

# Reproductive Cycle and Fish Hosts of the Rabbit's Foot Mussel, *Quadrula cylindrica strigillata* (Mollusca: Unionidae) in the Upper Tennessee River Drainage<sup>1</sup>

BRUCE L. YEAGER

Tennessee Valley Authority, Office of Natural Resources and Economic Development, Division of Services and Field Operations, Field Operations, Eastern Area, Norris, Tennessee 37829

and

RICHARD J. NEVES

Virginia Cooperative Fishery Research Unit, Department of Fisheries and Wildlife Sciences, Virginia Polytechnic Institute and State University, Blacksburg, 24061

**ABSTRACT:** The reproductive biology and fish host requirements of *Quadrula cylindrica* were studied in the upper Tennessee River drainage, Virginia and Tennessee, during 1982 and 1983. Gametogenesis in this summer breeder was characterized by three stages: active gamete formation in late summer, an inactive overwintering period, and rapid gamete maturation and release (spawning) from May to July. Gravid females were collected from mid-May through early July, and mean fecundity was approximately 115,000 embryos per female. Glochidia exhibited a relatively high degree of host specificity, metamorphosing on only three cyprinids of 34 fish species tested in the laboratory. Host species identified were *Notropis galacturus*, *N. spilopterus* and *Hypobopsis amblops*.

## INTRODUCTION

The rabbit's foot mussel, *Quadrula cylindrica* (Say 1817), is widely distributed throughout the Ohio, Cumberland and Tennessee river basins in the eastern United States and ranges westward in the Mississippi drainage to Arkansas, Kansas and Oklahoma (Ortmann, 1919). In the Tennessee River Valley, two subspecies are recognized. The headwaters form, *Q. c. strigillata* (Wright), is a compressed, highly tuberculate type in the Clinch, Powell and Holston rivers; and *Q. c. cylindrica* (Say), a more inflated, nodulate form with tubercles on the posterior ridge, occurs in the Duck River and mainstream Tennessee River below Pickwick Landing Dam.

In the early 20th century, the shells of several species of *Quadrula* were harvested by the commercial shell industry of the Mississippi River basin (Lefevre and Curtis, 1910; Coker, 1919; Coker *et al.*, 1921), and drastic reductions in their abundance prompted preliminary studies on reproduction and host fish identification (Surber, 1913; Howard, 1913, 1914; Coker *et al.*, 1921). On the basis of these early studies, the glochidia of *Quadrula* species were identified as gill parasites, and several species of sunfishes (Centrarchidae) and catfishes (Ictaluridae) were implicated as hosts for *Q. metanevra*, *Q. nodulata*, *Q. pustulosa* and *Q. quadrula* (Fuller, 1974). However, life history information on the rabbit's foot mussel has not been previously reported. Objectives of the present study were to describe the seasonal progression of gametogenesis, document the spawning and glochidial release periods, identify fish hosts and characterize the early life stages of *Q. c. strigillata*. This research was conducted as part of the Cumberlandian Mollusk Conservation Program of the Tennessee Valley Authority (Jenkinson, 1981) to provide life history information on the rabbit's foot mussel that would be applicable to biological studies proposed for *Quadrula intermedia* and other endangered mussel species.

<sup>1</sup>This is a work of the United States Government and is in the public domain. Copyright protection is not available.

<sup>2</sup>Reference to trade names does not imply government endorsement of commercial products.

## MATERIALS AND METHODS

All specimens of *Quadrula cylindrica strigillata* were collected from the upper Tennessee River drainage in 1982 and 1983 at the following sites and river mile (RM) locations: Grays Island (RM 233.5) and Pendleton Island (RM 226.3) on the Clinch River, Scott Co., Virginia; and McDowell Ford (RM 106.7) on the Powell River, Hancock Co., Tennessee. Except during periods of high flow and turbidity, Pendleton Island was sampled most often and served as the major source of *Q. c. strigillata* for reproductive studies. Mussels were obtained by snorkeling, and roughly five adult individuals were collected on each of the following sampling dates in 1982 and 1983: 21 and 30 April; 5, 10, 18, and 26 May; 16 June; 29 July; 24 August; 29 September; 7 January; 14 March; 21 April and 21 May. These specimens, collected for histological studies, were relaxed in propylene phenoxitol, fixed in 10% buffered formalin, and preserved in 70% ethyl alcohol. The gametogenic cycle was investigated by cutting serial sections (7  $\mu$ m thick) of gonadal tissue with a microtome and staining these glass slide preparations with standard hematoxylin-eosin techniques (Humason, 1972). The reproductive stage of each specimen was described and recorded. Absence of gametes immediately after a period of reproductive maturity was assumed to indicate that spawning had occurred (Zale and Neves, 1982a).

During spring and summer 1982, mussels from the Clinch and Powell Rivers were examined periodically by opening them slightly with modified O-ring expanders to check for gravidity. On each sampling date, nongravid mussels were returned to the river, and gravid specimens were placed in cloth bags for transport to a mobile laboratory (house trailer 3.7 m wide, 15.3 m long) downstream from Speer's Ferry (RM 211.8), Clinch River, Virginia. A 3.75-hp submersible sewage pump supplied ambient river water directly to aquaria and Living Streams<sup>2</sup> (Frigid Units Inc., Toledo, Ohio) in the laboratory at a maximum flow of ca. 72,000 liters/hr.

