J. N. Am. Benthol. Soc., 2002, 21(1):76–88

Life history and propagation of the endangered fanshell pearlymussel, *Cyprogenia stegaria* Rafinesque (Bivalvia:Unionidae)

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Abstract. Aspects of the reproduction, age, growth, fish hosts, and culture of juveniles were determined for the endangered fanshell pearlymussel, *Cyprogenia stegaria* Rafinesque, 1820, in the Clinch River, Tennessee. Glochidia of *C. stegaria* are contained in red, worm-like conglutinates that resemble oligochaetes. Conglutinates are 20 to 80 mm long and are released through the excurrent aperture. Estimated fecundity was 22,357 to 63,459 glochidia/mussel. Eighty-four valves of *C. stegaria* were thin-sectioned for aging; ages ranged from 6 to 26 y. Of 16 fish species tested, 9 hosts were identified through induced infestations of glochidia: mottled sculpin (*Cottus bairdi*), banded sculpin (*Cottus carolinae*), greenside darter (*Etheostoma blennioides*), snubnose darter (*Etheostoma simoterum*), banded darter (*Etheostoma zonale*), tangerine darter (*Percina aurantiaca*), blotchside logperch (*Percina burtoni*), logperch (*Percina caprodes*), and Roanoke darter (*Percina roanoka*). Newly metamorphosed juveniles were cultured in recirculating and nonrecirculating aquaculture systems within dishes containing sediments of 300 to 500 μm diameter (sand) or <105 μm diameter (silt), and fed either the green algae *Neochloris oleoabundans* or *Scenedesmus quadricauda* daily. Growth and survival of juvenile mussels were highest in the nonrecirculating aquaculture system, with a mean survival of 72% after 2 wk and 38% after 4 wk.

Key words: fanshell pearlymussel (*Cyprogenia stegaria*), endangered, life history, fish hosts, reproduction, propagation.

The fanshell pearlymussel, *Cyprogenia stegaria* Rafinesque, 1820, is endemic to the Ohio, Cumberland, and Tennessee river drainages (Ortmann 1918, 1919). Because of severe declines in populations, the species was listed as endangered by the US Fish and Wildlife Service (USFWS) in 1990. The fanshell inhabits medium to large rivers on shoals of coarse gravel and sand. At present, reproducing populations of this freshwater mussel are known from only 3 rivers: the upper Clinch River in Tennessee and Virginia, and the Green and Licking rivers in Kentucky. A small reproducing population may exist in the Tennessee River below Pickwick Landing Dam (Parmalee and Bogan 1998). Other presumed relict populations may persist in the Muskingum and Walhonding rivers in Ohio, Kanawha River in West Virginia, Wabash River in Illinois and Indiana, Barren River and Tygarts Creek in Kentucky, and the Tennessee and Cumberland rivers in Tennessee (USFWS 1991). An estimated 760 km of rivers throughout the United States currently contain populations of *C. stegaria*, which represents <10% of its historic range (R. Biggins, USFWS, Asheville, North Carolina, unpublished data). River alterations by dams, channel dredging, sand and gravel mining, coal mining, and sewage wastes have caused drastic declines in populations of *C. stegaria* in the 20th century. Even in reproducing populations, such as the Clinch River, declines of this species may still be occurring (Ahlstedt 1991).

This species was reported by Ortmann (1919) to be a long-term brooder; however, little else is known of its life history. The purpose of our research was to provide needed biological data specified in the federal recovery plan for this species (USFWS 1991). Specifically, *C. stegaria* life history, propagation, and culture were studied so this information could be applied to the restoration and recovery of the species.

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[2] The Unit is supported jointly by the US Geological Survey, Virginia Department of Game and Inland Fisheries, Wildlife Management Institute, and Virginia Polytechnic Institute and State University.

Methods

Fecundity

We estimated fecundity of 4 female *C. stegaria* from the number of mature glochidia in gravid

FIG. 1. Conglutinates of the fanshell pearlymussel, *Cyprogenia stegaria*.

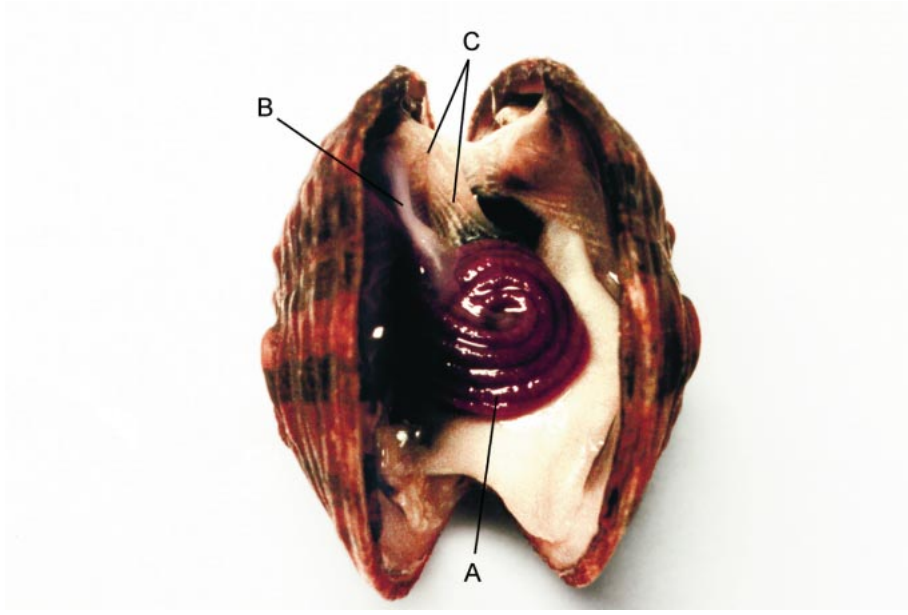

FIG. 2. A.—Conglutinates of the fanshell pearlymussel, *Cyprogenia stegaria*, coiled and contained in the marsupium of the outer gill. B.—Marsupium attached to the base of the inner lamina of the outer gill. C.—Inner and outer gills (left).

