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# Host fishes and reproductive biology of freshwater mussels in the Buttahatchee River, Mississippi

Final report submitted to the Mississippi Wildlife Heritage Fund,
2001 Research Grant Program

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November 2001

## Executive Summary

Diversity and endangerment of freshwater mussels is higher in the southeastern United States than any other place in the world. The Buttahatchee River, a Tombigbee River tributary, supports a diverse community of freshwater mussels (37 species), many of which are endemic to the Mobile Basin, and several of which are either recognized by the U.S. Fish and Wildlife Service for protection under the Endangered Species Act or by the American Fisheries Society for conservation action. The mussel fauna of the Mobile Basin, Tombigbee River drainage, and Buttahatchee River system have declined in this century, but conservation efforts for freshwater mussels are hampered by a lack of knowledge about their basic biology. In order to complete development, mussel larvae must undergo a brief period as external parasites on the gills or fins of fishes. Larvae of many mussel species can only complete develop on one or few, usually closely related fish species. Hence, knowledge of these host fishes and other aspects of reproductive biology can be critical in determining actions necessary to enhance or maintain freshwater mussel communities.

We report results of a study of host-fish relationships, reproductive periods, and modes of larval release for 7 mussel species occurring in the Buttahatchee River, Mississippi: southern clubshell, Alabama moccasinshell, Alabama spike, gulf pigtoe, Alabama orb, southern pocketbook, and Alabama hickorynut. These species are among the dominant members of the mussel community in the Buttahatchee River. The southern clubshell and Alabama moccasinshell are recognized as endangered and threatened, respectively, by the U.S. Fish and Wildlife Service, and the Alabama spike, southern pocketbook, Alabama hickorynut, and Alabama orb are deemed in need of conservation action by the American Fisheries Society.

We found that these 7 mussel species use a diverse array of fishes including representatives of at least 4 fish families, and that all specialize to varying degrees on particular fish species or related groups of fishes. The southern clubshell and gulf pigtoe exclusively used minnows as host fishes (family Cyprinidae), and produced semi-buoyant larval packets that resembled flatworms, a strategy we believe attracts minnows, like the blacktail shiner, to the larval packets. Three species, the Alabama moccasinshell, Alabama spike, and Alabama hickorynut exclusively used darters as host fishes (family Percidae). The Alabama moccasinshell attracts host fishes using a pulsating, modified mantle as a lure, but we did not observe lures for the other two species. The southern pocketbook exclusively used largemouth bass as a host, attracting that species using a large, mantle lure that resembles a small fish. The Alabama orb used only channel catfish as host, but no host-attracting mechanisms were observed. Even among species sharing a family of host fishes, we found overlap in fish species used as hosts was minimal.

We successfully determined host fishes for all 7 mussel species and documented information concerning periods of reproduction and mechanisms associated with larval transmission to host fishes. Much of this information was previously undocumented. Notably, all the host fishes for the target mussel species are common and widespread in the Mobile Basin, suggesting that factors other than loss or reduction of fish host populations are responsible for recent declines in the Buttahatchee River mussel community.

## Introduction

Freshwater mussels are one of the most endangered groups of organisms in North America. Diversity of freshwater mussels is higher in the southeastern United States than anywhere else in the world (Neves et al. 1997), but much of this fauna is at risk of extinction (Williams et al. 1993). The Buttahatchee River in eastern Mississippi supports one of the most important mussel communities remaining in the Mobile Basin. At least 37 species are known from the river (Hartfield and Jones 1990, Jones 1991), including 6 federally endangered or threatened species.

Lack of knowledge about basic biology of freshwater mussels hampers conservation efforts. In order to complete development, larvae (glochidia) of most mussel species must undergo a brief period as ectoparasites on the gills or fins of fishes. Larvae of many mussel species can develop on only a few, usually closely related host-fish species; larvae encountering an unsuitable host are rejected by the fish immune system. Knowledge of host fishes and other aspects of reproductive biology is lacking or incomplete for many North American mussels. In particular, there is a conspicuous absence of information for species endemic to the Mobile Basin, including the Buttahatchee River. Lack of recruitment in some mussel populations is attributed to unavailability of proper host fishes caused by human-induced changes in the fish community (Smith 1985, Khym and Layzer 2000). Conservation efforts for freshwater mussels, whether centered on captive propagation and reintroduction, relocation, or habitat improvement, require comprehensive information about host-fish use and reproductive biology of target species.

We identified host fishes, reproductive periods, and modes of glochidial release for 7 species of mussels from the Buttahatchee and Sipsey rivers: southern clubshell (Pleurobema decisum), Alabama moccasinshell (Medionidus acutissimus), Alabama spike (Elliptio arca), gulf pigtoe (Fusconaia cerina), Alabama orb (Quadrula asperata), southern pocketbook (Lampsilis ornata), and Alabama hickorynut (Obovaria unicolor). Along with two others (Quadrula rumphiana and Tritogonia verrucosa), these species dominate mussel communities in the Buttahatchee and Sipsey rivers (Haag and Warren, unpublished data). With the exception of F. cerina and L. ornata, all of these species are endemic to the Mobile Basin. Fusconaia cerina also occurs in the Amite, Pearl, and Pascagoula river systems (Williams and Fradkin 1999), and L. ornata is endemic to Gulf of Mexico drainages from the Amite River system east to the Escambia River drainage (Williams and Butler 1994). The U.S. Fish and Wildlife Service recognizes P. decisum and M. acutissimus as endangered and threatened, respectively. The American Fisheries Society considers E. arca threatened, and L. ornata, O. unicolor, and Q. asperata species of special concern (Williams et al. 1993). Published host information exists only for M. acutissimus, but this information is from a headwater population in the Black Warrior River drainage in Alabama (Haag and Warren 1997). No published information is available on host use for the other six mussel species.

## Methods

We determined host fishes by inducing glochidial infestations in laboratory trials and monitoring the rejection of glochidia or production of juvenile mussels. Our methods are described in detail in Haag and Warren (1997) and are based on a standard host-identification

protocol (Zale and Neves 1982). We identified suitable host-fish species as those that produced live juvenile mussels. We identified non-suitable host-fish species as those in which all mussel glochidia were rejected from all individual fishes without producing juvenile mussels. In some cases, some individuals of a particular fish species rejected all glochidia but others produced juvenile mussels; we regarded these species as marginal hosts, signifying that transformation of juvenile mussels was inconsistent with this species. For each mussel species, we ran replicate trials (20-22°C) using glochidia from 2 - 4 gravid females. We exposed glochidia from each mussel species to 15 - 34 fish species (1-10 individuals of each). We chose fish species to represent most families and genera and all common species present at sites inhabited by the mussels (Boschung 1989).

We collected gravid female mussels from the Buttahatchee River, Monroe Co., MS in late June and early July 2001 (Table 1 & 2). We were unable to obtain gravid specimens of Elliptio arca and Obovaria unicolor from the Buttahatchee River; these species were collected from the Sipsey River, Pickens/Greene Co., AL during the same time period (Table 2). Water temperature at the time of collection was 22-25°C. We also present data from host trials conducted with animals collected from the Sipsey River in June and July 1998 (Table 2). We collected mussels by diving and, in shallow areas, by searching the stream bottom using a glass-bottomed bucket. We assessed reproductive status of each individual of the target species by gently prying apart the valves and examining the gills. We recognized gravid females by the presence of distended gills indicating that the gills contained glochidia. We immediately returned male and non-gravid specimens to the stream. We brought gravid mussels into the laboratory and placed them into individual, aerated beakers at room temperature (21-25°C). Elliptio arca, Fusconaia cerina, Pleurobema decisum, and Quadrula asperata released glochidia

into the beakers within 24 to 48 hr; these glochidia were used in host trials immediately upon release. Lampsilis ornata, Medionidus acutissimus, and Obovaria unicolor did not release glochidia in the laboratory. With the exception of M. acutissimus, we harvested glochidia from these species by sacrificing the animal and dissecting the gills. We harvested glochidia from M. acutissimus by flushing the contents of the gills into a beaker using a hypodermic syringe and aged tap water. Because of their federal conservation status, M. acutissimus and P. decisum were released alive at their point of collection within 7 days of collection. Voucher specimens of M. acutissimus and P. decisum representing recently dead shells found at the study sites were deposited at the Mississippi Museum of Natural Science (MMNS), Jackson, MS. Live animals and shells of these species were collected under U.S. Fish and Wildlife Service Endangered Species Subpermitee Authorization Number SA-98-06, Mississippi Department of Wildlife, Fisheries, and Parks Scientific Collecting Permit, and Alabama Department of Conservation Scientific Collecting Permit Number 182. Voucher specimens of all other species used in host trials are deposited at both MMNS and the Illinois Natural History Survey, Champaign, IL.

