Am. Midl. Nat. 126:82–89

Reproductive Biology and Fish Hosts of the Tennessee Clubshell Pleurobema oviforme (Mollusca: Unionidae) in Virginia

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ABSTRACT.—The reproductive cycle and fish hosts of the freshwater mussel *Pleurobema oviforme* were determined during a 14-mo study (1979–1980) in Big Moccasin Creek, southwestern Virginia. Histological sectioning of mussel gonads collected throughout the year showed that gametogenesis for both sexes began in late spring and continued into early autumn; fertilization began in late March. Glochidial development in the outer gills of females required 3–5 wk. Judged by biweekly samples of stream drift, glochidia were released from mid-April through July. Six species of cyprinids, collected between May and September 1979, were naturally infested with amblemine glochidia. Induced infestations of putative fish hosts in the laboratory confirmed five host species: whitetail shiner (*Notropis galacturus*), common shiner (*N. cornutus*), river chub (*Nocomis micropogon*), stoneroller (*Campostoma anomalum*) and fantail darter (*Etheostoma flabellare*).

INTRODUCTION

The upper Tennessee River drainage in southwestern Virginia has a diverse fauna of freshwater mussels (Unionidae) and many species endemic to the Cumberland Plateau Region of eastern North America (Ortmann, 1918). However, precipitous declines in abundance and diversity of mussels have been reported in recent surveys, largely as a result of anthropogenic activities (Stansbery and Clench, 1974; Bates and Dennis, 1978; Ahlstedt, 1984). Many mussel species are in danger of extirpation, and 11 species in southwestern Virginia are federally listed as endangered.

The Tennessee clubshell (*Pleurobema oviforme*), a Cumberlandian species, once was widespread in the Tennessee and Cumberland river systems in Tennessee, Virginia and Kentucky (Ortmann, 1918). This species now is thought to be extirpated from the Cumberland River and is seemingly restricted to a few headwater streams in the Tennessee drainage (Ahlstedt, 1984). Because of its major decline in the last half century, the Tennessee clubshell was placed on the candidate list of species for possible addition to the List of Endangered and Threatened Wildlife (Federal Register 54(4):554–579). As with most endemic mussel species, we know little of its life history and ecological requirements. Our study was designed, therefore, to investigate the reproductive cycle and fish hosts of the Tennessee clubshell in Big Moccasin Creek, southwestern Virginia.

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[3] The Unit is jointly supported by the U.S. Fish and Wildlife Service, the Virginia Department of Game and Inland Fisheries, the Wildlife Management Institute, and Virginia Polytechnic Institute and State University

STUDY SITE

Big Moccasin Creek is a third-order stream flowing 88 km through Scott and Russell counties into the North Fork Holston River, Virginia (Neves and Zale, 1982). Our study site, 400 m long, above river km 82.6, was near the intersection of Virginia state highway routes 676 and 677 in Russell County. The creek at this point is bordered by pasture and cropland, averages 7 m wide and 0.2 m deep, and has a substratum of mainly cobble and gravel. Water quality characteristics during low flow (4 October 1979) were measured with a Hach[4] DR-EL/1 chemical kit and revealed temperature to be 14 C; turbidity, 5 FTU; conductivity, 250 mhos; pH, 8.2; dissolved oxygen, 9 mg/liter; hardness, 175 mg/liter as $CaCO_3$; and total alkalinity, 180 mg/liter. Weekly water temperatures, measured with a Ryan Model D-30 recording thermometer, ranged from nearly 0 C in February to 33 C in July (Zale and Neves, 1982a).

Seven mussel species, in decreasing order of relative abundance, occurred at the study site (Zale and Neves, 1982a): *Medionidus conradicus* (68.0%), *Villosa iris* (23.8%), *V. vanuxemi* (6.1%), *Pleurobema oviforme* (1.4%), *Lampsilis fasciola* (0.7%), *Fusconaia barnesiana* (>0.1%), and *Alasmidonta minor* (>0.1%). Two of these species, the Tennessee clubshell (*P. oviforme*) and Tennessee pigtoe (*F. barnesiana*), belong to the subfamily Ambleminae, which is characterized by the short-term brooding of young, small, unhooked glochidia and host fish specificity (Neves *et al.*, 1985).