Because conglutinates are often prematurely discharged (aborted) by females of *Quadrula* species (Lefevre and Curtis, 1910), two methods of transporting and maintaining gravid females were tested to avoid or reduce abortion of glochidia. Specimens in cloth bags were transported in insulated coolers with either ambient river water or crushed ice, and bags were examined for aborted conglutinates at the laboratory. Mussels transported in river water were placed directly in Living Streams with river substrate or in aerated aquaria. Specimens transported on ice were transferred to an 11-liter bucket with aerated, chilled river water (10 C), allowed to warm to room temperature (20 C), and then placed in Living Streams or aquaria. A sample of gravid females was aged by the external growth ring method (Chamberlain, 1931; Crowley, 1957). A fecundity estimate for gravid females was obtained by counting the number of eggs, embryos or glochidia in expelled or aborted conglutinates and multiplying by the mean number of water tubes used for brooding embryos in females.

Thirty-four fish species of eight families were exposed to glochidia of *Quadrula cylindrica strigillata* in laboratory trials for fish host identifications. Fish for these host experiments were collected at various river locations by backpack electroshocker or seine, segregated by species, and conveyed to the laboratory in insulated coolers. Collection areas for fish used in laboratory trials were as follows: Clinch River drainage—Copper Creek, Irving Branch and Stock Creek in Scott Co., Va.; Buffalo Creek, Bull Run Creek and Hinds Creek in Anderson Co., Tenn.; and Mill Creek in Union Co., Tenn.; Powell River drainage—RM 65.2 in Claiborne Co., Tenn.; and RM 106.5 in Hancock Co., Tenn.; North Fork Holston River drainage—RM 6.3 and 13.4 in Scott Co., Va.; Cumberland River drainage—RM 224 in Sumner Co., Tenn. Collection sites for each fish species are tabulated in results. Fish collections were made at mussel-free sites when possible to avoid prior exposure of specimens to glochidia, and specimens were checked during collection for parasitic infestations. Likely fish hosts for glochidia of the rabbit's foot mussel were selected by examining a list of fish species occurring where *Q. c. strigillata* also occur at numerous sites in the upper Tennessee River drainage and a list of fish

species with glochidal infestations at those sites. Numbers of fish in each trial depended on species availability and ranged from 1-25; each fish was exposed to glochidia only once. All fish were maintained in either 76-liter aerated aquaria or Living Streams with flow-through water. Laboratory confinement of fish prior to infestation varied from 1 hr to 14 days, depending on availability of mature glochidia for induced infestations. Frozen brine shrimp, commercial fish pellets, or forage fish were fed to experimental fish before and during infestation trials.

Mature glochidia were obtained from aborted conglutinates or by excision of the gills from gravid female mussels. Glochidia were tested for maturity by exposing a subsample to salt crystals; mature glochidia exhibit a closing response (Zale and Neves, 1982b). In the laboratory, fish were anaesthetized with tricaine methanesulfonate (MS-222), infested by pipetting several hundred glochidia into the left branchial cavity, and allowed to recover (15 min). Each species was placed in a separate aerated aquarium (19–114 liters) with filtered water and sections of plastic pipe for cover. Two fish species with a closely bound isthmus (*Camptostoma anomalum* and *Rhinichthys atratulus*) were exposed to glochidia in a 1-liter beaker for 1 min and then placed in separate aquaria.

Roughly 1.5 hr after infestation, specimens of each fish species were again anaesthetized and checked for glochidal attachment. Subsequent examinations occurred on day 2, day 3 or 4, and at irregular intervals thereafter to monitor the status of encysted glochidia. Nonhost fish usually rejected glochidia within the 1st 3 days. For all species, the last day of glochidal attachment was recorded. Material from the aquarium bottom was siphoned daily through a 125- $\mu$ m nylon mesh sieve beginning 1 day after infestation. Sloughed glochidia and dead juvenile mussels were examined with a stereomicroscope and preserved in 10% formalin. Live juveniles were kept for behavioral observations or placed in a shallow, stainless steel pan with sand-silt substrate in a flow-through Living Stream.

Measurements of glochidia and juveniles were obtained under a stereomicroscope with an ocular micrometer; length is the maximum anteroposterior dimension parallel to the hinge, and breadth is the maximum dorsoventral dimension perpendicular to the hinge.

#### RESULTS

The gonads of 35 males and 39 females, collected from the Clinch River on the 14 sampling dates in 1982 and 1983, were sectioned for histological study. Several gametogenic stages often occurred within and among individual mussels on each sampling date. Males began active spermatogenesis in August. In the males, the gonadal tissues were characterized by widely spaced acini with numerous spermatogonia, spermatocytes and nutritive granules (Fig. 1). These active spermatogenic stages were also present in late September. No specimens were collected from October through December, but the reproductive stages of males taken in January were similar to those of males collected in September. By March and April, the number of spermatids increased and acini were compact. Sperm were first noted in early May in 1982 and in mid-April in 1983. Sperm were closely packed in the lumen by mid-May of both years, with few spermatogonia and nutritive granules. In June, sperm occurred in the ducts, and acini were less tightly packed. All males examined in late July were spent. Based upon these gonadal sections, release of sperm (spawning) probably began in mid-May and was completed by July.

Females showed initial signs of oogenesis in late July. By mid-August, nutritive granules with imbedded oogonia and thick acinal walls were evident in ovarian tissue (Fig. 2). The appearance of gonadal tissues from specimens in the September sample was similar to that of mid-August. By January, many oocytes had developed, acinal walls were thinner, and oocytes had separated from nurse cells. The lumen was filled with eggs, acini were thin-walled, and some mature eggs occurred in the follicles. The number of mature eggs increased in April and May, but few eggs remained by mid-

June. Eleven females collected on 10 May had not spawned, although a single female examined on 3 May bore eggs in her gills. Gills of all females were partly or fully charged in late May and June, and acini were empty in July. These gonadal sections suggest that release of eggs (spawning) probably began in mid-May and was nearly completed by mid-June.