We collected most potential host fishes from the following streams in the western Mobile Basin: Clear Creek (Black Warrior River system), Winston Co., AL; Bull Mountain Creek (Tombigbee River system), Itawamba Co., MS; Noxubee River (Tombigbee River system), Winston Co., MS; Hashaqua Creek (Tombigbee River system), Noxubee Co., MS. We augmented fish collections with specimens from the following Mississippi River basin streams: Goodwin Creek (Yazoo River system), Panola Co., MS; Lee Creek (Yazoo River system), Lafayette Co., MS; and Little Tallahatchie River (Yazoo River system), Lafayette Co., MS. We collected all fishes from stream sites without mussels or with low mussel densities in order to avoid using fish with pre-existing glochidial infestations or acquired immunity to glochidia (Zale

and Neves 1982). We obtained Ictalurus punctatus, Micropterus salmoides, and Notemigonus crysoleucas from hatchery stock. We maintained all fishes in aerated aquaria in the laboratory and fed them bloodworms (minnows, darters, madtoms), earthworms and minnows (sunfishes), and pelletized fish food (channel catfish).

We were unable to completely document periods of gravidity for the target species in 2001 due to high water at the study sites for much of the late spring and summer. We based descriptions of gravid periods on a composite of field observations from 1996, 1998, 2000, and 2001. We also report observations of mantle displays of gravid mussels and release of glochidia in the field and laboratory.

## Results

In 2001, we conducted 11 host trials for 7 species. We conducted 7 trials with animals from the Buttahatchee River and 4 with animals from the Sipsey River. In addition, we included information from 12 host trials on 6 species conducted in 1998. We collected a total of 12 mussel species alive at the study site in the Buttahatchee River during this study (Table 3).

### Elliptio arca

Elliptio arca is a short-term brooder and is gravid in early summer. We observed gravid female E. arca from May 28 to July 28. We found mature glochidia from June 27 to July 28. In the laboratory, immature glochidia were released in well-formed, cohesive conglutinates. Mature glochidia were released freely and were not contained in conglutinates (Figure 1). Some

glochidia were released in small clusters, which represented degenerating pieces of conglutinate, but these pieces disassociated readily after release. Copious mucus was released with mature glochidia, and many glochidia were bound in this mucus. During release periods, long strands of mucus often issued from the excurrent siphon of releasing females.

Glochidia of E. arca transformed consistently on only two darter species (Percidae): Etheostoma artesiae and Percina nigrofasciata (Table 4). Glochidia transformed inconsistently on Ammocrypta meridiana. Three darter species were unsuitable hosts. An additional 19 species of fishes representing the families Catostomidae, Centrarchidae, Cyprinidae, and Ictaluridae were unsuitable hosts for E. arca.

**Fusconaia cerina**

Fusconaia cerina is a short-term brooder and is gravid in early summer. We observed gravid female F. cerina from May 28 to July 28. We found mature glochidia from June 8 to July 28. In the laboratory, both immature and mature glochidia were released in well-formed, cohesive conglutinates (Figure 2). Conglutinate color was pink, orange, or white. In the field, we frequently observed gravid female F. cerina releasing conglutinates. Releasing females were buried just under the surface of gravel substrates with only the siphons visible. Conglutinates were released in groups of 10 to 20 and were propelled upward approximately 15 to 20 cm into the water column, and then drifted with the current, well above the bottom. We commonly saw drifting conglutinates in the water column and observed schools of blacktail shiners, Cyprinella venusta, repeatedly approaching drifting conglutinates. Because of the fishes' rapid movements, it was not apparent whether shiners ingested conglutinates; in most cases, the fishes approached

conglutinates very closely then appeared to veer off just before contact. If shiners did ingest conglutinates, contact was brief, and conglutinates were expelled quickly. In the laboratory, we presented conglutinates to a wide variety of fishes: minnows (C. venusta, Nocomis leptocephalus, and Notropis ammophilus), darters (Etheostoma rupestre and Percina sciera), and sunfish (Lepomis macrochirus). All fish species responded to the presence of conglutinates by repeatedly approaching them closely as observed in the field, but again, in most cases, it was not apparent if individuals ingested conglutinates. We did observe one individual E. rupestre ingest and expel a conglutinate 3 times in rapid succession.

Glochidia of F. cerina transformed on a wide variety of minnow species (Cyprinidae) (Table 5). Glochidia transformed consistently on 6 minnow species: Cyprinella callistia, C. venusta, Hybopsis winchelli, Luxilus chrysocephalus, Lythrurus bellus, and Notemigonus crysoleucas. Glochidial transformation was inconsistent on six additional minnow species: Campostoma oligolepis, Nocomis leptocephalus, Notropis ammophilus, N. atherinoides, N. stilbius, and Pimephales notatus. Five minnow species were unsuitable hosts. An additional 14 species of fishes representing the families Catostomidae, Centrarchidae, Ictaluridae, and Percidae were unsuitable hosts for F. cerina.

**Lampsilis ornata**

Lampsilis ornata is a long-term brooder and is gravid from late summer to late spring. We found gravid individuals throughout most of the year with the exception of June to September when many females were spent. Glochidia were usually mature from approximately

October to June. Lampsilis ornata did not release glochidia in the laboratory. Gravid females displayed a large mantle lure in the laboratory and field (Figure 3).

Glochidia of L. ornata transformed only on largemouth bass, Micropterus salmoides (Centrarchidae)(Table 6). Four other centrarchid species were unsuitable hosts. In addition, 10 species of fishes representing the families Catostomidae, Cyprinidae, Esocidae, Ictaluridae, and Percidae were unsuitable hosts for L. ornata.

**Medionidus acutissimus**

Medionidus acutissimus is a long-term brooder and is gravid from approximately October to June. Most females are spent from early June to October. By late May, most gravid females were mostly spent and had only 2 to 3 gravid water tubes. Females did not release glochidia (Fig. 4) in the laboratory. In the field, we observed several gravid females in the Sipsey and Buttahatchee rivers displaying small, black mantle margins. When displaying, females were widely agape, completely unburied, lying on top of the substrate, and tethered to a pebble by a byssal thread. Displaying individuals were observed in a variety of orientations with the dorsal margin facing up, the ventral margin facing up, or lying on their side. The modified portion of the mantle margin extended along the ventral margin of the shell from the posterior tip to slightly anterior of the mid-point of the shell. The modified mantle was matte, inky black with a small (approximately 2 $mm^2$) white patch located at about the mid-point of shell, near the anterior-most portion of the modified mantle margin. The white patch flickered rapidly at approximately one second intervals; the motion was similar to the flickering of a television screen. With the exception of the flickering mantle patch, displaying females appeared moribund and showed

little response to handling. When removed from the water, females remained widely agape and did not attempt to close the shell or retract the mantle margins. The flickering motion of the white patch often continued for 15 seconds or more after being removed from the water. These behaviors were not observed in the laboratory.

Glochidia of M. acutissimus transformed on a wide variety of darter species (Percidae)(Table 7). Glochidia transformed consistently on 8 darter species: Ammocrypta beani, A. meridiana, Etheostoma nigrum, E. stigmaeum, E. swaini, E. artesiae, Percina nigrofasciata, P. vigil. One darter species, E. rupestre, was a marginal host. No darter species were identified as unsuitable hosts. Seven species of fishes representing the families Centrarchidae, Cyprinidae, and Ictaluridae were unsuitable hosts for M. acutissimus.

**Obovaria unicolor**

Obovaria unicolor is a long-term brooder and is gravid with mature glochidia from approximately November to June. Glochidial release appears to take place from April to June. We observed fully gravid and partially spent females from April to June. We did not observe fully gravid individuals after June 8. We found only partially spent individuals on June 17 and 26. After June 26, all females were completely spent. By late August, we found gravid females that were brooding embryos. We observed gravid females with mature glochidia by November. Females did not release glochidia (Figure 5) in the laboratory, and we observed no mantle displays in the field or laboratory.

Glochidia of O. unicolor transformed consistently on three darter species (Percidae): Ammocrypta beani, A. meridiana, and Etheostoma artesiae (Table 8). Four additional darter

species were marginal hosts: Etheostoma nigrum, E. swaini, Percina nigrofasciata, and P. sciera. Six darter species were unsuitable hosts. An additional 20 species of fishes representing the families Catostomidae, Centrarchidae, Cyprinidae, Esocidae, and Ictaluridae were unsuitable hosts for O. unicolor.

**Pleurobema decisum**

Pleurobema decisum is a short-term brooder and was gravid in early summer. We observed gravid females from May 28 to July 28. We found mature glochidia from June 8 to July 28. In the laboratory, both immature and mature glochidia were released in well-formed, cohesive conglutinates (Figure 6). Conglutinates were orange or white. In the field, we observed gravid female P. decisum releasing conglutinates in a manner similar to F. cerina (see previous) and commonly observed drifting conglutinates in the water column. In the field, we also observed schools of blacktail shiners, Cyprinella venusta, interacting with P. decisum conglutinates as described for F. cerina. In the laboratory, we presented conglutinates to minnows (Campostoma oligolepis, Cyprinella venusta, Luxilus chrysocephalus, Lythrurus bellus, Nocomis leptocephalus, Notropis atherinoides, and N. baileyi), and darters (Percina sciera). All fish species responded to the presence of conglutinates by repeatedly approaching them closely, but again, it was difficult to ascertain whether or not fishes ingested conglutinates.

Glochidia of P. decisum transformed consistently on one minnow species (Cyprinidae): Cyprinella venusta (Table 9). One additional minnow species was a marginal host: Luxilus chrysocephalus. Fourteen minnow species were unsuitable hosts. An additional 9 species

representing the families Centrarchidae, Ictaluridae, and Percidae were unsuitable hosts for P. decisum.