METHODS

Collections of *Pleurobema oviforme* were made by hand-picking or by using a water scope when water was high or turbid. We collected about five specimens weekly from April to October 1979 and bimonthly from November 1979 to May 1980. Species identification was made according to Ortmann (1921). Unequal numbers of each sex were collected because this species is not sexually dimorphic. Specimens were returned to the laboratory in cloth bags packed in crushed ice, relaxed with propylene phenoxitol (Spectrum Chemical Manufacturing Corporation, Gardena, California), fixed in 10% buffered formalin, and preserved in 70% ethyl alcohol. The gametogenic cycle was studied by histological sectioning of the gonads. Portions of gonadal material were removed, dehydrated, cleared and then embedded in paraffin. Serial 7-μm sections were cut, affixed to glass slides, and stained with standard hematoxylin and eosin (Humason, 1972). Slides were examined under a compound microscope for sex determination and stage of gametogenic development. Valves of juvenile and young adult mussels were aged by external annuli (Stansbery, 1961; Neves and Moyer, 1988). To follow embryo development in the outer marsupial gills of female mussels, we examined aborted conglutinates (aggregates of developing glochidia) collected in successive weeks.

We set three drift nets (930-cm^2 mouth area, 130-μm mesh, and removable cod ends) across a riffle area below the mussel assemblage for at least 1 h each week from 5 May–27 August 1979, and from 4 April–7 August 1980. Collections were not standardized because periodic high flows and accompanying turbid conditions sometimes resulted in the clogging of drift nets within 2 h. Drift samples were preserved in 10% buffered formalin and, before examination, filtered through 0.5-mm nylon mesh to remove large particulate matter. Rose bengal was added to each sample to stain glochidia and facilitate sorting. During the 1979

[4] Reference to trade names does not imply Government endorsement of commercial products

collection period, five subsamples of each week's sample were sorted, and amblemine glochidia were removed and preserved in 10% buffered formalin. In 1980, drift samples were sorted until either 50 amblemine glochidia were removed or the entire sample had been examined. Presence or absence of these glochidia in each sample was recorded.

We collected about 300 fish of as many species as possible weekly from 5 May to 18 September 1979, with either a Georator electroshocker or backpack electroshocker. Fish were anesthetized with tricaine methanesulfonate (MS-222) and inspected for attached amblemine glochidia. Infested fish were preserved in 10% buffered formalin, and uninfested fish were revived and returned to the stream. The species and numbers of infested and uninfested fish were recorded. From 1 May to 7 August 1980, only fish of the species infested with amblemine glochidia during the previous summer were collected. All were preserved in 10% buffered formalin for positive identification and checked for glochidial infestation in the laboratory.

Of the 23 fish species collected in the study area, 10 species were artificially infested with *Pleurobema oviforme* glochidia in laboratory experiments, with techniques similar to those of Zale and Neves (1982b). Specimens of *P. oviforme* were collected from the study area during late spring and early summer when females were gravid, returned to the laboratory in cloth bags, relaxed with a dilute solution (0.7 ml/liter) or propylene phenoxitol, and sexed. Gravid females were held in an aerated, temperature-regulated, 300-liter tank with recirculating water; males and nongravid females were returned to the stream. Tank temperatures approximated those in the stream study area to facilitate glochidial development in female mussels and prevent premature expulsion of glochidia. Powdered trout food or a commercial invertebrate diet (Hawaiian Marine Imports Inc., Houston, Texas) was introduced daily as food for the mussels.

Fish for induced infestations were collected by electroshocking from areas devoid of mussels in western Virginia (to avoid the possibility of including fish with acquired immunity; Arey, 1923), returned to the laboratory, and maintained in Living Streams (Frigid Units Inc., Toledo, Ohio) at temperatures of 16–20 C. About 10 fish of each species were acclimated to laboratory temperatures for at least 1 wk before they were infested with glochidia and maintained on a diet of commercial trout food (roughly 1% of body weight daily).