females. We used the end of a hypodermic needle to unravel and expose the distal ends of individual conglutinates. The red conglutinates of *C. stegaria* have the texture, shape, and color of oligochaetes (Fig. 1). Once a tip was exposed, forceps were used to pull the conglutinate free from the gill of the mussel. Each conglutinate was examined with a dissecting microscope to determine where mature glochidia were contained. The distal end of a conglutinate was generally comprised almost entirely of mature glochidia, whereas the basal end (the end attached to the gill) was comprised almost entirely of eggs or developing embryos. Therefore, we visually determined the point near the basal end of each conglutinate where the remaining material was ~90 to 100% embryos, usually ~60 to 75% of the length from the distal end of a conglutinate. We considered the segment that contained mostly mature glochidia to be the *viable* portion of the conglutinate. The total length and the viable length of each conglutinate were measured with calipers. Three conglutinates per mussel were selected; each conglutinate had a length with viable glochidia ≥30 mm. The segment of viable conglutinate was cut into three 10-mm long pieces (for ease of handling) from the distal to the basal end. Under a microscope, conglutinates were pinched and teased apart with tweezers to free the glochidia. This technique damaged some glochidia but released thousands unharmed. Released glochidia were enumerated to obtain a total count for each 10-mm long segment. Usually 100 to 300 glochidia could not be removed from the conglutinate, so they were counted or visually estimated to add to the total of each 10-mm long segment. The total number of glochidia from nine 10-mm long segments of conglutinate per mussel was used to obtain an average number of glochidia/segment. We divided this number by 10 to obtain the average number of glochidia per mm of conglutinate. This number was multiplied by the total viable length of conglutinate per female mussel to calculate the number of mature glochidia in each gravid female. Mean number of glochidia per female mussel also was calculated.

Our estimates of fecundity were only approximate because we neither randomly selected conglutinates, nor randomly selected 10-mm sections of sampled conglutinates. We used the larger conglutinates that contained more glochidia/mm than smaller conglutinates; therefore, the average number of glochidia/mm of conglutinate may be higher than average. Our selection of samples from the tip, middle, and end of the viable conglutinate probably underestimated the number of glochidia contained in larger conglutinates. For example, our method sampled only 50% of a section of viable conglutinate 60 mm in length. Furthermore, 20 mm of the sample came from sections of the conglutinate containing considerably fewer glochidia than sections near the distal end or tip of the conglutinate. Although the most accurate estimate would be obtained from counting all glochidia in viable conglutinates, total counts were not practical for this endangered species.

Age and growth

Relic valves, fresh dead shells, and live *C. stegaria* were collected from various Clinch River kilometer (CRKM) locations between Horton Ford (CRKM 321) and Swan Island (CRKM 277). Live *C. stegaria* were measured for length and either returned immediately to the site of collection or, if gravid, collected for juvenile mussel propagation. Measurements of 16 live mussels were obtained from quadrat sampling conducted by Ahlstedt and Tuberville (1997), and 39 specimens from collecting trips from 1998 to 2001. Relic valves and fresh dead shells of various lengths were collected to best represent the size range of the population in the river. We used relic valves if the periostracum and external growth rings of collected specimens were intact. Valves of *C. stegaria* contain relatively distinct external growth rings, which were enumerated visually. We thin-sectioned 84 valves, following procedures described by Clark (1980) and Neves and Moyer (1988), using a Buehler Isomet low-speed saw unit with a diamond-impregnated blade (Buehler Ltd., Evanston, Illinois). Valves were cut from the center of the umbo to the ventral shell margin. Cut valves were glued (2-Ton Clear Epoxy, Riviera Beach, Florida) to petrographic microslides (27 × 46 mm), then vacuum-sealed into a petrographic chuck attached to the cutting arm of the saw, and sectioned at a thickness of 280 μm (Neves and Moyer 1988). Because shells < age 6 were rare in the river, we obtained lengths at ages 1 to 5 y by back-calculating length-at-age based on internal annuli of older valves ($n = 5$) (Bruenderman and Neves 1993). Thin-sections

of valves were examined under 4× magnification, and internal growth lines were considered true annuli if they were continuous from the umbo region to the outer surface of the valve. Thin-sections and external growth rings of valves were examined separately by 3 experienced technicians to obtain consensus on the mean number of internal and external growth lines per valve. If a consensus was not obtained, then the thin-section in question was not used in the study. We assumed that 1 annulus/y is formed, based on the work of Neves and Moyer (1988) in temperate rivers.

Host fish

Gravid females of *C. stegaria* were collected by snorkeling in the Clinch River from Horton Ford downstream to Swan Island. Mussels were examined for gravidity by slowly opening the valves by hand to look for the red conglutinates characteristic of the species.

Most fish to be tested as potential hosts were collected from the upper North Fork Holston River, ~1 km above Saltville, Virginia. Banded sculpins (*Cottus carolinae)* were collected from the Middle Fork Holston River at Atkins, Smyth County, Virginia; and mottled sculpins (*C. bairdi*) were collected from Sinking Creek at Newport, Giles County, Virginia. All fish collection sites had few mussels and no populations of *C. stegaria*. Common and scientific names follow Robins et al. (1991) for fishes, and Turgeon et al. (1998) for mussels.