**Quadrula asperata**

Quadrula asperata is a short-term brooder and was gravid in spring and early summer. We observed gravid females from April 17 to July 28 and found mature glochidia from June 17 to July 28. In the laboratory, immature glochidia were released in well-formed, cohesive conglutinates. Mature glochidia were released freely and were not contained in conglutinates (Figure 7). Copious mucus was released with mature glochidia, and many glochidia were bound in this mucus. During release periods, long strands of mucus often issued from the excurrent siphon of releasing females.

Glochidia of Q. asperata transformed consistently on channel catfish, Ictalurus punctatus (Ictaluridae)(Table 10). One additional catfish species was a marginal host: Noturus leptacanthus. One catfish species, Ameiurus natalis, was an unsuitable host. An additional 32 species representing the families Catostomidae, Cyprinidae, Centrarchidae, Fundulidae, and Percidae were unsuitable hosts for Q. asperata.

## Discussion

The dominant mussel species in the Buttahatchee and Sipsey rivers use a taxonomically diverse array of fishes including representatives of at least 4 fish families. Each of these mussel species, however, is a host specialist to varying degrees, and host use varies widely among

species. Host use for each species is conserved taxonomically; glochidia of each species can transform successfully only on fishes belonging to a single family and in some cases, a single genus. Host use among mussels shows little overlap; few fish species served as host to more than one mussel species.

Two mussel species, Fusconaia cerina and Pleurobema decisum, used minnows exclusively (Cyprinidae) as hosts. Pleurobema decisum was a narrow specialist using only one minnow species consistently and another marginally. Fusconaia cerina also used these same two species but had broader host usage, using 6 species consistently and another 6 marginally. Both mussel species released glochidia in conglutinates that resemble flatworms or other small invertebrates. When released, these conglutinates drift well above the stream bottom where they are especially vulnerable to drift-feeding invertebrate feeders such as minnows.

Three mussel species, Elliptio arca, Medionidus acutissimus, and Obovaria unicolor, used darters exclusively as hosts. Elliptio arca and O. unicolor were narrow host specialists, but glochidia of M. acutissimus transformed on all darter species tested. Host use overlapped among these three species, but primary host use differed between E. arca and O. unicolor. Primary hosts of Elliptio arca were Percina nigrofasciata and Etheostoma artesiae; Ammocrypta meridiana was a marginal host. Primary hosts of O. unicolor were A. beani, A. meridiana, and E. artesiae; P. nigrofasciata was a marginal host. Gravid female M. acutissimus displayed a small, modified mantle margin that may be effective in initiating attacks from small, benthic fishes such as darters but may be relatively inconspicuous to mid-water feeding fishes such as minnows. No host-attraction mechanisms were observed for E. arca or O. unicolor.

Lampsilis ornata and Quadrula asperata were both narrow host specialists, and host use for these species did not overlap with other mussel species. Glochidia of L. ornata transformed

only on largemouth bass (Micropterus salmoides). Other species of Lampsilis can transform on several species of Micropterus (Zale and Neves 1982, Haag et al. 1999), and it is likely that L. ornata also may use spotted bass (M. punctulatus), which occurs in both the Buttahatchee and Sipsey rivers (Boschung 1989). Lampsilis ornata displayed a large, mantle lure similar to those of other species of Lampsilis. These lures elicit attacks from fishes and result in transmission of glochidia to fishes (Haag and Warren 1999). Host use of Q. asperata was limited to channel catfish (Ictalurus punctatus), but glochidia also transformed marginally on the speckled madtom (Noturus leptacanthus). No host attraction mechanisms were observed for Q. asperata.

Mussel populations in the Buttahatchee River have declined over the last 20 years (Hartfield and Jones 1990, Jones 1991). In some rivers, declines in mussel populations are attributed to disappearance or declines of host fishes, resulting in reduced mussel reproductive success (Smith 1985, Khym and Layzer 2000). This mechanism seems insufficient to explain declines of mussels in the Buttahatchee because all 7 species of mussels in this study used common, widespread fishes as hosts. Pleurobema decisum, a federally endangered species, consistently used only one fish species, Cyprinella venusta, as host. Although in some cases, use of only one fish species may be considered a conservation liability, C. venusta is one of the most widespread, abundant, and frequently observed fishes in western Mobile Basin (Boschung 1989, personal observation). Furthermore, this fish seems to be tolerant of highly degraded habitats including impounded and channelized streams (Mettee et al. 1996, personal observation). Similarly, Medionidus acutissimus, a federally threatened species, uses almost any darter species as host, including abundant and widespread species such as Percina nigrofasciata. Obovaria unicolor seems to have experienced the most dramatic recent decline of any species in the Buttahatchee River (Hartfield and Jones 1990, Jones 1991). Primary hosts for this species are

sand darters (Ammocrypta spp.). Although we have no data on current population levels of these fishes in the Buttahatchee River, they are currently abundant in the Noxubee River drainage and other western tributaries of the Tombigbee River in Mississippi (Warren and Haag, unpublished data). Factors other than loss of fish hosts must be sought to explain the decline of mussels in the Buttahatchee River.

## Acknowledgments

We thank the following individuals for their various contributions to this study: J.G. McWhirter, A. Greer, J.L. Staton, T. Fletcher, T. Slack, T. Mann, P. Hartfield, A. Sheldon, D. Carlson, C. Jenkins, F. McEwen. This study was supported by the Mississippi Wildlife Heritage Fund and the Southern Research Station, USDA Forest Service.

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Table 1. Collecting sites for gravid female mussels in the Buttahatchee River, MS, and Sipsey River, AL.

| |
|---|
| Buttahatchee River, approximately 100 m downstream of Cockerham Bridge (Barthatchie Road), 12.1 km N Caledonia, 22.4 km ESE Aberdeen. T15S, R17W, Sec 3. Monroe Co., MS |
| Sipsey River, Site 1, approximately 2.4 km downstream of Pickens Co Rd 2 bridge, 2.3 km SSE Benevola, 4.8 km NNW Mantua, 19.3 km ESE Aliceville. Pickens/Greene Co., AL. |
| Sipsey River, Site 2, at head of island just upstream of Pickens Co Rd 23, 2.4 km NW Lewiston, 13.4 km SE Aliceville. T24N, R1W, Sec 13. Pickens/Greene Co., AL. |
| Sipsey River, Site 3, approximately 0.8 km downstream of boat ramp along Greene Co Rd 156, 7.7 km NNE Mantua, 22.7 km E Aliceville. T22S, R13W, Sec 20. Pickens/Greene Co., AL. |

Table 2. Collecting localities and dates for mussels used in host trials.

| Species | Trial | Collecting locality and date |
|---|---|---|
| *Elliptio arca* | A | Sipsey River, Site 1. 6 July 1998 |
| | B | Sipsey River, Site 1. 27 June 2001 |
| *Fusconaia cerina* | A | Sipsey River, Site 1. 10 June 1998 |
| | B | Sipsey River, Site 1. 8 July 1998 |
| | C | Sipsey River, Site 1. 29 July 1998 |
| | D | Buttahatchee River. 22 June 2001 |
| *Lampsilis ornata* | A | Sipsey River, Site 1. 6 July 1998 |
| | B | Sipsey River, Site 1. 6 July 1998 |
| | C | Buttahatchee River. 28 June 2001 |
| | D | Buttahatchee River. 28 June 2001 |
| *Medionidus acutissimus* | A | Buttahatchee River. 21 June 2001 |
| | B | Buttahatchee River. 28 June 2001 |
| *Obovaria unicolor* | A | Sipsey River, Site 2. 17 June 1998 |
| | B | Sipsey River, Site 2. 26 June 1998 |
| | C | Sipsey River, Site 1. 27 June 2001 |
| | D | Sipsey River, Site 1 . 27 June 2001 |
| *Pleurobema decisum* | A | Sipsey River, Site 1. 14 July 1998 |
| | B | Sipsey River, Site 1. 29 July 1998 |
| | C | Buttahatchee River. 21 June 2001 |
| | D | Sipsey River, Site 3. 3 July 2001 |
| *Quadrula asperata* | A | Sipsey River, Site 1. 26 June 1998 |
| | B | Sipsey River, Site 1. 14 July 1998 |
| | C | Buttahatchee River. 22 June 2001 |

Table 3. Mussel species collected in the Buttahatchee River at Cockerham Bridge, June 2001. Number collected refers to live animals. Except for animals returned to lab for host trials, all live animals were released at the site.

| Species | Number collected |
|---|---|
| *Elliptio arca* | 1 |
| *Epioblasma penita* | 2 females (not gravid); 1 male |
| *Fusconaia cerina* | 14 |
| *Lampsilis ornata* | 2 |
| *Medionidus acutissimus* | 4 |
| *Obovaria unicolor* | 1 |
| *Pleurobema decisum* | 11 |
| *P. perovatum* | 1 |
| *Quadrula asperata* | 56 |
| *Q. rumphiana* | 1 |
| *Tritogonia verrucosa* | 7 |
| *Truncilla donaciformis* | 2 |

Table 4. Results of host trials for Elliptio arca. Letters A-B represent replicate trials using glochidia from 2 different female mussels. The sample size (N) is either the number of fish that produced juvenile mussels (hosts) or the number of fish that rejected all glochidia and produced no juvenile mussels (non-hosts). An asterisk denotes that all fish died before completion of the trial. A dash indicates that the fish species was not used in the trial.