Except for the female examined on 3 May, gravid individuals of *Quadrula cylindrica strigillata* were collected first from the Clinch River on 13 May 1982 and from the Powell River on 18 May 1982; water temperatures in these rivers were 20 C and 22 C, respectively (Table 1). Between 20 May and 26 June, 15-30% of the mussels examined from the Clinch River were gravid. Because the rabbit's foot mussel is not sexually dimorphic, percent gravidity is based on all mussels examined. However, since the randomly collected specimens for histological study (35 males:39 females) appear to indicate a roughly equal sex ratio in the population, percent gravidity among females is likely double the expressed values (Table 1). Most of the females examined ranged from 81-102 mm in length and 10-22 years in age. The percentage of gravid females peaked at 30-32% in late May and then gradually declined through June. The lower percentages of gravid specimens from the Clinch River observed on 16 and 23 June occurred after an extended period of high turbidity. The relatively greater number of gravid females collected on 26 June from the Clinch River was obtained primarily from another area at the Pendleton Island site. Mature glochidia were not abundant in conglutinates until June, females apparently expelled all glochidia by late July. All specimens examined after July were not gravid.

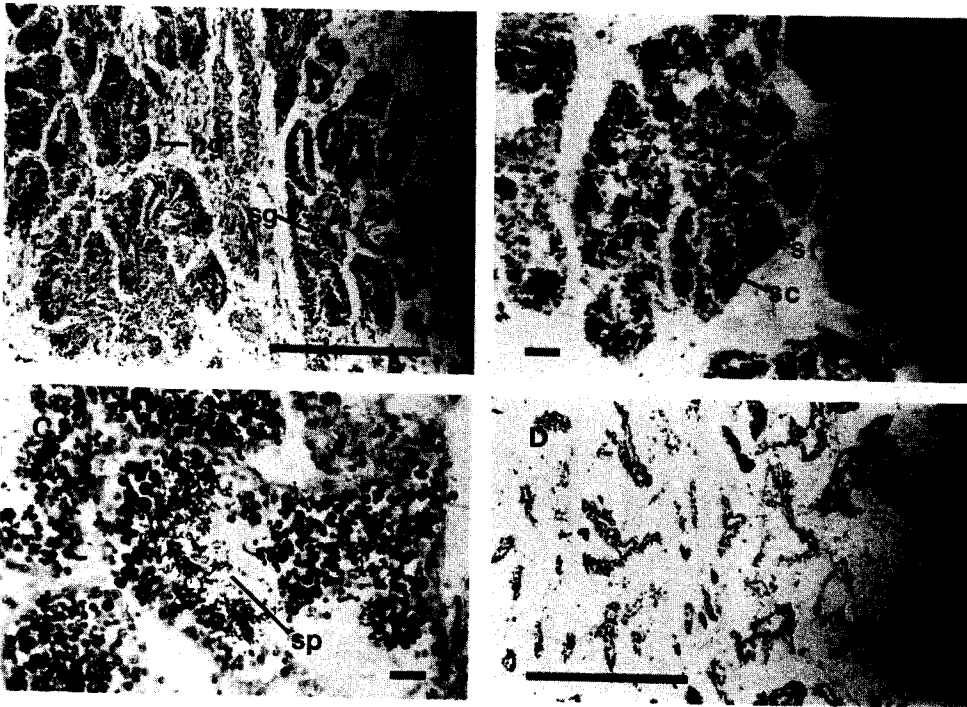


Fig. 1.—Histological sections of testes of *Quadrula cylindrica strigillata* collected 24 August (A), 1 January (B), 29 June (C) and 29 July (D). Abbreviations: ng, nutritive granules; sg, spermatogonia; sc, spermatocytes; st, spermatids; sp, sperm. Bar = 200  $\mu$ m

Fifty-three (65%) of the 82 gravid females examined from the Clinch River had glochidia in the outer demibranchs only, whereas the remaining 29 (35%) had at least part of all four gills serving as marsupia. Unfertilized eggs accounted for less than 5% of conglomerates during May and June. Fertilization success, the ratio of embryos to embryos plus unfertilized eggs, was high, therefore, at this time. In contrast, gravid females examined late in the reproductive season (7 July) contained only unfertilized eggs in their gills. Conglomerates with embryos and immature glochidia frequently were aborted by gravid females regardless of efforts to improve transportation and holding methods. Gravid females discharged at least a few conglomerates under all field and laboratory conditions. Expulsion of conglomerates generally occurred within 1 day of initial handling or examination for gravidity or after periods of high turbidity in the flow-through laboratory facilities.

Lanceolate-shaped conglomerates of immature and mature glochidia were expelled through the excurrent siphon. Seven conglomerates averaged  $11.5 (\pm 0.69 \text{ SD})$  mm long,  $1.7 (\pm 0.23 \text{ SD})$  mm wide, and  $0.9 (\pm 0.07 \text{ SD})$  mm deep. Embryos in the conglomerates were light yellow until they reached the late gastrula stage, then progressed through light peach to reddish brown in maturing glochidia. Fully mature glochidia were nearly transparent or light peach, except for a tinge of rust along the mantle margin. These glochidia were expelled in granular-appearing whitish to reddish brown conglomerates, or were expelled individually as the conglomerate matrix disintegrated. The eight conglomerates contained between 375 and 505 (mean,  $469 \pm 43.0 \text{ SD}$ ) embryos or glochidia. Mean fecundity (computed from the mean number of water tubes used for brooding embryos in five sacrificed specimens, field observations of gravid females, and the mean number of embryos per conglomerate) was  $114,246 (\pm 5,368 \text{ SD})$  embryos per female.