Infestive glochidia were removed from the marsupia of excised gills of female mussels into a water-filled watch glass. Viability of glochidia was tested before each infestation by placing a few individuals in a weak saline solution. Valves of mature glochidia immediately snapped shut, whereas the valves of immature glochidia remained open. Only mature glochidia were used for induced infestations; 100 to several hundred were pipetted directly onto the gill lamellae of fish anesthetized with MS-222. After recovery from anesthesia, fish of each species were placed in aerated, temperature-controlled 40-liter glass aquaria. One fish from each species was checked 2 h after exposure to determine that glochidia were attached. Daily checks of a subsample of each fish species continued for 5 days post-infestation. We considered the glochidia to have sloughed off and the fish species an unsuitable host if none were attached after 5 days. If glochidia remained attached after 5 days, daily siphoning of water in each aquarium through 130-μm nylon mesh was begun, and siphoned material was examined under a dissecting microscope (40×) for newly metamorphosed juvenile mussels. Siphoning of aquarium water continued for 3 days after the last juvenile mussel was found; all remaining fish then were sacrificed and checked for encysted glochidia. Gravid female *Pleurobema oviforme* were difficult to obtain, and only five were collected for artificial infestations. Because of this difficulty in finding gravid females and because bacterial and fungal infections killed many of the wild fish, we completed only one replication of each trial, except for stonerollers.

RESULTS AND DISCUSSION

Gonadal sections from 172 *Pleurobema oviforme* collected in Big Moccasin Creek indicated that 47% were males and 53% females; not statistically different from a 1:1 sex ratio (χ^2 test). Sexual maturity in males occurred by ca. 4 yr of age. One gonad of an age 3 male in October contained undeveloped acini and sparse gonadal material, whereas an age 4 male collected in September had a fully developed gonad. One age 5 female collected in May contained fully developed ova, but no younger females were collected to confirm age at maturity. These observed ages of sexual maturity concur with those of previous studies on other species (Matteson, 1948; Stein, 1969; Yokley, 1972; Zale and Neves, 1982a).

Active gametogenesis in males occurred from mid-May through early autumn. Gonads were characterized by reduced, widely spaced acini and few mature sperm in the lumen. Abundant spermatogonia, spermatids and nutritive granules were present. During winter, mature sperm were abundant and filled the closely packed, thin-walled acini. Release of sperm (spawning) began in late March and continued into May. Overlapping developmental stages were observed within and between individuals in the population—as also observed in *Elliptio complanata* by Matteson (1948).

Gametogenesis was similar in chronology among females but included a period of residual egg atresia. Active oogenesis (June through October) was characterized by thick alveolar walls with numerous nutritive granules and embedded oogonia. By late oogenesis, alveolar walls became thin and ova had migrated to the lumen. Alveoli were packed with eggs during the overwintering period and the gonado-visceral mass was enlarged. Once egg release began (late March through May), alveoli became thin-walled again and few ovocytes were visible in the lumen. Residual eggs deteriorated thereafter, and only atretic material remained in the ovary.

Progressively more advanced features of embryo development became evident between late March and late June: the four-cell stage within the vitelline membrane by 24 March; the hinge line of developing embryos by 11 April; the semicircular shape and early shell formation by 24 April; the fully formed shell, mantle cells and adductor muscle by 12 June; and mature glochidia free of the vitelline membrane by late June. Glochidia required a development period of ca. 3–5 wk before they were ready to be released into the water column and capable of attachment to a fish host. This time period is similar to the 4–6 wk periods estimated for *Elliptio complanata* and *Pleurobema cordatum* (Matteson, 1948; Yokley, 1972). Before release, glochidia were bound together in conglutinates within the gills. At the time of release, conglutinates became less rigid with a granular appearance. At this stage, the mean and standard deviation of measurements of 50 mature glochidia were as follows: length, 169 $\pm$ 4.6; breadth, 162 $\pm$ 4.2; and hinge length, 123 $\pm$ 5.0 μm. These dimensions are very similar to those reported by Kitchel (1985) for *P. oviforme* in the North Fork Holston River and for other amblemine glochidia (Ortmann, 1921).