| | Mean No. juveniles/fish (N)<br>Days to transformation | | Days to rejection (N) | |
|---|---|---|---|---|
| | A | B | A | B |
| **Hosts** | | | | |
| Percina nigrofasciata | 8 (3)<br>19-29 | 9 (3)<br>22-35 | na | na |
| Etheostoma artesiae | 5 (2)<br>15-19 | - | na | - |
| **Marginal hosts** | | | | |
| Ammocrypta meridiana | - | 3 (1)<br>33 | - | 4 (1)<br>na |
| **Non-hosts** | | | | |
| Campostoma oligolepis | na | - | 2 (3) | - |
| Cyprinella venusta | na | na | 2-20 (5) | 4 (4) |
| Luxilus chrysocephalus | na | - | 2-7 (4) | - |
| Lythrurus bellus | - | na | - | 4 (4) |
| L. umbratilis | na | - | 2 (2) | - |
| Notemigonus crysoleucas | na | na | 2-7 (3) | 4 (3) |
| Notropis ammophilus | na | na | 2 (4) | 4 (3) |
| N. atherinoides | na | - | 2-20 (2) | - |
| N. baileyi | na | - | 2-7 (2) | - |
| N. stilbius | - | na | - | 4 (3) |
| N. volucellus | na | - | 2 (2) | - |
| Pimephales notatus | na | - | 2 (4) | - |

| | | | | |
|---|---|---|---|---|
| Ictiobus bubalus | na | - | 2 (2) | - |
| Ictalurus punctatus | na | na | 2 (2) | 5 (2) |
| Noturus leptacanthus | na | - | 2-14 (2) | - |
| Lepomis cyanellus | na | na | 14 (2) | 5 (2) |
| L. macrochirus | na | - | 7-14 (2) | - |
| L. megalotis | na | na | 2-7 (3) | 4-5 (2) |
| Micropterus salmoides | na | na | 7-14 (3) | 13-19 (3) |
| Ammocrypta beani | - | na | - | 4 (2) |
| Etheostoma rupestre | na | na | 2 (3) | 4 (1) |
| E. stigmaeum | - | na | - | 4-19 (3) |

Table 5. Results of host trials for Fusconaia cerina. Letters A-D represent replicate trials using glochidia from 4 different female mussels. The sample size (N) is either the number of fish that produced juvenile mussels (hosts), or the number that rejected all glochidia and produced no juvenile mussels (non-hosts). An asterisk denotes that all fish died before completion of the trial. A dash indicates that the fish species was not used in the trial.

| | Mean No. juveniles/fish (N)<br>Days to transformation | | | | Days to rejection (N) | | | |
|---|---|---|---|---|---|---|---|---|
| | A | B | C | D | A | B | C | D |
| **Hosts** | | | | | | | | |
| Cyprinella callistia | - | 2 (1)<br>15 | - | 6 (4)<br>24-32 | - | na | - | na |
| C. venusta (Mobile) | * | 2 (2)<br>19-21 | 15 (7)<br>19-26 | 22 (1)<br>24 | * | na | na | na |
| C. venusta (Miss.) | - | - | - | 1 (4)<br>24-34 | - | - | - | na |
| Hybopsis winchelli | - | - | - | 1 (1)<br>20 | - | - | - | na |
| Luxilus chrysocephalus | * | 1 (3)<br>19-21 | 0*(3)<br>15 | 3 (3)<br>24-31 | * | na | na | na |
| Lythrurus bellus | - | - | - | 2 (3)<br>27-31 | * | - | - | na |
| Notemigonus crysoleucas | * | - | 2 ( 4)<br>14-21 | 1 (1)<br>18-27 | * | - | na | na |
| **Marginal hosts** | | | | | | | | |
| Campostoma oligolepis | * | na | 2 (3)<br>14-16 | 1 (4)<br>18-27 | * | 2 (4) | 5-7 (2) | 3 (1) |
| Nocomis leptocephalus | na | na | 1*(1)<br>14 | 2 (1)<br>18 | 2-5 (1) | 5 (2) | na | 3 (1) |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Notropis ammophilus | na | na | 4 (3)<br>14-19 | 1 (2)<br>20-24 | 2 (2) | 2-14 (3) | 13 (1) | 3 (1) |
| N. atherinoides | - | 1 (1)<br>21 | - | 0(0)<br>na | - | 2 (2) | - | 3 (1) |
| N. stilbius | - | - | - | 2 (3)<br>18-26 | - | - | - | 3-6 (2) |
| Pimephales notatus | * | 0 (0)<br>na | 1* (3)<br>14 | - | * | 2-20 (3) | 12 (1) | - |
| **Non-hosts** | | | | | | | | |
| Hybognathus nuchalis | na | - | - | na | 5 (1) | - | - | 18 (1) |
| Notropis baileyi | na | - | na | - | 1-5 (6) | - | 7-12 (3) | - |
| N. texanus | na | - | - | - | 2 (5) | - | - | - |
| N. volucellus | - | na | - | na | - | 2 (3) | - | 3 (3) |
| Pimephales vigilax | - | - | - | na | - | - | - | 3 (3) |
| Ictiobus bubalus | - | na | - | - | - | 2 (2) | - | - |
| Ameiurus natalis | na | - | - | - | 5 (2) | - | - | - |
| Ictalurus punctatus | na | - | - | na | 5 (3) | - | - | 3 (3) |
| Noturus leptacanthus | na | - | - | - | 5 (3) | - | - | - |
| Lepomis cyanellus | na | - | - | - | 2-5 (3) | - | - | - |
| L. macrochirus | na | na | - | - | 5-12 (3) | 2 (2) | - | - |
| L. megalotis | na | na | - | na | 2 (3) | 2 (2) | - | 3 (2) |
| Micropterus salmoides | na | - | na | na | 2-5 (3) | - | 2-7 (2) | 3 (2) |
| Pomoxis annularis | na | - | - | - | 5 (1) | - | - | - |
| Etheostoma rupestre | na | na | na | - | 1-2 (5) | 2 (3) | 7 (5) | - |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| E. stigmaeum | - | - | - | na | - | - | - | 3 (3) |
| E. artesiae | na | - | na | - | 5 (3) | - | 7 (3) | - |
| Percina nigrofasciata | na | - | - | na | 2 (4) | - | - | 3-18 (3) |
| P. sciera | - | na | - | - | - | 2-7 (2) | - | - |

Table 6. Results of host trials for Lampsilis ornata. Letters A-D represent replicate trials using glochidia from 4 different female mussels. The sample size (N) is either the number of fish that produced juvenile mussels (hosts), or the number that rejected all glochidia and produced no juvenile mussels (non-hosts). An asterisk denotes that all fish died before completion of the trial. A dash indicates that the fish species was not used in the trial.

| | Mean No. juveniles.fish (N)<br>Days to transformation | | | | Days to rejection (N) | | | |
|---|---|---|---|---|---|---|---|---|
| | A | B | C | D | A | B | C | D |
| **Hosts** | | | | | | | | |
| Micropterus salmoides | 3 (3)<br>39-57* | 2 (3)<br>54* | 12 (4)<br>42-81 | 6 (4)<br>39-94 | na | na | na | na |
| **Non-hosts** | | | | | | | | |
| Esox americanus | na | - | - | - | 2 (1) | - | - | - |
| Campostoma oligolepis | na | na | - | - | 2 (2) | 2 (2) | - | - |
| Cyprinella venusta | na | na | - | - | 2 (3) | 2 (3) | - | - |
| Notemigonus crysoleucas | na | na | - | - | 2 (3) | 2 (3) | - | - |
| Notropis ammophilus | na | na | - | - | 2 (3) | 2 (3) | - | - |
| Pimephales notatus | na | na | - | - | 2 (3) | 2 (3) | - | - |
| Ictiobus bubalus | na | - | - | - | 2 (2) | - | - | - |
| Ictalurus punctatus | na | na | - | - | 2 (2) | 2 (2) | - | - |
| Ambloplites ariommus | - | na | - | - | - | 2-14 (2) | - | - |
| Lepomis cyanellus | na | na | na | - | 7-14 (2) | 2-9 (2) | 10 (1) | - |
| L. macrochirus | na | na | na | na | 7 (3) | 2 (3) | 10 (2) | 15-22 (3) |
| L. megalotis | na | na | na | na | 2-7 (3) | 2 (3) | 4-10 (3) | 7-15 (4) |
| Etheostoma rupestre | na | na | - | - | 2 (3) | 2 (3) | - | - |
| Percina nigrofasciata | na | na | - | - | 2 (2) | 2 (2) | - | - |

Table 7. Results of host trials for Medionidus acutissimus. Letters A-B represent replicate trials using glochidia from 2 different female mussels. The sample size (N) is either the number of fish that produced juvenile mussels (hosts), or the number that rejected all glochidia and produced no juvenile mussels (non-hosts). An asterisk denotes that all fish died before completion of the trial. A dash indicates that the fish species was not used in the trial.

