Reproduction and Fish Hosts of Unionids From the Ozark Uplifts

M. Christopher Barnhart and Andrew Roberts
Southwest Missouri State University, Department of Biology, Springfield, Missouri

Abstract. Observations of reproductive status and potential fish hosts are reported for *Ptychobranchus occidentalis* (Ouachita kidneyshell), *Lampsilis reeveiana brevicula* (Ozark brokenray), *L. rafinesqueana* (Neosho mucket), and *Anodonta suborbiculata* (flat floater). Kidneyshells in the North Fork of the White River (Douglas County, Missouri) released mimetic glochidial packets resembling larval fish between 6 March and 8 April. Transformation of glochidia to juveniles occurred in 26-31 days at 21°C on darters (*Etheostoma blennioides, E. juliae, E. caeruleum*, and *E. spectabile*). Brokenrays in White River tributaries displayed the mantle lure between March 6 and August 8. Transformation occurred in 22-34 days at 21°C on smallmouth bass (*Micropterus dolomieu*), green sunfish (*Lepomis cyanellus*), and banded sculpin (*Cottus carolinae*). Neosho muckets in the Elk River (McDonald County, Missouri) displayed the mantle lure in July and August (previously reported to display in September and October). Transformation occurred in 27-32 days at 21°C on *M. dolomieu* and on largemouth bass (*M. salmoides*). Flat floaters (Marais des Cygnes drainage, Linn County, Kansas) released glochidia between 19 December and 25 February. Transformation occurred in 51-63 days at 10°C on golden shiners (*Notemigonus crysoleucus*), warmouth (*Lepomis gulosus*), white crappie (*Pomoxis annularis*), and largemouth bass (*M. salmoides*); transformation was generally unsuccessful at 21°C.

Introduction

Knowledge of the fish hosts that can support mussel glochidia is a prerequisite for management actions that involve introduction or reintroduction of threatened mussels to natural habitats. Host information is also essential should captive propagation become necessary to provide a refuge from the zebra mussel. A recent literature search concluded that host fish species have been reported for only about 25% of North American unionids (Hoggarth 1992). Over the past few years several reports have added to this total (e.g., Weaver et al. 1991; Bruenderman and Neves 1993; Michaelson and Neves 1995; Hove 1996). However, the hosts and reproductive habits of most unionid species remain unknown.

As more species have been investigated, it has become apparent that unionids employ a variety of strategies that enhance the probability of glochidia reaching suitable hosts (Kat 1984). For example, the mantle-flap lures of lampsilines (Kraemer 1970) and the super-conglutinates of *Lampsilis perovalis, L. australis*, and *L. subangulata* (Hagg et al. 1995; Hartfield and Butler 1997; O'Brien et al. 1997) apparently mimic small fish and act to lure a piscivorous host. Recently, it was reported that the movements of glochidia within the wormlike placentae of *Strophitus undulatus* cause movements of the entire structure, which may attract host fish (Hove 1997).

Another remarkable aspect of unionid reproduction that has received relatively little attention is the release of the sperm in masses called spermatozeugmata (O'Foighil 1989; Lynn 1994). Each spermatozeugma consists of several thousand individual sperm cells attached by their heads to a lamina surrounding a spherical globe. The sperm tails beat in synchrony and drive the spermatozeugma through the water, much like a colonial protist. Very little is known of the behavior and function of the spermatozeugmata of unionids.

In the present study, lab tests were used to investigate the potential fish hosts of three unionids endemic to the Ozark uplifts (Johnson 1980): *Ptychobranchus occidentalis* (Conrad, 1836) (Ouachita kidneyshell), *Lampsilis reeveiana brevicula* (Call, 1887) (Ozark brokenrays), and *L. rafinesqueana* Frierson, 1927 (Neosho mucket). The mimetic glochidia packets of *Ptychobranchus occidentalis* are described and illustrated. We also report suitable fish hosts and describe the spermatozeugmata and seasonal reproduction of a lowland unionid species, *Anodonta suborbiculata* Say, 1831 (flat floater).