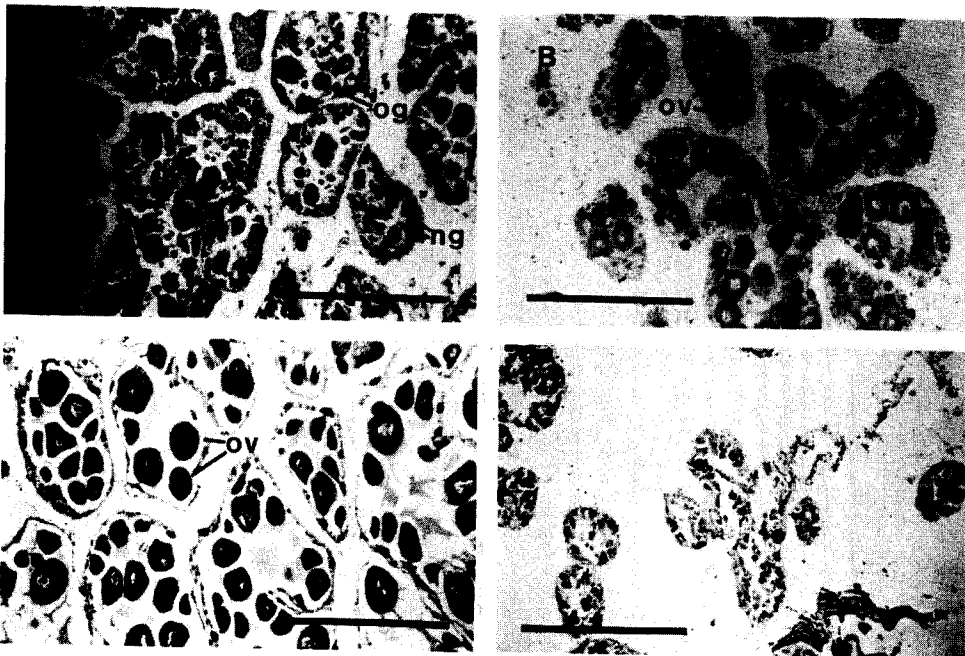


Fig. 2. — Histological sections of ovaries of *Quadrula cylindrica strigillata* collected 24 August (A), 6 November (B), 21 May (C) and 29 July (D). Abbreviations: ng, nutritive granules; og, oogonia; ov, ovocytes. Bar = 200  $\mu\text{m}$

Within each conglomerate, all glochidia were at the same stage of development. Gravid females, determined to contain mature glochidia by excising and agitating a gill, consistently had mature glochidia in all conglomerates. However, one female collected on 13 May had several developmental stages in different conglomerates. She aborted several nearly mature glochidia with well-formed valves on 14 May, but expelled conglomerates of immature embryos (light yellow to peach) on 18 May.

Conglomerates with mature glochidia were easily teased or shaken apart to release their glochidia. Colorless glochidia were not used for induced infestation trials because they exhibited a slow closing response when exposed to salt crystals. Mean dimensions of 100 mature glochidia from four females were as follows: length, 0.22 ( $\pm 0.03$  SE) mm; breadth, 0.22 ( $\pm 0.02$  SE) mm; depth, 0.16 ( $\pm 0.02$  SE) mm; and hinge length, 0.14 ( $\pm 0.02$  SE) mm. Glochidia were subcircular with a truncated dorsal hinge line (Fig. 3); the single adductor muscle was readily visible.

Glochidia pipetted onto the gills of potential hosts typically attached to the distal portion of gill lamellae, although some glochidia occasionally attached to epithelial tissue lining the branchial cavity. Degrees of infestations were light to moderate, depending on the relative size of the fish species. As few as 5-10 glochidia were seen on cyprinids and as many as several hundred on large centrarchids.

Glochidia were sloughed from the gills of 31 of the 34 species tested at mean water temperatures between 20.0 and 23.1 C (Table 2). In these infestation trials, 19 species sloughed glochidia within the 1st day after exposure. Several species of cyprinids re-

TABLE 1. — Reproductive condition of *Quadrula cylindrica strigillata* examined from the Clinch and Powell rivers during 1982

Stream and date	Water temperature (C)	No. examined	Percent gravid
Clinch River			
21 April	15.6	35	0
5 May	16.7	10	0
10 May	20.0	11	0
13 May	20.0	1	100
14 May	21.1	46	0
20 May	21.4	33	15
26 May	22.2	70	30
27 May	22.2	51	29
16 June	20.6	260	5
23 June	21.1	106	6
26 June	23.0	96	15
7 July	24.0	106	8
29 July	24.0	30	0
Powell River			
5 May	17.8	9	0
18 May	22.2	31	32
25 May	21.7	13	31
18 June	22.2	3	0

tained glochidia somewhat longer than species in other fish families. For example, the striped shiner *Notropis chrysocephalus* retained glochidia for up to 12 days, but no transformed juveniles were found. All specimens of this species used in these tests had a moderate infestation of encysted trematodes, which may have affected our experimental results. Glochidia of *Quadrula cylindrica strigillata* were sloughed from all rock bass (*Ambloplites rupestris*) within 1 day after exposure, but a light infestation of elongate glochidia (subfamily Lampsilinae) was discovered on two of these fish after 2 days. Juveniles of this undetermined mussel species were collected 11-15 days later.