| | Mean No. juveniles/fish (N)<br>Days to transformation | | Days to rejection (N) | |
|---|---|---|---|---|
| | A | B | A | B |
| **Hosts** | | | | |
| Ammocrypta beani | 13 (2)<br>28-51 | 0 (4)<br>25* | na | na |
| A. meridiana | 4 (2)<br>32-38* | - | na | - |
| Etheostoma nigrum | 3 (2)<br>32-34* | - | na | - |
| E. stigmaeum | 2 (5)<br>32-45 | 0 (3)<br>35* | na | na |
| E. swaini | 19 (1)<br>32-51 | 2 (2)<br>52 | na | na |
| E. artesiae | 20 (1)<br>38-42 | - | na | - |
| Percina nigrofasciata | 9 (3)<br>25-45 | 2 (5)<br>31* | na | na |
| P. vigil | 36 (1)<br>38-65 | - | na | - |
| **Marginal hosts** | | | | |
| Etheostoma rupestre | 2 (1)<br>25-38 | 0 (1)<br>31* | 7 (2) | 4 (2) |

**Non-hosts**

| | | | | |
|---|---|---|---|---|
| Cyprinella venusta | na | - | 2-4 (4) | - |
| Notemigonus crysoleucas | na | - | 4 (2) | - |
| Ictalurus punctatus | na | - | 4 (4) | - |
| Lepomis cyanellus | na | na | 7 (1) | 4 (1) |
| L. macrochirus | na | na | 7-11 (3) | 4 (1) |
| L. megalotis | na | na | 11 (1) | 4 (1) |
| Micropterus salmoides | na | - | 7 (3) | - |

Table 8. Results of host trials for Obovaria unicolor. Letters A-D represent replicate trials using glochidia from 4 different female mussels. The sample size (N) is either the number of fish that produced juvenile mussels (hosts), or the number that rejected all glochidia and produced no juvenile mussels (non-hosts). An asterisk denotes that all fish died before completion of the trial. A dash indicates that the fish species was not used in the trial.

| | Mean No. juveniles.fish (N)<br>Days to transformation | | | | Days to rejection (N) | | | |
|---|---|---|---|---|---|---|---|---|
| | A | B | C | D | A | B | C | D |
| **Hosts** | | | | | | | | |
| Ammocrypta beani | - | - | 2 (3)<br>21-53* | 3 (6)<br>20-24* | - | - | na | na |
| A. meridiana | - | - | 2 (3)<br>21-37* | 0 (5)<br>11* | - | - | na | na |
| Etheostoma artesiae | 3 (2)<br>18-22 | 10 (3)<br>21-41 | 3 (1)<br>18-26 | - | na | na | na | - |
| **Marginal hosts** | | | | | | | | |
| Etheostoma nigrum | - | - | 0 (25)* | 0 (0) | - | - | na | 11 (1) |
| E. swaini | - | 0 (0)<br>na | 0 (0)<br>na | 1 (1)<br>24 | - | 6 (1) | 11 (2) | 11 (1) |
| P. nigrofasciata | 1 (1)<br>22 | 0 (0)<br>na | 8 (1)<br>26-28 | 2 (1)<br>24 | 11-19<br>(6) | 6-14 (3) | 11-19<br>(3) | 11-18<br>(7) |
| P. sciera | na | 1 (1)<br>23 | na | - | 19-22<br>(3) | 23 (1) | 11 (1) | - |
| **Non-hosts** | | | | | | | | |
| Esox americanus | 4 (1) | - | - | - | na | - | - | - |
| Campostoma oligolepis | na | na | - | - | 2 (4) | 2 (2) | - | - |
| Cyprinella venusta | na | na | - | - | 2 (3) | 2 (3) | - | - |
| Luxilus chrysocephalus | na | na | - | - | 2 (3) | 2 (2) | - | - |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Notemigonus crysoleucas | na | | - | - | 2 (3) | | - | - |
| Notropis ammophilus | na | na | - | na | 2 (4) | 2 (4) | - | 5 (4) |
| N. atherinoides | - | na | - | - | - | 2 (2) | - | - |
| N. stilbius | na | - | - | na | 4 (2) | - | - | 5 (2) |
| N. texanus | na | - | - | - | 2 (2) | - | - | - |
| N. volucellus | - | na | - | - | - | 2 (2) | - | - |
| Pimephales notatus | na | na | - | - | 2 (4) | 2 (5) | - | - |
| P. vigilax | - | na | - | - | - | 2 (1) | - | - |
| Ictiobus bubalus | na | - | - | - | 2 (3) | - | - | - |
| Ameiurus natalis | - | na | - | - | - | 2 (1) | - | - |
| Ictalurus punctatus | na | na | - | - | 2 (2) | 2 (1) | - | - |
| Noturus leptacanthus | na | - | - | - | 2 (2) | - | - | - |
| Lepomis cyanellus | - | - | na | - | - | - | 11 (2) | - |
| L. macrochirus | na | na | na | - | 4-7 (4) | 2-6 (2) | 11 (2) | - |
| L. megalotis | na | na | na | - | 2-4 (3) | 2 (3) | 11 (2) | - |
| Micropterus salmoides | na | na | na | - | 7 (3) | 6 (2) | 11 (3) | - |
| Etheostoma caeruleum | na | - | - | - | 2 (1) | - | - | - |
| E. ruflineatum | - | na | - | - | - | 2 (2) | - | - |
| E. rupestre | na | na | - | na | 2-5 (5) | 2-6 (4) | - | 5 (1) |
| E. stigmaeum | na | na | na | na | 4 (2) | 2 (1) | 11 (4) | 5-11 (5) |
| Percina kathae | na | na | - | - | 2 (2) | 2 (1) | - | - |
| P. vigil | na | - | - | - | 2 (1) | - | - | - |

Table 9. Results of host trials for Pleurobema decisum. Letters A-D represent replicate trials using glochidia from 4 different female mussels. The sample size (N) is either the number of fish that produced juvenile mussels (hosts), or the number that rejected all glochidia and produced no juvenile mussels (non-hosts). An asterisk denotes that all fish died before completion of the trial. A dash indicates that the fish species was not used in the trial.

| | Mean No. juveniles.fish (N)<br>Days to transformation | | | | Days to rejection (N) | | | |
|---|---|---|---|---|---|---|---|---|
| | A | B | C | D | A | B | C | D |
| **Hosts** | | | | | | | | |
| Cyprinella venusta | 2 (3)<br>24 | 2 (10)<br>16-22 | 0 (5)<br>5* | 2 (5)<br>23-37 | na | na | na | na |
| **Marginal hosts** | | | | | | | | |
| Luxilus chrysocephalus | 0 (3)<br>23* | na | 2 (5)<br>17-21 | 1 (1)<br>17 | na | 2-13 (4) | na | 10 (2) |
| **Non-hosts** | | | | | | | | |
| Campostoma oligolepis | na | na | na | - | 2-9 (4) | 2-6 (5) | 6 (2) | - |
| Cyprinella callistia | na | na | na | na | 2 (1) | 2 (1) | 6-11 (3) | 3-17 (4) |
| Hybopsis winchelli | - | na | - | - | - | 2 (1) | - | - |
| Lythrurus bellus | na | na | na | na | 2 (2) | 2 (2) | 3-6 (4) | 3 (4) |
| Nocomis leptocephalus | na | na | - | na | 2 (2) | 2 (2) | - | 10 (1) |
| Notemigonus crysoleucas | na | na | - | na | 2-15 (4) | 2-9 (4) | - | 3 (3) |
| Notropis ammophilus | - | na | na | - | - | 2 (2) | 3 (5) | - |
| N. atherinoides | na | na | na | - | 2 (2) | 2 (2) | 3 (3) | - |
| N. baileyi | na | na | - | na | 2 (4) | 2 (4) | - | 3 (1) |
| N. stilbius | na | - | na | na | 2 (2) | - | 3 (5) | 2-3 (4) |
| N. texanus | - | na | na | na | - | 2 (2) | 3 (2) | 3 (2) |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| N. volucellus | na | na | na | - | 2 (4) | 2 (3) | 3 (2) | - |
| Pimephales notatus | na | na | na | - | 2 (3) | 2 (1) | 3 (1) | - |
| P. vigilax | - | na | - | - | - | 2 (1) | - | - |
| Noturus leptacanthus | na | - | - | - | 2 (2) | - | - | - |
| Lepomis cyanellus | - | na | - | - | - | 6 (3) | - | - |
| L. macrochirus | na | - | - | - | 2 (2) | - | - | - |
| L. megalotis | na | na | - | - | 2 (2) | 2 (1) | - | - |
| Micropterus salmoides | na | na | - | - | 2-15 (3) | 2-6 (4) | - | - |
| E. rupestre | na | na | - | - | 2 (4) | 2 (5) | - | - |
| E. artesiae | na | na | - | - | 9-15 (2) | 2-6 (3) | - | - |
| Percina nigrofasciata | - | - | na | - | - | - | 3 (1) | - |
| P. sciera | na | - | - | - | 2 (2) | - | - | - |

Table 10. Results of host trials for Quadrula asperata. Letters A-C represent replicate trials using glochidia from 3 different female mussels. The sample size (N) is either the number of fish that produced juvenile mussels (hosts), or the number that rejected all glochidia and produced no juvenile mussels (non-hosts). An asterisk denotes that all fish died before completion of the trial. A dash indicates that the fish species was not used in the trial.