Methods

Field sites

Ptychobranchus occidentalis was observed in the North Fork of the White River (Douglas County, Missouri). *Lampsilis reeveiana* was observed in the *rafinesqueana* was observed in two tributaries of the Arkansas River in southwestern Missouri: the Spring River (Newton County) and Elk River (McDonald County). *Anodonta suborbiculata* was studied in a small lake near the Marias des Cygnes River (Linn County, Kansas).

Most observations of reproductive status were made in the field. Lure display of lampsilines was observed by snorkeling and SCUBA. Gravidity was checked by gently opening the valves with a tubing-stretcher tool. Eggs, embryos, and glochidia of gravid individuals were sampled by syringe and preserved in 70% ethanol. Adult *A. suborbiculata* were collected monthly in the fall and held in aerated aquaria in order to observe sperm release and to ascertain whether individuals that released sperm would also produce eggs.

Host tests

Methods were similar to those described by Zale and Neves (1982). Glochidia were obtained by flushing the marsupia with water via syringe, or by excision. Glochidia were examined by light microscopy and tested for maturity by observing the shell-closing response to salt solution. Approximately 10 potential fish hosts were tested for each mussel species, with the fish species chosen based upon availability, local faunas, and upon the known host specificity of related mussel species. Fish were obtained from commercial suppliers or by seining and electro-shocking, and were quarantined with antibiotics for approximately 1 week before use.

Six to twelve individuals of each fish species were infected. Each individual was briefly anaesthetized with MS-222 and infected by pipetting glochidia onto the gills on the left side. The infected fish were held in 30-gallon aquaria, pooling individuals but keeping each species separate. Encystment was checked after 4 and 8 days by anaesthetizing and examining specimens of each host species. Unsuitable host fish typically shed attached glochidia within 1 week. If infection persisted beyond 1 week, each tank containing infected fish was vacuumed daily and the water filtered to retain glochidia and juvenile mussels.

Results and Discussion

Ptychobranchus occidentalis (Ouachita kidneyshell)

Kidneyshells in the North Fork of the White River apparently released glochidia in March. Four gravid individuals with mature glochidia were collected on 6 March 1994. The following spring none of 10 individuals examined was gravid on 8 April. Egg production evidently occurs well into fall; none of 12 kidneyshells examined was gravid on 28 September 1994. One of four individuals was gravid with embryos on 20 December.

The reproductive products of *P. occidentalis* and other *Ptychobranchus* species are unusual in that the glochidia from each brood chamber are enclosed within a membranous sheath, rather than embedded in a conglutinate. We suggest that these structures be referred to as glochidia "packets," in order to recognize their distinctive structure. In contrast, the terms "placenta" and "conglutinate" are used to refer to glochidia embedded in a matrix (c.f. Ortmann 1911). The packets are cylindrical and mimic a larval fish, with a bulbous distal "head" end (1 mm x 1 mm) and a tapered proximal "tail" end (0.5 mm x 10 mm) (Figure 1; figures are on p. 15). Coloration is light buff with dark lateral bars that suggest segmentation or myomeres. The "head" end is marked with four darkly pigmented "eyespots" and with lines reminiscent of skull sutures. It contains a rose-colored vesicle (color lost on preservation), which may mimic the heart and gills of a larval fish.

Glochidia are free within the mature packets. Pressure on the packet results in rupture of the "eyespots" and ejection of glochidia through these openings. This arrangement appears well-suited to infect small predatory hosts, such as darters, that would seize the packet by its "head." The packets do not appear to be well suited to infect larger hosts that might swallow them whole without first crushing them.

The tip of the tapered proximal end is adhesive and was observed to anchor glochidial packets to the substrate. Similar adherence has been observed in glochidial packets of *P. greenii* (P. Hartfield, pers. comm.). This function presumably reduces the probability that the packets will drop into gravel interstices and out of view of hosts.

An individual female *P. occidentalis* of typical size (shell length=8 cm) possessed 170 brood chambers/ctenidium. Each glochidial packet contained 270 ± 61.4 glochidia (mean $\pm$ S.D., n=10). Therefore, we estimate that this individual produced approximately 91,800 glochidia (the number would presumably vary with body size).