Stream drift was sampled 56 times at the site between May 1979 and August 1980, but sampling was concentrated during spring and summer when species of the Ambleminae are known to release glochidia (Coker *et al.*, 1921). Amblemine glochidia were collected only from mid-April through July, with the peak release in June. Kitchel (1985) reported peak densities of glochidia of *Pleurobema oviforme* in the North Fork Holston River, Virginia, during mid-July.

About 4100 fish were sampled on 14 occasions from May to September 1979 in Big Moccasin Creek and checked for amblemine glochidia on gill lamellae (Table 1). Infested fish included only the following species of Cyprinidae: stoneroller (*Campostoma anomalum*), river chub (*Nocomis micropogon*), warpaint shiner (*Notropis coccogenis*), whitetail shiner (*N. galacturus*), striped shiner (*N. chrysocephalus*) and bluntnose minnow (*Pimephales notatus*).

TABLE 1.—Fish species composition and infestation by amblemine glochidia, May to September 1979

Fish species	No. examined	Percent infested
Cyprinidae		
Campostoma anomalum, stoneroller	1281	0.2
Hybopsis amblops, bigeye chub	1	0.0
Nocomis micropogon, river chub	270	4.1
Notropis coccogenis, warpaint shiner	104	1.9
N. chrysocephalus, striped shiner	337	0.6
N. galacturus, whitetail shiner	48	12.5
N. leuciodus, Tennessee shiner	27	0.0
N. rubellus, rosyface shiner	177	0.0
N. telescopus, telescope shiner	188	0.0
Pimephales notatus, bluntnose minnow	54	7.4
Rhinichthys atratulus, blacknose dace	2	0.0
Catostomidae		
Catostomus commersoni, white sucker	1	0.0
Hypentelium nigricans, northern hog sucker	90	0.0
Moxostoma duquesnei, black redhorse	1	0.0
Ictaluridae		
Ictalurus natalis, yellow bullhead	27	0.0
Centrarchidae		
Ambloplites rupestris, rock bass	178	0.0
Lepomis auritus, redbreast sunfish	350	0.0
Micropterus dolomieui, smallmouth bass	8	0.0
Percidae		
Etheostoma blennioides, greenside darter	75	0.0
E. flabellare, fantail darter	423	0.0
E. rufilineatum, redline darter	237	0.0
E. simoterum, Tennessee snubnose darter	82	0.0
Cottidae		
Cottus carolinae, banded sculpin	112	0.0

Whitetail shiners and bluntnose minnows were the most frequently infested, and no amblemine glochidia were observed on fish after July.

Cyprinids were sampled weekly on 15 dates from May to August 1980, because only members of this family were implicated as hosts in 1979, and Neves and Widlak (1988) reported amblemine glochidia only on cyprinid species. Glochidial infestations on cyprinids peaked during the week of 5 June (13.6% infestation), but continued until 7 August (Table 2). As judged by infestation data, river chubs and whitetail shiners were the most frequently infested. Wiles (1975) suggested that the prevalence of infestations on fish was correlated with abundance of glochidia in the water column, and peak infestations of amblemine glochidia on cyprinids reportedly occur shortly after maximum densities of glochidia in stream drift (Neves and Widlak, 1988). We suspect, therefore, that densities of glochidia in the water column peaked in late May or early June.

Ten species of fish were infested with glochidia from five female *Pleurobema oviforme* in

TABLE 2.—Number of fish examined (E) and percent infested with amblemine glochidia, 1 May through 7 August 1980