| | Mean No. juveniles/fish (N)<br>Days to transformation | | | Days to rejection (N) | | |
|---|---|---|---|---|---|---|
| | A | B | C | A | B | C |
| **Hosts** | | | | | | |
| Ictalurus punctatus | 4 (6)<br>25-27 | 0* (3)<br>22* | 2 (5)<br>17-34 | na | na | na |
| **Marginal hosts** | | | | | | |
| Noturus leptacanthus | na | na | 1 (1)<br>31 | 3 (2) | 2 (2) | 26 (1) |
| **Non-hosts** | | | | | | |
| Campostoma oligolepis | na | - | - | 3 (4) | - | - |
| Cyprinella callistia | na | - | - | 3 (2) | - | - |
| C. camura | - | na | - | - | 2 (3) | - |
| C. venusta | na | na | na | 3-6 (5) | 2 (2) | 4 (3) |
| Luxilus chrysocephalus | na | - | - | 3 (4) | - | - |
| Lythrurus bellus | na | - | - | 3 (2) | - | - |
| Nocomis leptocephalus | na | - | - | 3 (2) | - | - |
| Notemigonus crysoleucas | na | na | na | 3-6 (3) | 2 (3) | 4-7 (3) |
| Notropis ammophilus | na | - | - | 3 (4) | - | - |
| N. atherinoides | na | na | - | 3 (3) | 2 (2) | - |
| N. baileyi | na | na | - | 3-6 (3) | 2 (2) | - |
| N. stilbius | na | - | - | 3 (1) | - | - |
| N. texanus | na | - | - | 3 (2) | - | - |
| N. volucellus | na | na | - | 3 (3) | 2 (2) | - |

| | | | | | | |
|---|---|---|---|---|---|---|
| Pimephales notatus | na | - | - | 3 (4) | - | - |
| P. vigilax | - | - | - | - | - | - |
| Ictiobus bubalus | na | na | - | 3-6 (5) | 2 (2) | - |
| Moxostoma poecilurum | na | - | - | 3 (1) | - | - |
| Ameiurus natalis | - | na | na | - | 6* (2) | 4-18 (3) |
| Fundulus olivaceus | na | - | - | 18 (1) | - | - |
| Lepomis cyanellus | na | - | na | 3 (2) | - | 4 (2) |
| L. macrochirus | na | na | - | 3 (2) | 2 (2) | - |
| L. megalotis | na | na | na | 3 (2) | 2 (2) | 4 (2) |
| Micropterus salmoides | na | na | na | 3 (3) | 2 (2) | 4 (3) |
| Pomoxis annularis | na | - | - | 3 (2) | - | - |
| Ammocrypta meridiana | - | - | na | - | - | 4 (2) |
| Etheostoma rupestre | na | na | - | 3-6 (4) | 2 (3) | - |
| E. stigmaeum | - | - | - | - | - | - |
| E. artesiae | - | - | - | - | - | - |
| Percina kathae | na | - | - | 3 (1) | - | - |
| P. nigrofasciata | na | - | na | 3 (3) | - | 4 (2) |
| P. sciera | na | - | - | 3-6 (2) | - | - |

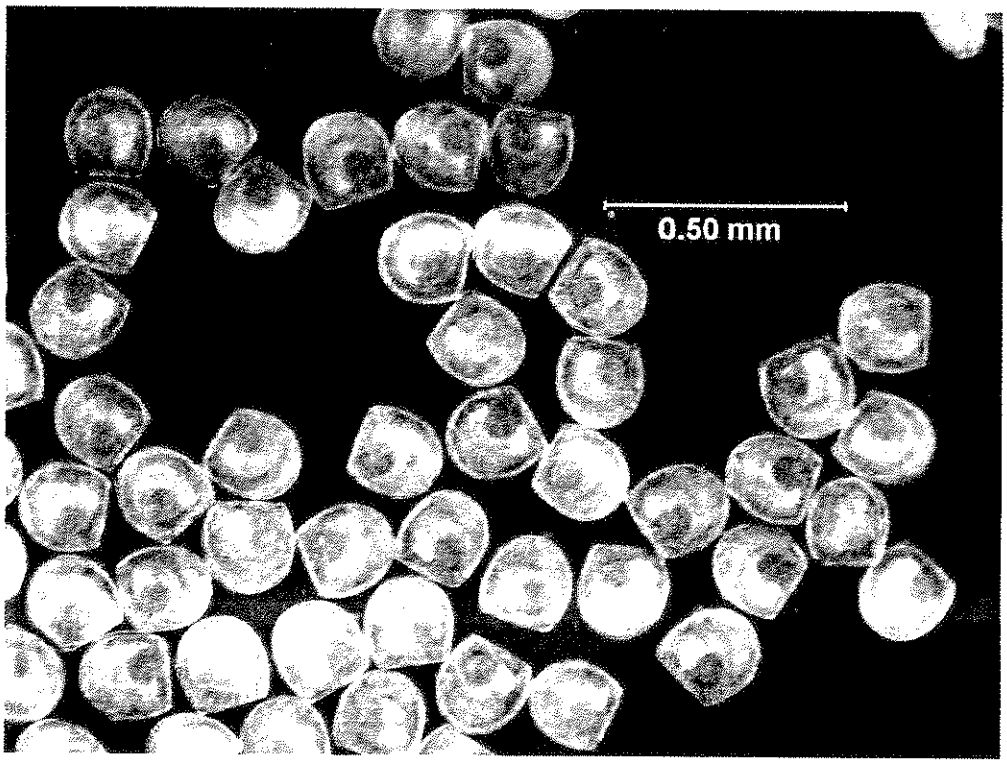


Figure 1. Glochidia of *Elliptio arca* from the Sipsey River, Pickens/Greene Co., AL

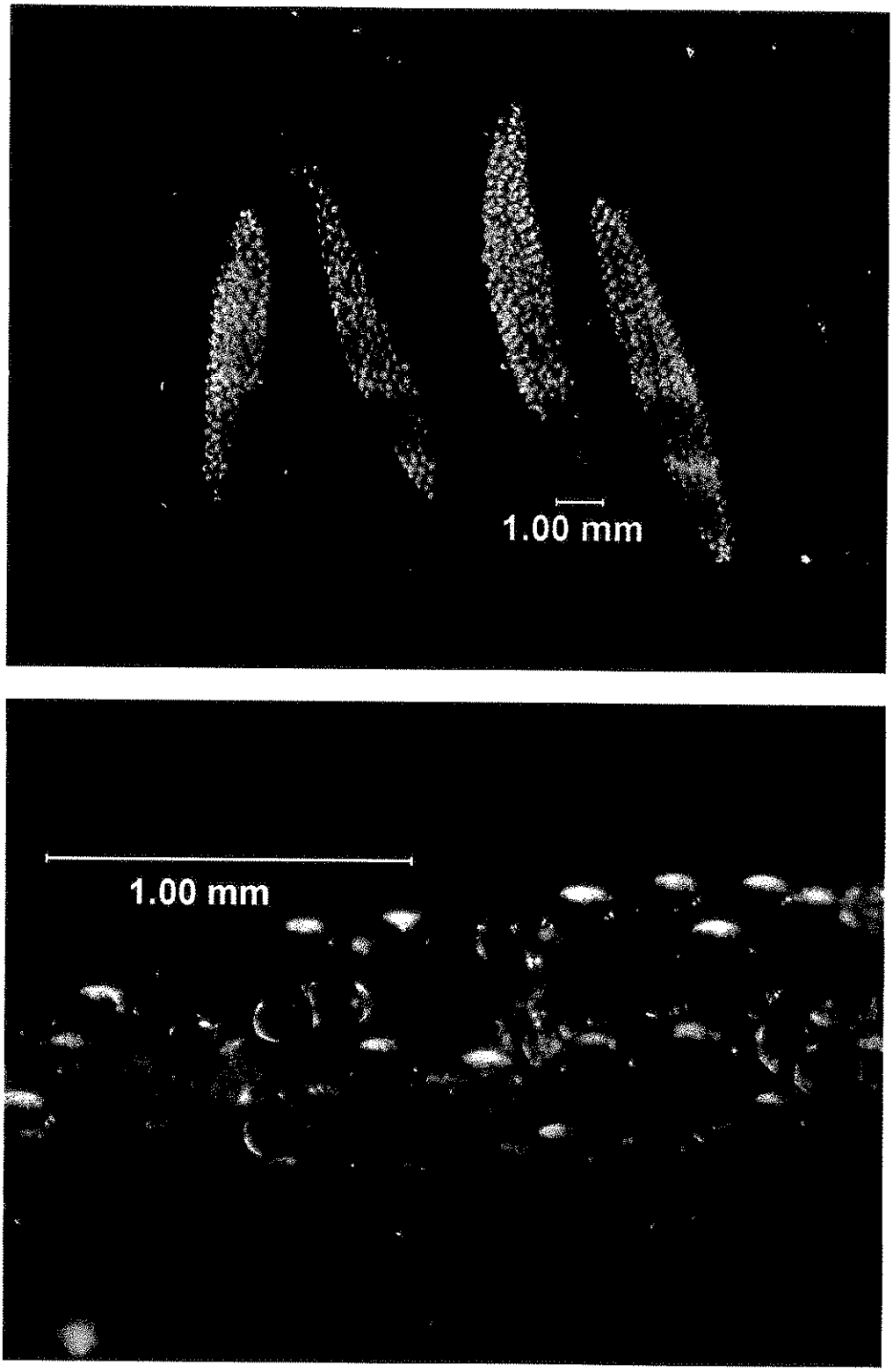


Figure 2. Conglutinates of *Fusconaia cerina* from the Buttahatchee River, Monroe Co., MS. Conglutinates in upper frame contain developing embryos. Lower frame shows detail of a conglutinate with mature glochidia.