Three species of Cyprinidae were confirmed as suitable fish hosts for the glochidia of the rabbit's foot mussel (Table 3). In three trials with a total of 13 whitetail shiners (*Notropis galacturus*), 17 juvenile mussels were collected in the siphonate of aquaria. The period of metamorphosis was 13-23 days at mean water temperatures between 20.5 and 21.6 C. One juvenile each was recovered from glochidial infestations on 12 spotfin shiners (*Notropis spilopterus*) in 20 days and from 12 bigeye chubs (*Hybopsis amblops*) in 17 days.

Three newly metamorphosed juveniles of *Quadrula cylindrica strigillata*, probably several hours old, averaged 0.23 mm in length, 0.23 mm in breadth and 0.16 mm in depth, with a hinge length of 0.15 mm. In size and shape, they were nearly identical to glochidia. Juveniles in a glass petri dish moved by slowly extending the foot a distance roughly equivalent to 50% of body length, then quickly drawing the body to the foot. The foot was extremely adhesive, and juveniles could hold position when nudged by a dissecting probe. In the laboratory, 0.5 cm of fine silt had settled in the shallow pan with juveniles, and no live individuals were recovered after 28 days.

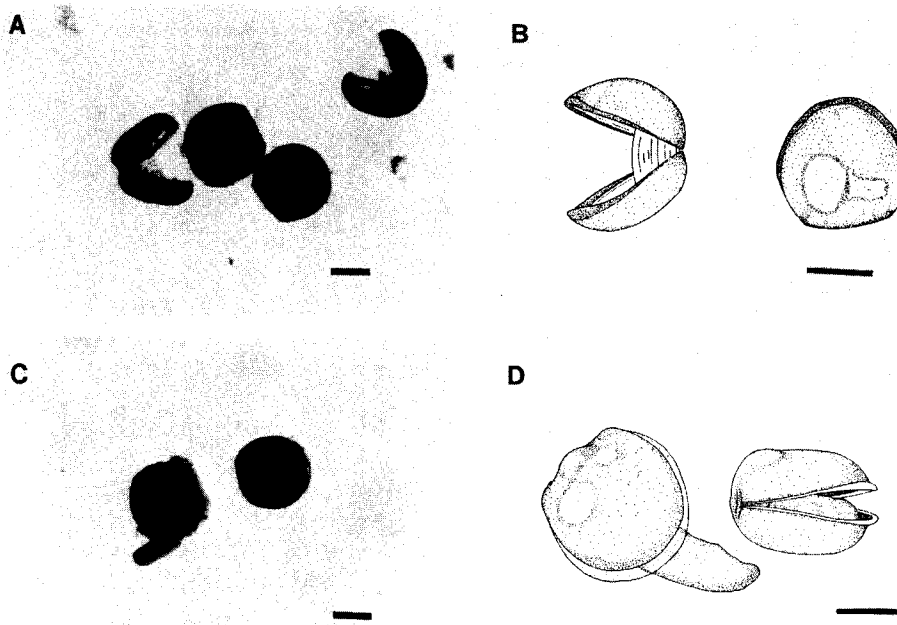


Fig. 3.—Mature glochidia of *Quadrula cylindrica strigillata* in (a) lateral and dorsal (100X) and (b) schematic view (155X). Metamorphosed juvenile (c) lateral (82X); (d) schematic view (165X). Bar = 100  $\mu\text{m}$

TABLE 2. — Number of fish exposed (N), maximum periods of attachment, mean water temperatures and sources of specimens in laboratory trials with glochidia of *Quadrula cylindrica strigillata* on nonhost fish species

Species	N	Period (days)	Temperature (C)	Source
Catostomidae				
<i>Catostomus commersoni</i>	1	3	20.8	Bull Run Creek
<i>Hyopentelium nigricans</i>	5	<1	21.2	Bull Run Creek
<i>Moxostoma erythrurum</i>	3	<1	22.8	Bull Run Creek
Centrarchidae				
<i>Ambloplites rupestris</i>	9	<1	22.4	Buffalo Creek
<i>Lepomis macrochirus</i>	5	2	22.2	Hinds Creek
<i>L. megalotis</i>	6	<1	22.7	Buffalo Creek
<i>Micropterus dolomieu</i>	2	<1	22.2	Bull Run Creek
<i>M. punctulatus</i>	4	<1	21.8	Bull Run Creek
Cottidae				
<i>Cottus carolinae</i>	7	<1	22.6	Bull Run Creek
Cyprinidae				
<i>Campostoma anomalum</i>	12	6	22.6	Buffalo Creek
<i>Hybopsis dissimilis</i>	7	<1	22.2	Powell River
<i>Nocomis micropogon</i>	1	<1	20.2	Powell River
<i>Notropis chrysocephalus</i> <sup>a-b</sup>	8	9-12	22.5	Hinds Creek
<i>N. coccogenis</i>	7	4	20.0	Stock Creek
<i>N. leuciodus</i>	9	2	21.9	Copper Creek
<i>N. rubellus</i>	9	2	21.9	Powell River
<i>N. serrulatus</i>	8	<1	22.4	North Fork Holston River
<i>N. volucellus</i>	3	<1	21.7	Powell River
<i>Phenacobius uranops</i>	3	<1	22.8	Powell River
<i>Pimephales notatus</i> <sup>b</sup>	25	4-7	22.7	Mill Creek
<i>Rhinichthys atratulus</i>	12	2	21.8	Irving Branch
Cyprinodontidae				
<i>Fundulus catenatus</i>	7	<1	22.6	Stock Creek
Ictaluridae				
<i>Ictalurus punctatus</i>	6	<1	22.7	Cumberland River
<i>Noturus eleutherus</i>	1	<1	20.8	Powell River
Lepisosteidae				
<i>Lepisosteus osseus</i>	1	<1	20.8	Stock Creek
Percidae				
<i>Etheostoma blennioides</i>	6	<1	21.6	Hinds Creek
<i>E. jessiae</i>	12	3	22.8	Hinds Creek
<i>E. rufilineatum</i>	6	<1	22.7	North Fork Holston River
<i>E. simoterum</i>	11	2	22.6	Hinds Creek
<i>E. zonale</i>	17	2	21.2	North Fork Holston River
<i>Percina caprodes</i>	3	<1	20.5	Hinds Creek