Methods for infesting fish with mussel glochidia generally followed those of Zale and Neves (1982). A plastic container 29 cm long, 19 cm wide, and 12 cm deep was used to hold fish while they were being infested. Usually ~15 to 25 fish of various species were held in ~0.5 L of water, and glochidia from 3 to 10 conglutinates were added to the container and agitated with an airstone for 1 h. After infestation, fish were separated by species and placed in 38 L aquaria. Fish were held at low densities (~1 to 8/aquarium), depending on size and availability of fish. The contents from the bottoms of aquaria were siphoned every 2 to 3 d until juvenile mussels were collected. About 10 to 15 L of conditioned water were added to aquaria after siphoning to maintain water levels. If juveniles were collected, they were counted and put in a culture dish, and aquaria were then siphoned every 1 to 2 d. The collection of mobile juveniles indicated a fish species was capable of transforming glochidia to juveniles, and was a potential host of *C. stegaria*.

Culture of juveniles

Our recirculating aquaculture system for juvenile mussels resembled that of O'Beirn et al. (1998). In 1999, juvenile mussels were held in containers constructed of PVC pipe 7.5 cm in height and diameter; a piece of 75-μm mesh screen was glued to one end of the pipe as a bottom. In 2001, we used containers 23 cm in diameter and 6 cm high. Juvenile mussels were cultured in either fine sediment or sand to compare survival and growth of juveniles held in different substrata. Fine sediment was collected from the banks of the Clinch River at Wallens Bend (CRKM 310), and sieved to particle sizes <105 μm. In 1999, 2 to 3 cm of fine sediment were placed into the containers and allowed to settle for 3 d to become firm. In 2001, ~5 mm of fine sediment were placed in containers and allowed to settle for 1 d. Sand was collected from the New River, Montgomery County, Virginia, and was prepared by sieving to particle sizes of 300 to 500 μm. In both years, 0.25 to 0.5 cm of sand was put into the containers. We used a 225 L plastic livestock feed trough as our raceway to hold containers with juvenile mussels. A 50:50 mixture of conditioned (dechlorinated) municipal water and well water was used in the recirculating culture system, with hardness ranging from 250 to 350 mg/L $CaCO_3$. The recirculating culture system was set up in a greenhouse at the Virginia Tech Aquaculture Center, where ambient water temperatures in the raceway ranged from 18 to 21°C during February and March 1999 and 19 to 24°C during March and April 2001.

In 1999, juvenile mussels were fed a daily ration of 3 L of the green alga *Scenedesmus quadricauda* at a mean concentration of 2 to 4 × 10^6 cells/mL. This feed ration provided a mean of 18,943 cells/mL (SE 789) of water in the recirculating culture system. A total of 2561 juvenile mussels were cultured in sand in the raceway. These juveniles were placed into 15 containers, with a mean of 170 juveniles/container (range = 70–372). A total of 1333 juvenile mussels were cultured in fine sediment. These juveniles were placed into 12 containers, with a mean of 111

TABLE 1. Reproductive status of *Cyprogenia stegaria* collected in the Clinch River from 1998 to 2001.

	Month					
	October–November	December–January	February–March	April–May	June–July	August–September
Number examined	10	2	6	9	18	3
Number gravid	5	1	4	4	0	0

juveniles/container (range = 23–265). In 2001, juvenile mussels were fed a daily ration of 8 L of the green alga *Neochloris oleoabundans* at a mean concentration of 8 to 12 $\times$ 10^6 cells/mL. This feed ration provided a mean of 30,256 cells/mL (SE 2891) of water in the recirculating culture system. A total of 406 juvenile mussels were cultured in sand in the raceway. These juveniles were placed into 4 containers, with a mean of 101 juveniles/container (range = 51–173). A total of 382 juvenile mussels were cultured in fine sediment. These juveniles were placed into 4 containers, with a mean of 95 juveniles/container (range = 33–154). Containers with juvenile mussels in both sand and fine sediment substrata were placed side by side on the bottom of the raceway and cultured together. The dishes were cleaned weekly to remove excess algae and potential predators such as *Hydra* and flatworms. Containers with fine sediment were cleaned by gently rinsing off the juvenile mussels and accumulated algae from the top of containers in a 150-μm mesh sieve. The contents then were washed in the sieve with conditioned water until most of the algae were removed. Containers with sand were cleaned by rinsing the entire contents into a 150-μm mesh sieve. The contents, including the juvenile mussels, were gently washed. For all trials, algae that accumulated on the bottom of the raceway were siphoned out weekly, and 50% of the water was exchanged in the system each week.

Juvenile mussels also were held in small containers to compare survival and growth of juveniles cultured in a nonrecirculating system to that of our recirculating culture system. A total of 1690 juvenile mussels were placed in 12 small tupperware containers, with a mean of 141 juveniles/container (range = 61–258). Each container was 6 cm square and 5 cm deep. Juvenile mussels were placed in containers with 50 mL of conditioned water and 50 mL of algae, at a cell density of 2 $\times$ 10^6 cells/mL; therefore, algal density was many times greater than in the troughs. In addition, ~0.5 mL of fine sediment (particle size <105 μm) was pipetted into each dish. The water, algae, and sediment were exchanged every 2 d. Water temperatures were maintained at ambient levels (20–21°C). In both the recirculating and nonrecirculating culture systems, juvenile mussels were counted at 2 wk and 4 wk to determine survival.

Data analysis

A von Bertalanffy growth equation was computed from length-at-age data and was fit by nonlinear procedures to derive the parameters of the equation (SAS Institute. 2001. Statistical analysis system, SAS Institute, Cary, North Carolina). Counts of external growth-rings and internal annuli were compared using a *t*-test, and survival and growth of cultured juveniles were compared using repeated-measures analysis of variance (SAS Insitute 2001).