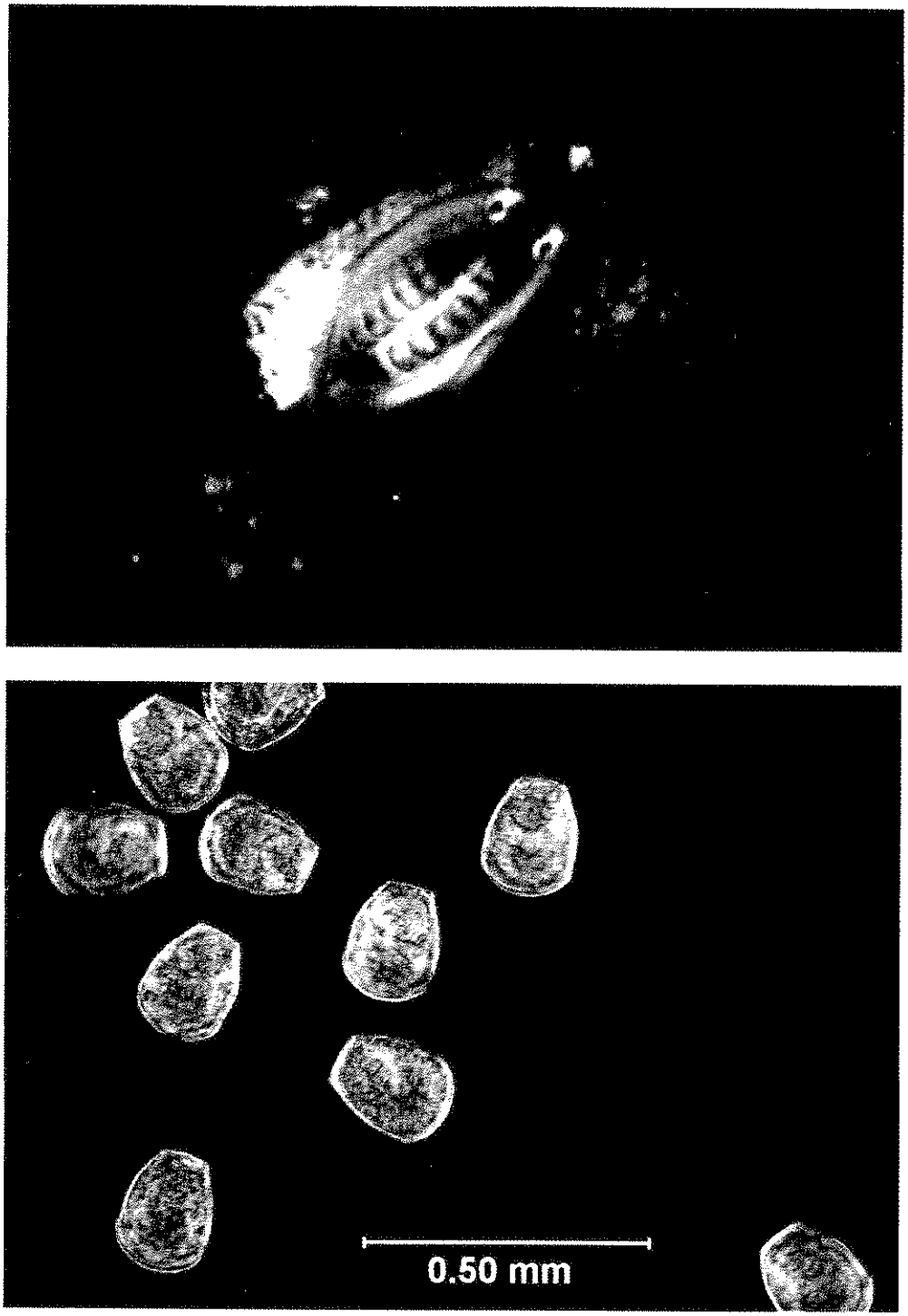


Figure 3. Gravid female *Lampsilis ornata* displaying mantle lure in the Sipsey River, Pickens/Greene Co., AL (upper frame), and glochidia of *L. ornata* from the Buttahatchee River, MS (lower frame).

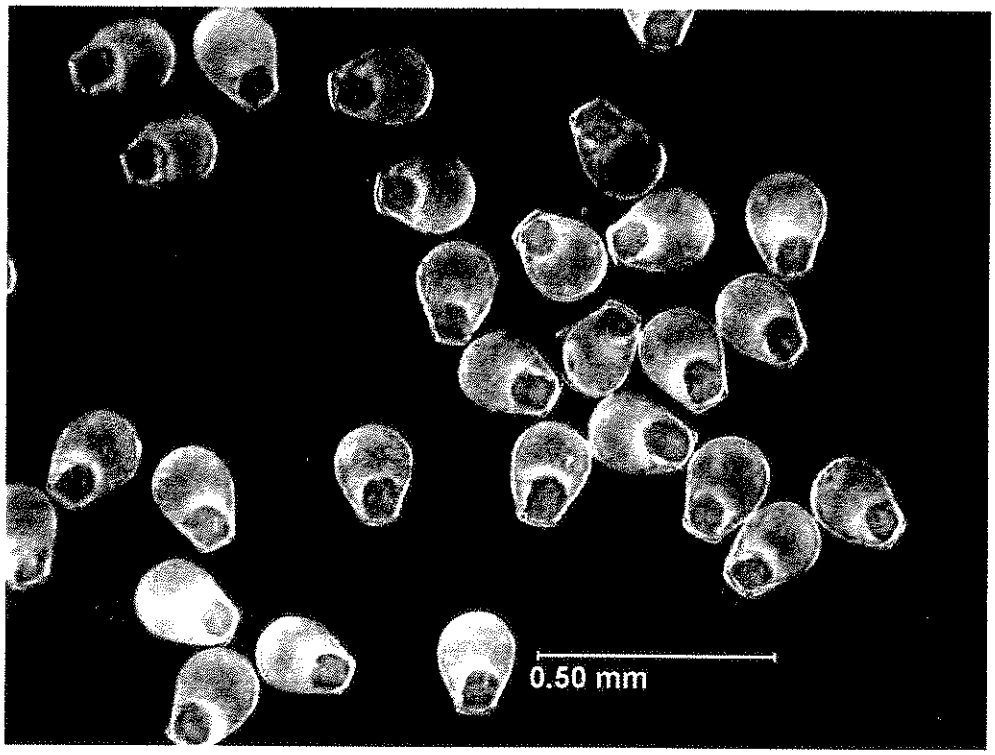


Figure 4. Glochidia of *Medionidus acutissimus* from the Buttahatchee River, Monroe Co., MS

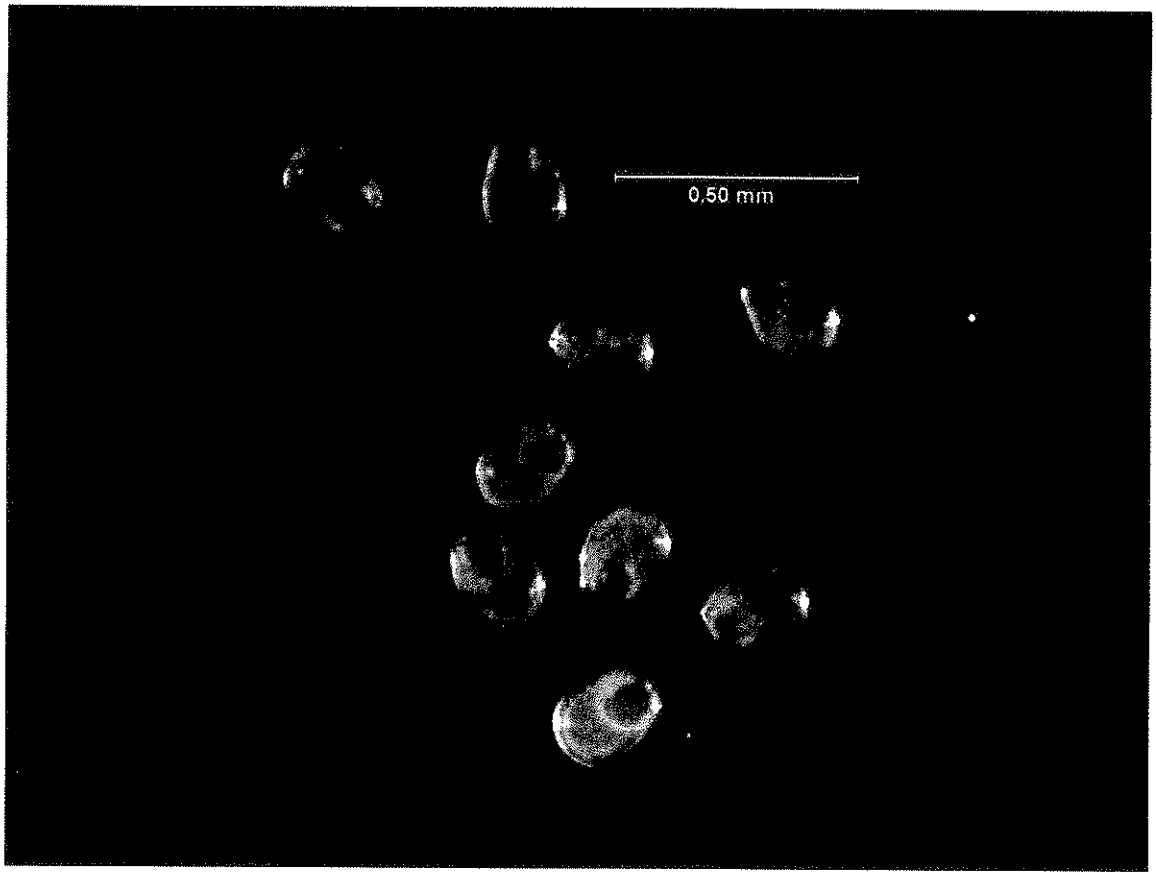


Figure 5. Glochidia of *Obovaria unicolor* from the Sipsey River, Pickens/Greene Co., MS.