<sup>a</sup> Fish with trematode infestation

<sup>b</sup> Two infestation trials on this species



## DISCUSSION

In the upper Tennessee River drainage, *Quadrula cylindrica strigillata* is a short-term summer breeder (tachytictic) that spawns and releases glochidia from May to July. Although this reproductive period agrees with periods described previously for other short-term breeders (Matteson, 1948; Yokley, 1972; Weaver, 1981), the seasonal gametogenic cycle is different. Gonadal development in the rabbit's foot mussel was characterized by three general stages; active gametogenesis in late summer, a relatively inactive overwintering period, and rapid gamete maturation and release from May to July. Yokley (1972) reported early gametogenic stages in male *Pleurobema cordatum* from the Tennessee River during summer and sperm by autumn. Mature sperm were present year-round in *Elliptio complanatus* from the Great Lakes region (Matteson, 1948) and in *Pleurobema oviforme* from the Tennessee River drainage (Weaver, 1981), increasing in abundance as the spawning period approached. Females of these three species also contained mature ova during winter months, with little if any additional maturation reported before the spring spawning. The formation of intermediate gametic stages during winter and rapid maturation of sex products during spring in the rabbit's foot mussel thus differed considerably from the seasonal progression of gametogenesis in other summer breeders that have been studied.

The fertilization period for *Quadrula cylindrica strigillata* apparently began in mid-May and continued into June. Gravid females were collected from mid-May through early July. This period coincides with the occurrence of gravid specimens in June and July in the Cumberland River (Wilson and Clark, 1914) and with 22 May to 8 July records of gravid females in Pennsylvania (Ortmann, 1919). The breeding period of this and other *Quadrula* species is apparently similar throughout the Mississippi River basin (Lefevre and Curtis, 1910; Howard, 1914), but some differences in reproductive traits are evident. Females of *Quadrula* were reported to carry developing glochidia in all four gills (Howard, 1914; Ortmann, 1919), but we found this trait varied in the rabbit's foot mussel throughout the period of gravidity. The high percentage (65%) of females with glochidia in the outer gills only is apparently atypical for the genus (Howard, 1914).

Unfertilized eggs are commonly found in summer breeders and are especially common among *Quadrula* species (Lefevre and Curtis, 1910). However, we observed high fertilization success (>95%) through late June, particularly at the Pendleton Island site. The abundance of *Quadrula cylindrica strigillata* at this location was greater than at other known sites in the upper Tennessee drainage, and this highly successful fertilization may be attributed to high population density. Coker *et al.* (1921) suggested that release of sperm of male mussels and their entry into females may stimulate ovulation, although no documentation has yet been presented. The females with unfertilized eggs in their gills on 7 July apparently released eggs after the major spawning period of males (late May until July). Lefevre and Curtis (1910) intimated that their collection records

TABLE 3.—Metamorphosis of glochidia of *Quadrula cylindrica strigillata* from induced laboratory infestations on whitetail shiners (*Notropis galacturus*), spotfin shiners (*N. spilopterus*) and big-eye chubs (*Hybopsis amblops*)

Fish species	No. of fish exposed	Period of metamorphosis (days)	Mean temperature (C)	No. of juveniles recovered
<i>N. galacturus</i> <sup>a</sup>	2	18	21.6	1
	10	15-23	20.6	12
	1	17	21.9	4
<i>N. spilopterus</i> <sup>a</sup>	12	20	21.0	1
<i>H. amblops</i> <sup>b</sup>	12	17	21.3	1

<sup>a</sup> Collected from Bull Run Creek

<sup>b</sup> Collected from Powell River

of *Quadrula* species provided evidence of a double spawning, first in June-July and again in July-August; however, our field and laboratory examination of specimens provided no evidence of the bimodal occurrence of gravid females.

The gills of gravid females were clearly distended and assumed the coloration of enclosed conglutinates. Ortmann (1919) described the conglutinates of the rabbit's foot mussel as being a yellow brown or pale orange, and we noted similar hues depending on the stage of development. The coloration of conglutinates provided a useful means of determining when mature glochidia were present and ready for release. Our measurements of mature glochidia of *Quadrula cylindrica strigillata* were slightly larger than previously reported (length and height ca. 0.19 mm) by Ortmann (1919) and similar in size and shape to those of *Q. metanevra* (Lefevre and Curtis, 1910; Surber, 1912). The most diagnostic character for mature glochidia of the rabbit's foot mussel was the tinge of reddish brown on the mantle.

Sensitivity of gravid *Quadrula cylindrica strigillata* to handling and holding stresses confirmed earlier observations of other *Quadrula* species. Lefevre and Curtis (1910) reported that each of the *Quadrula* species they studied tended to abort embryos and glochidia to varying degrees when removed from the river. Within a few hours after collection, at least partial abortion occurred in all specimens in spite of precautionary measures. However in our flow-through holding tanks, some females retained glochidia for up to 8 days under conditions of high aeration, low turbidity and adequate current. This tendency to abort was a major problem in our study and will probably handicap efforts to conduct life history research on congeneric species, particularly the endangered *Q. intermedia* and *Q. sparsa*.