Results

Fecundity

Mussels were gravid from late October to late May (Table 1). Most gravid *C. stegaria* contained 3 to 7 conglutinates between the inner and outer gills. Two small females, 32 to 36 mm long, contained only 1 to 3 short conglutinates/pair of gills. Conglutinates were 20 to 80 mm long and 1 to 4 mm wide. Conglutinates were aligned parallel to each other and enclosed and coiled within a membrane or external *marsupium* associated with the outer gill (Fig. 2). This membrane is attached to the base of the inner lamina of the outer gill, positioning conglutinates between the inner and outer gills (Fig. 2). Our observations concur with Ortmann (1911) in that conglutinates are typically only attached from the central to anterior portion of the gill, leaving the posterior half nonmarsupial.

TABLE 2. Number of mature glochidia/female for 4 specimens of *Cyprogenia stegaria*.

	Specimen no.			
Measurements	1	2	3	4
Length (mm)	37.3	48.2	47.2	34.6
Date collected	3 November 1998	15 November 1998	15 December 1998	7 April 1999
Number of conglutinates	8	14	6	6
Glochidia per mussel	24,999	63,544	63,011	22,393

Most of each conglutinate was not contained in the water tubes of the outer gill. Each individual conglutinate was contained within the marsupial membrane separately; therefore, when a conglutinate was extracted from the mussel, an enclosed tube within the membrane remained. Ortmann (1912) provided a diagram and description of how the conglutinates are attached to the gills of *C. stegaria*. Each conglutinate was modified at the basal end with several ribs and slots for attachment between filaments of the outer gill and perhaps to the gill axis (Fig. 1). The modified basal end was generally ~5 mm long and 4 mm in diameter, and was hollow. The glochidia at this end of a conglutinate were the least mature and generally were only eggs or developing embryos. The yolks of embryos were brick red, giving the conglutinate its color. The embryos forming the basal end of a conglutinate were elastic and difficult to break apart. As embryos mature into glochidia, the reddish color fades. The color fade could be seen at the tip or distal end of a conglutinate where glochidia were mature and transparent, giving this section an opaque color. Chamberlain (1934) made nearly identical observations on the color, structure, and release of conglutinates produced by the western fanshell (*C. aberti*), stating that the conglutinate produced by this mussel was a ''bright red, wormlike object''.

One gravid female, released 4 conglutinates into the trough of the recirculating system used to hold adult mussels. Conglutinates were released one at a time from the excurrent aperture over a period of 2 wk. The modified basal end of each conglutinate exited first. Conglutinates were first released into the suprabranchial cavity before exiting through the excurrent aperture. March was the earliest conglutinates were expelled from the marsupium into the river. Some of the females collected in April and May had only 1 or 2 conglutinates remaining, suggesting that most females released their conglutinates by late spring.

Mean fecundity of 4 female *C. stegaria* was 43,494 mature glochidia/mussel (Table 2). The 2 largest *C. stegaria* contained the most glochidia because of the greater size and numbers of conglutinates in these individuals. We estimated that ~30 to 50% of all conglutinate contents in the 4 females examined were embryos or unfertilized eggs. These embryos or eggs may serve a functional role in keeping the conglutinate together and making it alluring to fish hosts.

Age and growth

Ages of *C. stegaria* ranged from 6 to 26 y. Mean lengths by age were fitted to a von Bertalanffy growth equation ($L(mm)_t = 53.2\ (1 - e^{-0.1461(t-0.053)})$) and used to predict values for length ($r^2 = 0.97$). Annual growth averaged 4.1 mm/y through age 10, and decreased to ~0.7 mm/y thereafter (Table 3).

A comparison of ages derived from counts of external annuli versus internal annuli showed that counts of external annuli disagreed greatly with the number of internal annuli in thin-sections (Fig. 3). Counts of external growth rings consistently underestimated ages of *C. stegaria* valves determined by internal annuli to the extent that mussel ages determined by the 2 methods were significantly different ($p < 0.001$). Therefore, external shell characteristics, such as growth rings and shell length, are not reliable means to determine ages of *C. stegaria* in the Clinch River.

The mean age at death of *C. stegaria* was ~12 to 13 y, based on predicted length-at-age data presented in Table 3. The smallest individuals found gravid were 28 to 34 mm long, implying that most individuals are mature at ages 5 to 9 y.

TABLE 3. Observed and predicted lengths-at-annuli (mm) of *Cyprogenia stegaria* shells collected from the Clinch River, Hancock County, Tennessee, 1998 to 1999.

Annulus	Number of individuals	Observed length		Predicted length	Growth increment
		Mean	Range		
0	1	0.21	0.21	−0.41	0
1	5	7.1	5.2–9.0	6.9	6.9
2	5	11.9	8.9–15.0	13.1	6.2
3	5	16.5	13.0–20.5	18.6	5.5
4	5	22.1	18.1–25.0	23.3	4.7
5	5	27.0	23.4–29.2	27.3	4.0
6	3	34.3	32.5–37.0	30.8	3.5
7	2	37.5	33.9–41.2	33.9	3.1
8	5	38.6	33.5–41.4	36.5	2.6
9	7	37.5	32.2–41.0	38.8	2.3
10	3	40.9	40.0–41.9	40.7	1.9
11	8	42.3	39.9–47.4	42.4	1.7
12	8	40.9	36.9–47.2	43.9	1.5
13	9	46.7	40.0–52.1	45.1	1.2
14	4	43.7	40.1–48.0	46.2	1.1
15	9	48.8	43.3–55.5	47.2	1.0
16	6	48.4	41.9–56.7	48.0	0.8
17	4	42.6	38.1–46.1	48.7	0.7
18	1	45.2	45.2	49.3	0.6
19	3	50.6	48.5–54.1	49.8	0.5
20	2	54.0	53.4–54.6	50.3	0.5
21	3	50.4	41.2–55.7	50.7	0.4
22	4	53.9	44.3–59.9	51.0	0.3
23	1	50.6	50.6	51.3	0.3
24	1	50.0	50.0	51.6	0.3
25	1	51.2	51.2	51.8	0.2
26	1	56.0	56.0	52.0	0.2