Ortmann (1911) argued that the placentae of *Ptychobranchus* were released from the distal edge of the ctenidium. It is possible to withdraw them from the edge of the ctenidium with forceps. However, in *P. occidentalis*, the packets may normally be released via the excurrent siphon. Although we have not observed this release directly, we have observed mature packets partly withdrawn dorsally in the water tubes, and also lying in the suprabranchial chamber of a sacrificed specimen. Similar observations have been made in *P. greenii* (P. Hartfield, pers. comm.).

Of the twelve species of fish tested as hosts, only the four darters tested (all in the genus *Etheostoma*) supported encystment and transformation (Table 1A). All four darter species tested co-occur with *P. occidentalis* in the North Fork. Both *Etheostoma caeruleum* and *E. spectabile* readily attacked kidney-shell placentae when these were presented in aquaria.

***Lampsilis reeveiana brevicula* (Ozark brokenray)**
Brokenrays are often the most abundant unionids in headwater streams of the White River system. The period of glochidia release appears to be of relatively long duration. We observed gravid females displaying their mantle flap lures on six occasions ranging from 6 March to 8 August (1992-1995). Nondisplaying females gravid with functional glochidia were noted on 28 September 1994 (8/8 females) and on 20 December (3/3 females). The periods of sperm release and egg production are not yet clear but probably fall in August-September.

The mantle flap lure of *Lampsilis reeveiana brevicula* is extremely fishlike (Figure 2). To our eyes, the size and markings of the lure are suggestive of juvenile orange-throat and rainbow darters (*Etheostoma spectabile* and *E. caeruleum*), both of which are commonly found with brokenrays. Of 16 fish species tested, the 3 successful hosts were all piscivores that prey upon darters in White River tributaries (*Cottus carolinae, Lepomis cyanellus,* and *Micropterus dolomieu*; Table 1B).

***Lampsilis rafinesqueana* (Neosho mucket)**
The reproductive period of the Neosho mucket is not well known. In the Spring River, Neosho muckets were observed displaying mantle lures on 28 July 1994 and 8 August 1993. Gravid individuals with mature glochidia were found on these dates and also on 20 June 1995. Three of three females examined on 9 October 1993 were not gravid. Of the 13 fish species tested, only *Micropterus dolomieu* and *M. salmoides* were suitable hosts (Table 1C).

***Anodonta suborbiculata* (flat floater)**
Suitable fish hosts of flat floaters at 10°C include warmouth (*Lepomis gulosus*), white crappie (*Pomoxis annularis*), largemouth bass (*Micropterus salmoides*), and golden shiners (*Notemigonus crysoleucas*)(Table 1D). Interestingly, transformation was unsuccessful in three of these four host species at 21°C. Only the white crappie were successful hosts at the higher temperature.

Egg deposition and fertilization in flat floaters apparently occurs in mid- to late September. Five of seven individuals collected 17 October 1994 had functional glochidia, but these were still enclosed in egg membranes. Six of eight individuals collected 13 September 1995 released sperm within the next 2 weeks (15-27 September) while held in aquaria in the laboratory. The two individuals that failed to release sperm were found to be gravid with embryos on 27 October. This observation (that individuals produced either sperm or eggs but not both) suggests that flat floaters in this population are gonochoristic and not hermaphroditic.

The spermatozeugmata of *A. suborbiculata* were briefly described previously by Utterback (1915, 1931). We found the diameter of the lamina of 3-hour-old spermatozeugmata to be 57 ± 2.1 microns (mean $\pm$ S.D., N=10). Freshly released spermatozeugmata were completely covered by roughly 3,600 sperm and made no directional progress in swimming. Within a few hours the sperm were localized on one side of the spermatozeugma, and effective directional swimming was observed. Localization of the sperm on one side of the spermatozeugmata appears due at least in part to swelling, which increases the surface area of the spermatozeugmata. Sperm also occasionally detach and leave the spermatozeugmata so that the number remaining attached decreases with time. Interestingly, the remaining attached sperm are usually adjacent to one another rather than dispersed over the surface. We suggest that the sperm are able to move laterally to maintain this clumped distribution, which permits directional swimming.