The mechanics of attachment and encystment by glochidia on host fish followed early descriptions of natural infestations (Arey, 1921, 1924, 1932). Sloughing of glochidia by nonhost fish within a few hours after attachment appeared to involve some degree of cytolytic disintegration. Open valves of sloughed glochidia in aquaria were generally without internal tissues. The few closed glochidia with body tissues intact found in the siphonate 1 day after infestation apparently had not attached successfully to gill lamellae. Meyers *et al.* (1980) noted a failure of host cells to form cyst walls around glochidia of *Margaritifera margaritifera* as an initial rejection response by coho salmon (*Oncorhynchus kisutch*) but did not describe the condition of rejected glochidia. Seemingly normal glochidia of *M. margaritifera* were rejected after infestation by a tissue response of cyst formation and sloughing of tissue (Fustish and Millemann, 1978). Similarly, glochidia of *Quadrula cylindrica strigillata* partly enclosed in nonhost fish tissue were sloughed intact with no evidence of disintegration after 9-12 days on the cyprinid *Notropis chrysocephalus*. However, we were unable to document the manner of rejection from unsuitable hosts because of the relatively short sloughing period.

Glochidia of *Quadrula cylindrica strigillata* exhibited a relatively high degree of host specificity, metamorphosing on only three (all cyprinids) of the 34 fish species tested. Association of the rabbit's foot mussel with members of the Cyprinidae may be related to the co-occurrence of the mussel with these fish species in riverine habitats. Adult mussels were usually observed lying on their sides on a mixed cobble and gravel substrate or were only partly burrowed in the river bottom. Water velocity appeared to influence the instream distribution of specimens, since this species was most abundant in eddies along the periphery of midstream currents or adjacent to emergent or submersed vegetation. It was not found in river sections with considerable current or stagnant water. Individuals or small schools of whitetail shiners are commonly collected in riffle areas with coarse substrates immediately above and below pools or in eddies along the periphery of midstream currents (Outten, 1958). On several occasions, we collected gravid *Q. c. strigillata* with individuals of *Notropis galacturus* hovering in proximity. The co-occurrence of mussel and host species in the same habitat obviously would enhance the degree of infestation and reproductive success for the mussel.

The degree of overlap between the rabbit's foot mussel and the whitetail shiner in

their southern (Tennessee-Cumberland rivers) and western (Black, White, Saline, Quachita and Neosho rivers) changes is noteworthy (Fig. 4). Numerous other cyprinids of the subgenus *Cyprinella*, including partly sympatric species, border the southern range of *Quadrula cylindrica*, but the rabbit's foot mussel appears to occur only in those river drainages populated by the whitetail shiner. Ranges of *Notropis spilopterus* and *Hybopsis amblops* (Lee *et al.*, 1980) encircle the northern portion of the range of *Q. cylindrica*, and these fish may serve as more frequent hosts in those northern drainages. The occurrence of other forms of *Q. cylindrica* in the mainstream Tennessee and Ohio Rivers where *N. galacturus* and *H. amblops* typically do not occur, and in regions not occupied by *N. spilopterus* (*e.g.*, northern Arkansas), suggests that additional hosts remain to be identified. One likely candidate fish host in these other rivers would be *N. whipplei*, formerly considered a subspecies of *N. spilopterus*, which has a range enclosing that of the rabbit's foot mussel (Lee *et al.*, 1980).

*Acknowledgments.*—We thank H.E. Kitchel for preparing histological sections and photographs; G. Owens for maintaining laboratory facilities; and S. Ahlstedt, J. Feeman and C. Saylor for assisting with the collection of mussels and fish. The Virginia Cooperative Fishery Research Unit is jointly supported by the U.S. Fish and Wildlife Service, the Virginia Commission of Game and Inland Fisheries, and Virginia Polytechnic Institute and State University.

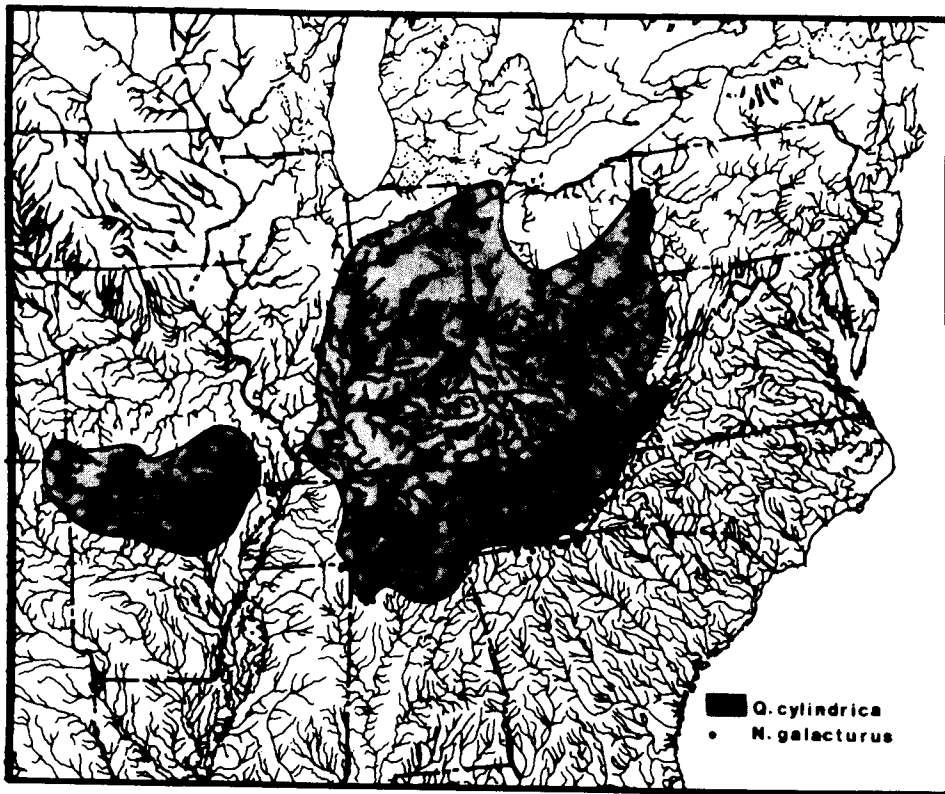


Fig. 4.—Distribution of *Quadrula cylindrica* (shaded area) and of the host fish *Notropis galacturus* (dots) in the South-central United States. Host distribution was redrawn from Lee *et al.* (1980)