Fish hosts

We tested 16 species of fish as hosts for *Cyprogenia stegaria* (Table 4). Seven species of darters (Percidae) and 2 species of sculpin (Cottidae) were identified as hosts from induced infestations of glochidia. Eight of the fish species are native to the Tennessee River system (Jenkins and Burkhead 1993) and sympatric with *C. stegaria* in all or part of its range. The Roanoke darter (*Percina roanoka*) is native to the Roanoke River system (Jenkins and Burkhead 1993) and is the only fish host identified from another drainage. All gilt darters (*P. evides*) held in aquaria died by day 26 because of bacterial infections. Therefore, gills from preserved specimens of gilt darters were excised and examined with a microscope for attached glochidia. None were found, but whether glochidia were sloughed off in aquaria or during preservation could not be determined. Our induced infestation results showed that *C. stegaria* used a range of host species in the genus *Percina*, so the potential fish host status of *P. evides* is undetermined. The excystment of juvenile mussels varied in timing and duration among fish hosts identified in our study (Table 4, Fig. 4).

Culture of juveniles

The average size of a 1-d-old juvenile was 0.21 mm long, 0.18 mm high, and 0.13 mm wide. Thus, newly excysted juveniles are only slightly larger than the dimensions of glochidia obtained by Hoggarth (1999). Fully developed juveniles are rounded in shape, slightly agape, light brown to gray in color, and actively pedal-feed. Many of the initial 50 to 100 juveniles to excyst from a host did not appear completely developed. The valves of these juveniles were more compressed and closed, and no pedal-feeding was observed. Juvenile mussels that excysted early and appeared premature experienced high mortality during efforts to culture them.

Survival of juvenile mussels among treatments was significantly higher ($p < 0.001$) in the nonrecirculating containers than in containers held in the recirculating system (Table 5). Survival of juveniles fed *N. oleoabundans* and held in the recirculating system was significantly higher ($p < 0.001$) in fine sediment than sand. Survival of juveniles fed *S. quadricauda* and held in the recirculating system in containers with either sand or fine sediment was not significantly different. Growth among treatments was not significantly different. Most of the remaining 8380 juvenile mussels propagated during this study were cultured for 1 to 2 wk in the nonrecirculating containers, and 7392 juveniles (survival = 88.2%) were released into the Clinch River in spring 1999.

Discussion

Formation of conglutinates and glochidial release

We infer that formation of conglutinates in *C. stegaria* begins when the first eggs or embryos are transported from the gonads to the base of

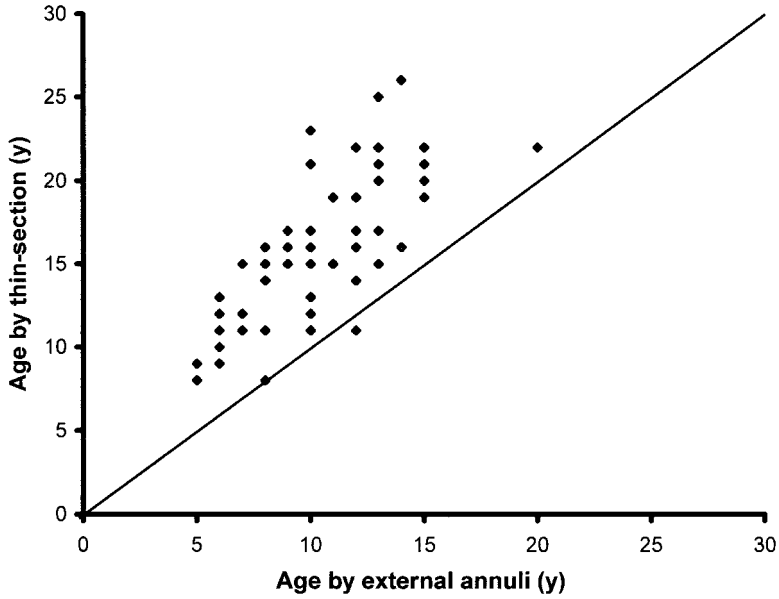

FIG. 3. Comparison of age estimates obtained from *Cyprogenia stegaria* valves by thin-sectioning and by counts of external growth rings. Data points above the 1:1 line represent underestimates of specimen ages by the external growth rings.

the outer gill, where they aggregate between the gill filaments along the gill axis. These initial embryos begin to fill in the marsupial membrane and form the distal ends or tips of conglutinates, while subsequent embryos allow conglutinates to grow in length, pushing the distal ends outward from the base of the gill. We speculate that the marsupial membrane grows around the embryos as conglutinates are formed. The last embryos transported to the gills form the modified basal end of the conglutinate, anchoring it to the base of the outer gill. We have observed that many more embryos develop into mature glochidia near the distal than basal ends of conglutinates. The increased number of glochidia at the distal end of the conglutinate may be a factor of the amount of time embryos need to develop, i.e., the embryos are transported to the marsupium earlier in the brooding season. Moreover, if these embryos are transported earlier, the warmer water temperatures in the summer and early fall may facilitate embryonic development.