The sperm flagella beat slowly until the sperm are released from the spermatozeugma. After release the sperm become "activated" and swim rapidly for a short period (O'Foighil 1989). Interestingly, we were able to trigger the abrupt dispersal of *A. suborbiculata* sperm by exposing spermatozeugmata to 25 mm or higher concentrations of NaCl. All sperm simultaneously released and swam rapidly away. Osmotically comparable glucose solutions did not trigger dispersal. It appears possible that some chemical cue might act to trigger similar abrupt activation of sperm when the spermatozeugma enters the female.

Only a few observations of spermatozeugmata appear in the unionid literature (Utterback 1931; Edgar 1965; Pekkarinen 1991; Lynn 1994). Most recent studies of unionid reproduction have relied upon histological observations and do not mention the presence or absence of spermatozeugmata. The ecology and physiology of spermatozeugmata may be relevant to management of threatened species. In bivalves, spermatozeugmata are negatively buoyant

Table 1. Potential fish hosts of unionids tested by laboratory infections. Approximate time to transformation and test temperature are noted for each species. Results are indicated as follows: No encystment (-), encystment but no transformation (+), or encystment and transformation (++). From 6 to 12 individuals of each potential host were tested. Fish families are arranged phylogenetically according to Nelson (1994).

A. *Ptychobranchus occidentalis*
26-31 days, 21°C

Hosts tested	Results
Cyprinidae	
Luxilus pilsbryi	-
Pimephales notatus	-
Notemigonus crysoleucas	-
Ictaluridae	
Ameiurus melas	-
Noturus exilis	-
Cottidae	
Cottus carolinae	-
Centrarchidae	
Lepomis cyanellus	-
Lepomis macrochirus	-
Lepomis megalotis	-
Percidae	
Etheostoma blennioides	++
Etheostoma caeruleum	++
Etheostoma juliae	++
Etheostoma spectabile	++

B. *Lampsilis reeveiana brevicula*
22-34 days, 21°C

Hosts tested	Results
Cyprinidae	
Campostoma anomalum	-
Luxilus pilsbryi	-
Notropis rubellus	-
Pimephales notatus	-
Semotilus atromaculatus	-
Catostomidae	
Hypentelium nigricans	-
Ictaluridae	
Ameiurus melas	-
Noturus exilis	-
Fundulidae	
Fundulus olivaceus	+
Cottidae	
Cottus carolinae	++
Centrarchidae	
Lepomis cyanellus	++
Lepomis macrochirus	-
Lepomis megalotis	-
Micropterus dolomieu	++
Percidae	
Etheostoma caeruleum	-
Etheostoma spectabile	-

C. *Lampsilis rafinesqueana*
27-32 days, 21°C

Hosts tested	Results
Cyprinidae	
Semotilus atromaculatus	-
Campostoma anomalum	-
Catostomidae	
Hypentelium nigricans	-
Ictaluridae	
Ictalurus punctatus	-
Pylodictis olivaris	-
Moronidae	
Morone saxatilis	-
Centrarchidae	
Ambloplites rupestris	-
Lepomis macrochirus	-
Lepomis megalotis	-
Lepomis cyanellus	+
Micropterus dolomieu	++
Micropterus salmoides	++
Percidae	
Percina caprodes	-

D. *Anodonta suborbiculata*
51-63 days, 10°C; 14-16 days, 21°C

Hosts tested	Results 10°C	21°C
Cyprinidae		
Notemigonus crysoleucas	++	+
Cyprinus carpio	+	+
Semotilus atromaculatus	-	
Ictaluridae		
Ictalurus punctatus	+	-
Centrarchidae		
Lepomis macrochirus	+	-
Lepomis megalotis	+	-
Lepomis gulosus	++	-
Pomoxis annularis	++	++
Micropterus salmoides	++	-
Amphibia: Ranidae		
Rana clamitans	+	

and may function to concentrate sperm at the substrate/water interface where they are likely to be encountered by conspecifics (O'Foighil 1989). This function seems plausible in lentic species, such as *A. suborbiculata*, but perhaps less so for unionids inhabiting flowing water. Given that spermatozeugmata are capable of locomotion, the possibility of taxis toward chemical signals and triggering of sperm dispersal by species-specific cues should be investigated.

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