## LITERATURE CITED

- AREY, L. B. 1921. An experimental study of glochidia and the factors underlying encystment. *J. Exp. Zool.*, **33**:463-499.
- . 1924. Glochidial cuticulae, teeth, and mechanics of attachment. *J. Morphol. Physiol.*, **39**:323-355.
- . 1932. The formation and structure of the glochidial cyst. *Biol. Bull.*, **62**:212-221.
- CHAMBERLAIN, T. K. 1931. Annual growth of freshwater mussels. *Bull. U.S. Bur. Fish.*, **46**:713-739.
- COKER, R. E. 1919. Freshwater mussels and mussel industries of the United States. *Ibid.*, **36**:13-89.
- , A. F. SHIRA, H. W. CLARK AND A. D. HOWARD. 1921. Natural history and propagation of fresh-water mussels. *Ibid.*, **37**:77-181.
- CROWLEY, T. E. 1957. Age determination in *Anodonta*. *J. Conchol.*, **24**:201-207.
- FULLER, S. L. H. 1974. Clams and mussels (Mollusca:Bivalvia), p. 215-273. In: C. W. Hart, Jr. and S.L.H. Fuller (eds.). *Pollution ecology of freshwater invertebrates*. Academic Press, New York.
- FUSTISH, C. A. AND R. E. MILLEMANN. 1978. Clochidiosis of salmonid fishes. II. Comparison of tissue response of coho and chinook salmon to experimental infection with *Margaritifera margaritifera* (L.) (Pelecypoda: Margaritanidae). *J. Parasitol.*, **64**:155-157.
- HOWARD, A. D. 1913. The catfish as a host for fresh-water mussels. *Trans. Am. Fish. Soc.*, **42**:65-69.
- . 1914. Experiments in propagation of fresh-water mussels of the *Quadrula* group. Rep. U.S. Comm. Fish. 1913, Appendix IV: 1-52.
- HUMASON, G. L. 1972. *Animal tissue techniques*. W. H. Freeman Co., San Francisco, Calif. 641 p.
- JENKINSON, J. J. 1981. The Tennessee Valley Authority Cumberlandian Mollusk Conservation Programs. *Am. Malacol. Union Inc. Bull.*, **1980**:62-63.
- LEE, D. S., C. R. GILBERT, C. H. HOCUTT, R. E. JENKINS, D. E. MCALLISTER AND J. R. STAUFFER, JR. 1980. *Atlas of North American freshwater fishes*. N.C. State Mus. Nat. Hist., Raleigh, N.C. 854 p.
- LEFEVRE, G. AND W. C. CURTIS. 1910. Reproduction and parasitism in the Unionidae. *J. Exp. Zool.*, **9**:79-116.
- MATTESON, M. R. 1948. Life history of *Elliptio complanatus* (Dillwyn, 1817). *Am. Midl. Nat.*, **40**:690-723.
- MEYERS, T. R., R. E. MILLEMANN AND C. A. FUSTISH. 1980. Glochidiosis of salmonid fishes. IV. Humoral and tissue responses of coho and chinook salmon to experimental infection with *Margaritifera margaritifera* (L.) (Pelecypoda:Margaritanidae). *J. Parasitol.*, **66**:274-281.
- ORTMANN, A. E. 1919. A monograph of the naiades of Pennsylvania. Part III. Systematic account of the genera and species. *Mem. Carnegie Mus.*, **8**:1-384.
- OUTTEN, L. M. 1958. Studies of the life history of the cyprinid fishes *Notropis galacturus* and *rubricroceus*. *J. Elisha Mitchell Sci. Soc.*, **74**:122-134.
- SURBER, T. 1912. Identification of the glochidia of freshwater mussels. U.S. Bur. Fish. Doc. No. 771. 10 p.
- . 1913. Notes on the natural hosts of fresh-water mussels. *Bull. U.S. Bur. Fish.*, **32**:110-116.
- WEAVER, L. R. 1981. Life history of *Pleurobema oviforme* (Mollusca:Unionidae) in Big Moccasin Creek, Virginia, with emphasis on early life history, species associations, and age and growth. M.S. Thesis, Va. Polytech. Inst. State Univ., Blackburg. 89 p.
- WILSON, C. B. AND H. W. CLARK. 1914. The mussels of the Cumberland River and its tributaries. U.S. Bur. Fish. Doc. No. 781. 63 p.
- YOKLEY, P., JR. 1972. Life history of *Pleurobema cordatum* (Rafineque 1820) (Bivalvia:Unionacea). *Malacologia*, **11**:351-364.
- ZALE, A. V. AND R. J. NEVES. 1982a. Reproductive biology of four freshwater mussel species (Mollusca:Unionidae) in Virginia. *Freshwater Invertebr. Biol.*, **1**:17-28.
- AND ———. 1982b. Fish hosts of four species of lamproline mussels (Mollusca: Unionidae) in Big Moccasin Creek, Virginia. *Can. J. Zool.*, **60**:2535-2542.