Cyprogenia stegaria has unusual reproductive characteristics compared to other species in the subfamily Lampsilinae. *Cyprogenia* is one of the few genera in the subfamily that has modified conglutinates that are released through the suprabranchial cavity. Ortmann (1909, 1910, 1911) considered the release of glochidia through the suprabranchial cavity and excurrent aperture to be a more primitive mechanism of glochidial release, which is more typical of amblemine and anodontine mussels. The females of supposedly more advanced lampsilines release glochidia through pores along the edge of the ventral margin of the gill, have a modified mantle that displays to attract fish hosts, and are bradytictic or long-term brooders. *Cyprogenia* is bradytictic, but does not have modified mantle tissue to display and has a thick shell more typical of amblemines; therefore, this genus has traits characteristic of both amblemines and lampsilines. In addition, *C. stegaria* and *C. aberti* are the only North American unionids that brood most of their larvae in an external marsupium attached to the outer gill.

Age and growth

The thin-sectioning of shells of *C. stegaria* corroborated previous findings of the unreliability of external growth rings for age determination (Neves and Moyer 1988). As specimens age, the external growth ring method becomes more unreliable and underestimates actual age (Fig. 3).

TABLE 4. Results of fish-host tests using glochidia of *Cyprogenia stegaria*.

Fish species	No. of fish	No. of fish alive	No. of days	No. of juveniles recovered	Days to transform (peak)	Mean temp. (°C)
Centrarchidae						
Micropterus dolomieu[b]	20	18	45	0	0	18.0
Ambloplites rupestris[b]	20	17	45	0	0	18.0
Cottidae						
Cottus carolinae[a]	5	2	22	7	22 (22)	20.0
C. carolinae[d]	11	7	60	8	28–46 (38)	18.0
C. carolinae[d]	2	1	60	16	20–24 (22)	23.0
C. bairdi[e]	11	8	92	176	24–88 (43–55)	18.0
Cyprinidae						
Nocomis leptocephalus[b]	3	3	45	0	0	20.0
Ictaluridae						
Noturus insignis[b]	2	2	45	0	0	18.0
Percidae						
Etheostoma blennioides[a]	4	4	65	69	27–57 (40)	20.0
E. blennioides[c]	6	5	66	324	28–62 (40–42)	20.0
E. blennioides[e]	9	6	92	666	24–84 (45–65)	18.0
E. blennioides[e]	3	2	72	368	24–68 (37–47)	20.0
E. blennioides[d]	3	3	65	216	25–62 (43–47)	19.0
E. blennioides[f]	13	10	53	476	22–53 (42–46)	18.0
E. blennioides[h]	15	13	70	757	47–70 (56–63)	17.5
E. flabellare[e]	10	5	29	0	0	18.0
E. flabellare[h]	20	14	70	0	0	18.0
E. rufilineatum[a]	5	5	40	0	0	20.0
E. simoterum[d]	8	6	50	34	24–44 (31)	23.0
E. zonale[c]	10	7	50	55	29–45 (41)	20.0
E. zonale[d]	4	4	53	0	0	18.0
E. zonale[c]	24	21	56	68	21–48 (38)	18.0
Percina aurantiaca[e]	2	1	98	325	69–94 (74–81)	21.0
P. burtoni[d]	1	1	48	398	31–48 (44–46)	21.0
P. burtoni[x]	1	1	39	423	19–39 (36–37)	24.0
P. caprodes[f]	20	9	68	187	26–63 (44–58)	20.5
P. evides[e]	13	0	26	0	0	21.0
P. roanoka[e]	6	6	80	439	45–80 (59–63)	18.0
P. roanoka[g]	110	98	70	2913	35–70 (43–62)	18.0
P. roanoka[i]	120	104	58	6289	24–56 (30–38)	21.0
P. roanoka[j]	114	55	54	805	34–51 (40–48)	20.0

[a] *C. carolinae, E. rufilineatum,* and *E. blennioides* infested together for 1 h with glochidia from 2 conglutinates on 3 November 1997

[b] *M. dolomieu, A. rupestris, N. leptocephalus,* and *N. insignis* infested together for 1 h with glochidia from 5 conglutinates on 10 November 1997

[c] *E. blennioides* and *E. zonale* infested together for 2 h with glochidia from 3 conglutinates on 8 December 1997

[d] *C. carolinae, E. blennioides, E. simoterum, E. zonale,* and *P. burtoni* infested together for 2 h with glochidia from 10 conglutinates on 12 December 1997

[e] *C. bairdi, E. blennioides, E. flabellare, E. zonale, P. aurantiaca, P. evides,* and *P. roanoka*, infested together for 2 h with glochidia from 10 conglutinates on 19 December 1997

[f] *E. blennioides* and *P. caprodes* infested together for 2 h with glochidia from 10 conglutinates on 19 November 1998

[g] *P. roanoka* infested for 2 h with glochidia from 6 conglutinates on 20 January 1999

[h] *E. blennioides* and *E. flabellare* infested together for 2 h with glochidia from 6 conglutinates on 20 January 1999

[i] *P. roanoka* infested for 2 h with glochidia from 13 conglutinates on 7 April 1999

[j] *P. roanoka* infested for 2 h with glochidia from 6 conglutinates on 26 February 2001