	Stoneroller		River chub		Warpaint shiner		Common shiner		Whitetail shiner		Bluntnose minnow		Total	
Sample date	E	%	E	%	E	%	E	%	E	%	E	%	E	%
May 1	28		24	4.0	5		9		1		0		67	1.5
May 8	38		38		4		15		1		9		105	
May 15	74		14		2		32		6		22		150	
May 21	59		31	16.1	20		24	4.2	4	50.0	5		143	5.6
May 29	43		20	10.0	14		28		5	40.0	26	15.4	136	5.9
June 5	54		26	46.2	20		16	6.3	3	66.7	6	33.3	125	13.6
June 12	62	11.3	31	35.5	26	3.8	44	6.8	5	20.0	24	8.3	192	13.0
June 19	63	1.6	32	15.6	22		57	1.8	17	17.7	22	4.6	213	5.2
June 26	71	8.5	25	32.0	20		13		8		7		144	9.7
July 3	136	2.9	32	28.1	12	16.7	62	3.2	26	11.5	7		275	7.3
July 10	65	7.7	8	50.0	6		9	11.1	5	20.0	0		93	11.8
July 18	142	12.7	15	20.0	9		51	2.0	13	30.8	17		247	10.5
July 24	92	4.3	34	8.8	16		18		9		5	20.0	174	4.6
July 31	109	0.9	9		3		15		17	5.9	15	6.7	168	1.8
Aug 7	81		31		19		22		17		9		179	
Total	1117	4.1	370	17.0	198	1.5	415	2.4	137	13.9	174	6.3		

TABLE 3.—Periods of attachment (days), water temperatures and number of juvenile mussels recovered from host fish species of the Tennessee clubshell

Fish species	n	Period of attachment range	Peak	Water temperature (C)	No. recovered
		Hosts			
Notropis cornutus	5	17–22	19	19.0–23.0	48
N. galacturus	2	11–13	11	20.0–24.5	14
Nocomis micropogon	1	17–18	18	19.0–23.0	3
Campostoma anomalum	2	13–16	15	20.5–24.0	2
	5	9–12	9	23.0–26.5	8
Etheostoma flabellare	6	9–12	10	21.5–24.5	17
		Non-hosts			
Etheostoma rufilineatum	10	3		21.5–24.0	
E. blennioides	11	2		22.5	
Amploplites rupestris	10	2		22.5	
Pimephales notatus	3	5*		23.0–26.5	
Notropis coccogenis	4	4*		20.5–24.0	

* All fish died

the laboratory (Table 3). Glochidia metamorphosed on five of the 10 species tested: striped shiner, whitetail shiner, river chub, stoneroller and fantail darter. Glochidia on stonerollers metamorphosed in 13–16 days at water temperatures of 20.5–24 C, and in 9–12 days at 23–26.5 C. In a previous study, glochidia of *P. cordatum* metamorphosed on the rosefin shiner (*Notropis ardens*) in 14–18 days at 21 C (Yokley, 1972). Glochidia attached to warpaint shiners, but all fish died by day 4 from fungal infection, and this trial was not successfully repeated. However, glochidia remained attached at the time of death. Bluntnose minnows also were infested successfully with glochidia, and attachment was verified through day 5, but no juvenile mussels were collected. These cyprinids may therefore be hosts for the Tennessee clubshell.

Because the fantail darter was not naturally infested with amblemine glochidia in Big Moccasin Creek, its suitability as host was unexpected. This darter serves as host for the Cumberland moccasinshell (*Medionidus conradicus*) in this stream and is heavily infested with glochidia of *M. conradicus* in all months except August (Zale and Neves, 1982b). Reuling (1919) suggested that fish may acquire immunity to glochidia over time and slough glochidia of one species if already or previously infested by other species. This immunological mechanism may explain the observed absence or at least low incidence of amblemine glochidia on the fantail darter in Big Moccasin Creek.

Acknowledgments.—We thank E. F. Benfield for reviewing the manuscript; Al Zale, Jane Bain and Wanda Morehead for assisting in data collection and laboratory work; and Dr. Paul Parmalee, University of Tennessee, and Dr. D. H. Stansbery, Ohio State Museum of Zoology, for assisting with mussel identifications. This research was supported by the Virginia Department of Game and Inland Fisheries (Project No. E-F-1) and the Virginia Cooperative Fish and Wildlife Research Unit.

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SUBMITTED 6 DECEMBER 1990 ACCEPTED 19 MARCH 1991