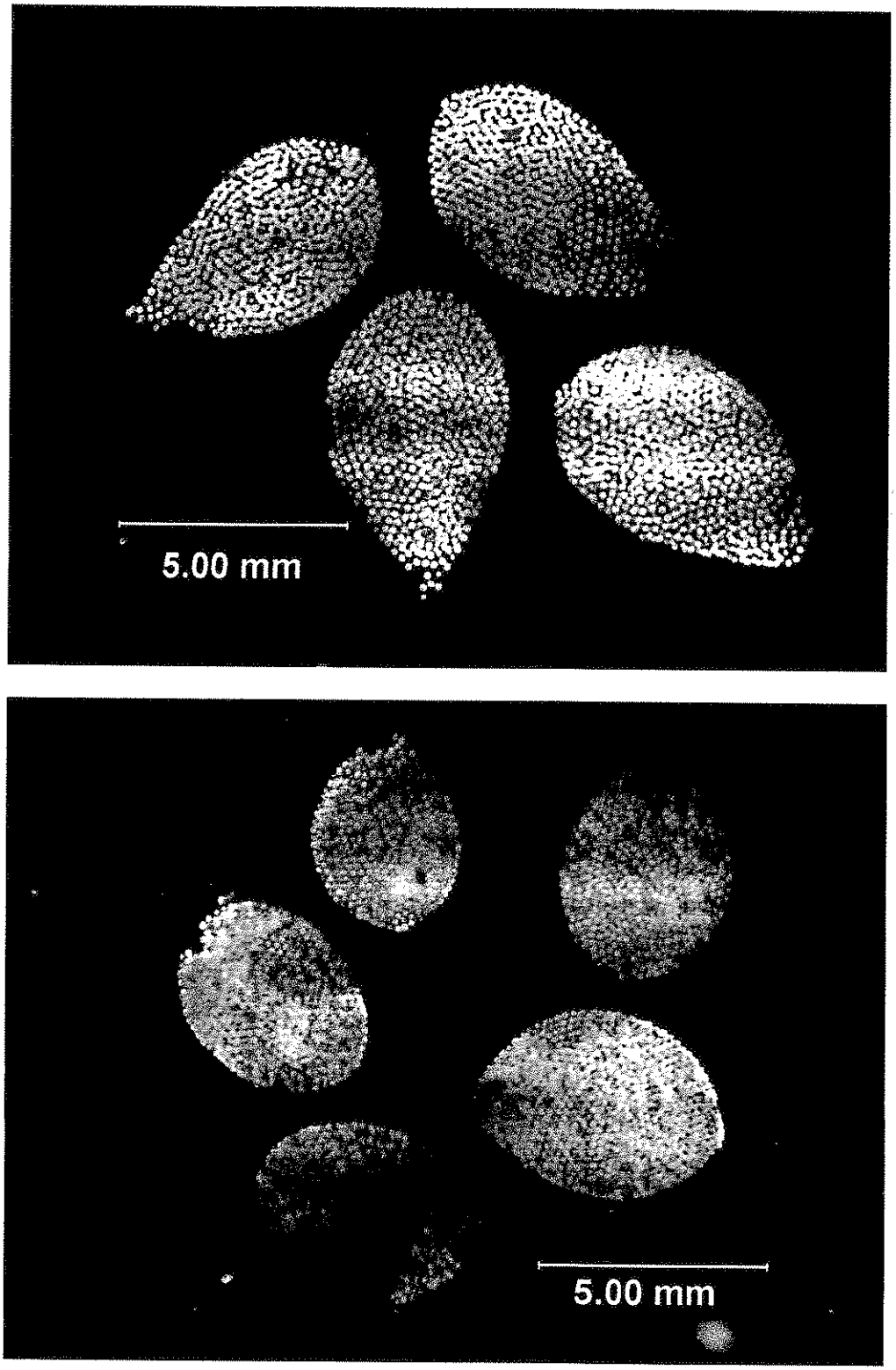


Figure 6. Conglutinates of *Pleurobema decisum* from the Buttahatchee River, Monroe, Co., MS.

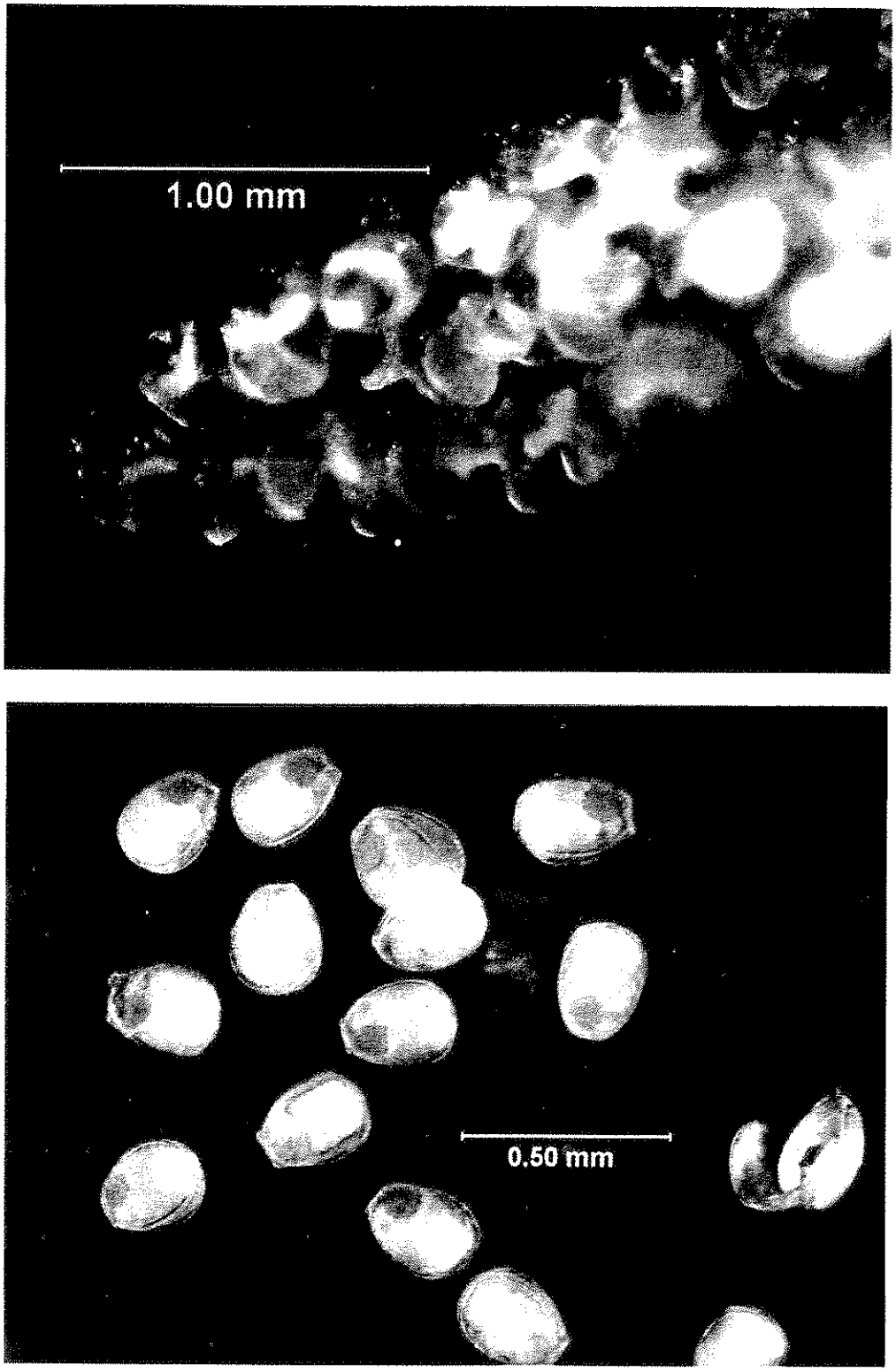


Figure 7. Glochidia of *Quadrula asperata* from the Buttahatchee River, Monroe Co., MS. Upper frame shows immature glochidia bound in a conglutinate. Lower frame shows mature glochidia.