[x] Infestation methods were not recorded

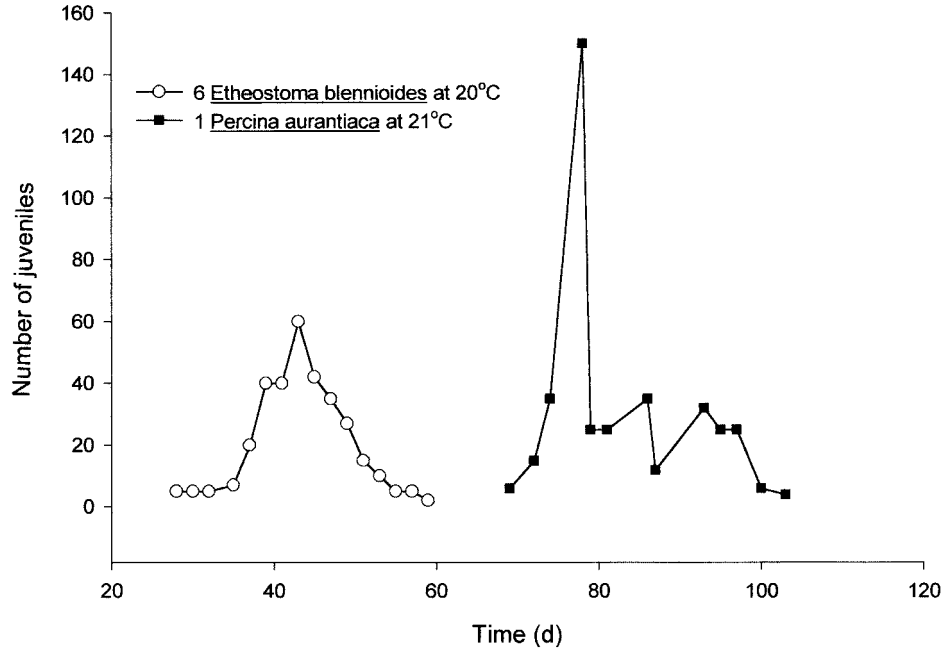

FIG. 4. Timing of excystment of juvenile fanshell pearlymussel, *Cyprogenia stegaria*, from *Etheostoma blennoides* and *Percina aurantiaca*.

Fanshells >10 y of age decrease substantially in shell growth (Table 3), and the external growth rings become indistinguishable. However, for individuals ≲40 mm, the external growth ring method can provide a reasonably accurate estimate of ages.

The mean length of live *C. stegaria* collected from the Clinch River was 38.3 mm, implying ages of 8 to 10 y. The live individuals collected represented only the part of the population that could be easily sampled, which is individuals 5 to 6 y or older. Therefore, the actual mean age of the current population is younger than 8 to 10 y. The mean length of thin-sectioned shells was 44.6 mm (~12 to 13 y) and the species is known to live to at least 26 y in the river, so we believe that the current population is comprised mostly of young and middle-age cohorts. Many

TABLE 5. Survival and growth of *Cyprogenia stegaria* juveniles cultured in recirculating and nonrecirculating culture systems. Juveniles were 0.2 mm long upon excystment at time 0. Number of juveniles alive at 2 wk in the nonrecirculating culture system was significantly higher ($p < 0.05$) than in either substrate of the recirculating system.

	% of juvenile mussels alive (length, mm)	
Diets and methods (*n*)	2 wk	4 wk
Neochloris oleoabundans		
Recirculating, sand (406)	11.3 (0.29)	0.7 (0.29)
Recirculating, fine sediment (382)	37.2 (0.30)	5.0 (0.31)
Scenedesmus quadricauda		
Recirculating, sand (2561)	3.2 (0.28)	0.3 (0.28)
Recirculating, fine sediment (1333)	3.8 (0.28)	0.2 (0.31)
Nonrecirculating (1690)	72.0 (0.29)	37.3[a] (0.31)

[a] Estimated from counting 4 of the 12 containers

C. stegaria found in the river from 1998 to 2001 were small and in excellent condition, suggesting that the population is currently experiencing recruitment.

Fish hosts

Percina aurantiaca, *P. burtoni*, *P. roanoka*, and *E. blennioides* were excellent hosts for *C. stegaria* in that more glochidia transformed to juvenile mussels on them than other species. However, *P. aurantiaca* and *P. burtoni* are difficult species to collect because of low abundance or uncommon occurrence. *Percina burtoni* is a species of special concern in Virginia and, in our experience, *P. aurantiaca* can be difficult to keep alive in captivity. Thus, it is not practical to use these 2 fish species for propagation of *C. stegaria*. Conversely, *P. roanoka* is common, easy to collect, and does well in captivity. This fish species occurs in the Roanoke River drainage (Jenkins and Burkhead 1993), so it cannot be released back to its natal river if used for propagation because residual glochidia may remain encysted on the gills. Last, *E. blennioides* is common, easy to collect, and also does well in captivity. This fish species is sympatric in parts of its range with *C. stegaria*, thus eliminating problems associated with the release of exposed fish. The remaining 5 hosts identified in our study (*C. bairdi*, *C. carolinae*, *E. simoterum*, *E. zonale*, and *P. caprodes*) were marginal hosts on which few juvenile mussels per fish transformed.

Glochidia on all tested species of *Percina* transformed to juvenile mussels, so other species of *Percina* may be suitable hosts. Species in the genus *Etheostoma*, such as swannanoa darter (*E. swannanoa*), variegate darter (*E. variatum*), and rainbow darter (*E. caeruleum*) also may be worth investigating as potential hosts.

The excystment period of juvenile mussels on *P. aurantiaca* was protracted when compared to other fish species; juvenile mussels began excysting at 69 d and finished at 104 d postinfestation. This excystment period was ~20 to 40 d longer than juvenile excystment patterns observed on other fish hosts. It is difficult to explain this result because *P. aurantiaca* was infested at the same time as other tested hosts (Table 4). Furthermore, this species was held in aquaria at a mean temperature of 21°C, which was the highest temperature used for the transformation trial. This protracted excystment shows that water temperature is just one of several factors regulating the rate of juvenile mussel transformation.

