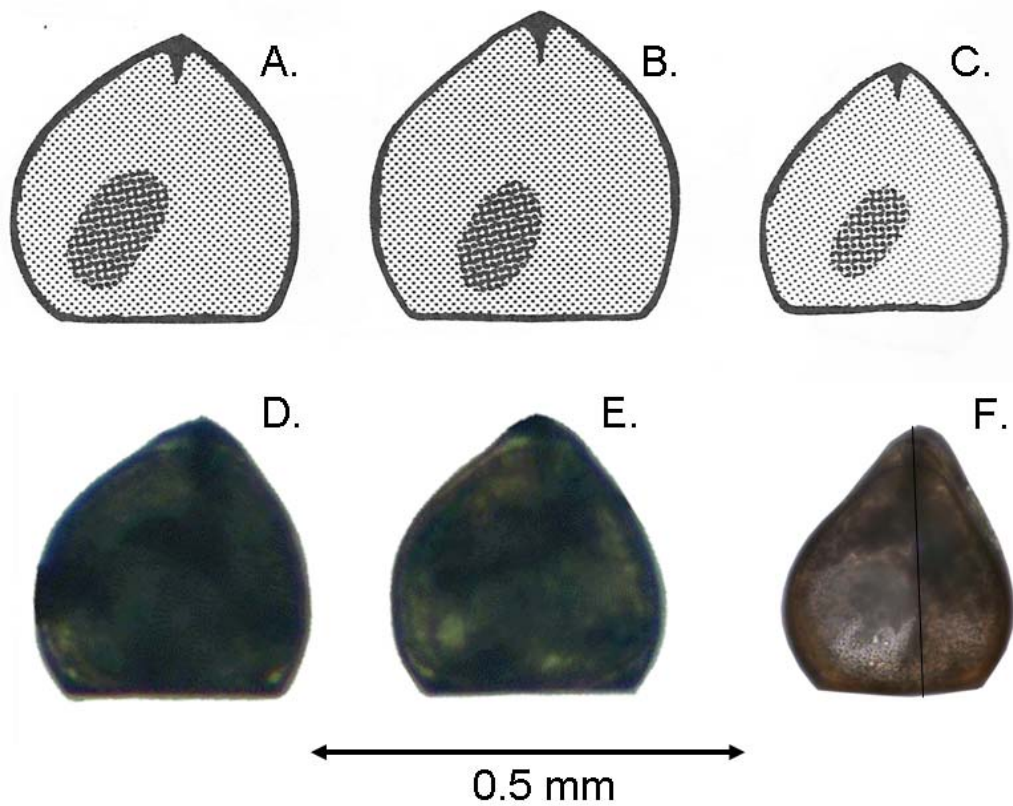


Figure 7. Lateral views of the glochidia of *Pyganodon grandis* (A, B) *Utterbackia imbecillis* (C) the two juvenile mussels (D, E) illustrated by Seagraves (2006) and a glochidium of *Arkansia wheeleri* (F) (present study). A, B, C are from Hoggarth (1999). The scale line refers only to A, B, C, and F. No scale is available for the juveniles.