Culture of juveniles

Of the 2 culture methods tested, the static culture system was the more effective to sustain *C. stegaria* juveniles for the first 15 to 30 d. This system may be effective because it significantly increased food availability while juvenile mussels were pedal-feeding. This method ensures that dense concentrations of algae and organic material from sediment settle into the substratum with juvenile mussels. Algae were readily visible in the guts of juveniles after 1 d of being cultured in these conditions. The key to obtaining good results with this method is to keep juvenile mussels at cool temperatures (18–21°C); change algae, water, and sediment every 2 to 3 d; and feed algae of sufficient quantity ($1 \times 10^{5-6}$ cells/mL) and quality (e.g., *N. oleoabundans* or other nutritious algae species <10 μm). These techniques facilitated control of predators (e.g., flatworms), bacteria, and fungi, all of which can decrease survival and growth of juvenile mussels during culture. The static culture system was an effective, short-term culture method for *C. stegaria* juveniles, and is currently the best method to ensure that many newly metamorphosed juveniles are available for stocking in rivers. Depending on water temperature, this method is effective at culturing juveniles for 2 to 4 wk, with survival rates ranging between 40% and 80%. However, we recommend culturing juveniles for only 1 to 2 wk to ensure maximum survival and condition before release. This method is likely to be less effective for older juvenile mussels. Growth beyond 1 mo of age may decline or stagnate because juvenile mussels rely more on filter-feeding (Yeager et al. 1994), and mortality is likely to increase. Last, the method is labor intensive.

The low survival of juvenile mussels cultured in the recirculating aquaculture system may be related to food quantity and quality, hygiene, tolerance to fine sediment, or container size. The greater volume of water in the recirculating aquaculture system diluted alga and therefore decreased food availability to juveniles, compared to the high density of algae used in the static aquaculture system. Neither species of algae promoted good growth and survival of ju-

venile *C. stegaria* in our recirculating aquaculture systems. Juveniles of *C. stegaria* may not tolerate fine sediment, but juveniles cultured in a sand substratum also had low survival. In general, we have had very low survival of juveniles of other endangered mussel species, such as the oyster mussel, *Epioblasma capsaeformis*, that were cultured in a fine-sediment substratum. In contrast, juvenile mussels of some common mussel species do relatively well in a fine substratum. For example, survival of juvenile wavy-rayed lampmussels (*Lampsilis fasciola*) at 1 mo in a recirculating aquaculture system with fine sediment was 50% (Steg 1998). Survival at 1 mo for the rainbow mussel, *Villosa iris*, juveniles cultured in fine sediment and fed various diets averaged 46% (Gatenby et al. 1997). From 1995 to 2000, we have consistently cultured *V. iris* juveniles to ≥3 to 6 mo of age, and to sizes >4000 μm by feeding *N. oleoabundans* and using the current recirculating aquaculture technology. These common mussel species may be more robust and have wider food and habitat tolerances than juveniles of some endangered species and, therefore, are easier to culture.

Hygiene may also affect juvenile survival in several ways. First, little water flow occurred over the sediment surface in the smaller (7.5 cm × 7.5 cm) culture containers used in 1999. Reduced flow allowed excess algae, possibly bacteria, fungi, chironomid larvae, and predators such as flatworms to accumulate in the containers, all of which can affect juvenile mussel survival. These relatively small containers may not allow adequate flow to circulate food and eliminate waste products. Hanlon (2000) found significant differences in survival and growth of juvenile *L. fasciola* held in small containers with dimensions of ~7 cm, versus containers more than twice that size. However, we still had poor survival and no significant differences in growth of juveniles cultured in larger containers in 2001. Thus, the specific requirements to culture *C. stegaria* juveniles in recirculating aquaculture systems are poorly understood. It seems that water quality, flow, and substratum must be optimum, and alternative foods such as river sediments, bacteria, and detritus may be necessary to effectively culture this mussel species in captivity.

In conclusion, the population of *C. stegaria* in the Clinch River above Norris Reservoir in Hancock County, Tennessee, has been stable for at least the last 20 y. Live individuals representing multiple year classes, ranging in length from 10 to 60 mm, have been collected regularly in the river between 1979 and 2000 (Ahlstedt 1991, Ahlstedt and Tuberville 1997, JWJ and RJN, unpublished data). Furthermore, *C. stegaria* has been consistently collected at low levels of abundance (0.02–0.04 individuals/m^2) in quadrat samples over the years (1979, 1988, 1994, and 1997) (Ahlstedt 1991, Ahlstedt and Tuberville 1997, S. Ahlstedt, US Geological Survey, Knoxville, Tennessee, personal communication). Thus, the *C. stegaria* population in the Tennessee portion of the river has maintained similar abundance among sample years. In addition, darter populations in the genera *Percina* and *Etheostoma*, the primary, natural fish hosts of the fanshell, also appear stable and robust in the Clinch River (C. Saylor, Tennessee Valley Authority, Norris, Tennessee, personal communication). Therefore, population size of *C. stegaria* is small but seemingly stable at sites of occurrence, and reproduction is expected to continue.

Acknowledgements

We thank everyone who helped us complete the laboratory and field work for this study. The following individuals helped make our study possible: Steve Ahlstedt, Braven Beaty, Richard Biggins, Scott Cooney, Joe Ferraro, Jeff Flynn, Steve Fraley, Chris Good, Tom Hampton, Bill Henley, Rachel Mair, Monte McGregor, Matt Patterson, Mike Pinder, Charlie Saylor, Rebecca Winterringer, and Lora Zimmerman. This study was funded by Tennessee Wildlife Resources Agency, Nashville, Tennessee, and US Fish and Wildlife Service, Asheville, North Carolina.

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Received: 7 November 2000
Accepted: 31 October 2001