GLOCHIDIAL HOSTS AND OTHER ASPECTS OF THE LIFE HISTORY OF THE CUMBERLAND PIGTOE (*PLEUROBEMA GIBBERUM*)

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ABSTRACT - The endangered Cumberland pigtoe is a small mussel endemic to the upper Caney Fork River drainage in middle Tennessee, USA. Gravid individuals were collected from late June through August. Conglutinates obtained from these individuals contained few glochidia and were composed primarily of unfertilized eggs. In the laboratory, 18 species of fish were exposed to glochidia, and metamorphosis occurred on telescope shiners and striped shiners 8 to 10 days later. Although conglutinates contained few (< 50) glochidia, ≥ 30% of the telescope shiners collected from the Collins River were infested with glochidia, and juvenile Cumberland pigtoes excysted from naturally-infested telescope shiners returned alive to the laboratory. The consistent predominance of unfertilized eggs in conglutinates of the Cumberland pigtoe and the high incidence of glochidia encysted on telescope shiners collected from the Collins River support the hypothesis that a high proportion of unfertilized eggs in conglutinates may be adaptive rather than an anomaly in certain species.

INTRODUCTION

The life cycle of most freshwater mussels includes a parasitic larval phase. Typically, larvae (glochidia) must attach and become encysted on the gills or fins of a fish to metamorphose into juveniles; however, all species of fish cannot serve as a host for a given mussel species. The degree of host specificity varies greatly among mussels; glochidia of some mussel species can metamorphose only on a single fish species, whereas glochidia of other species can metamorphose on numerous species. Glochidial hosts are known only for about one-third of the mussel species in North America (O'Dee and Watters 2000). The life history, including glochidial host(s), of the Cumberland pigtoe (*Pleurobema gibberum* (Lea, 1838)) is unknown (Fig. 1). The Cumberland pigtoe is endemic to the Cumberlandian region, seemingly restricted to the upper Caney Fork River drainage in middle Tennessee, USA (Parmalee and Bogan 1998). The Cumberland pigtoe is 1 of 62 species of North American mussels listed as endangered by the federal government. The imperilment of mussels in North America is the result of many factors including dams, pollution, siltation, and habitat destruc-

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tion. These same factors caused the extirpation of 62 % of the 60 species of mussels that once occurred in the Caney Fork River drainage (Layzer et al. 1993). Presently, only five populations of the Cumberland pigtoe are known, and all occur in tributaries to the Caney Fork River above Great Falls. Prior to construction of Rock Island Dam in 1916 at Great Falls, additional populations of the Cumberland pigtoe may have occurred in the upper Caney Fork River system but few historic mussel collections were made in this area (U.S. Fish and Wildlife Service 1991). In contrast to the diverse mussel fauna (51 species) known from the Caney Fork River drainage below Great Falls, only 10 species have been reported from the river upstream of Great Falls (Anderson 1990, Layzer et al. 1993, Layzer unpublished data).

Knowledge of the life history of the Cumberland pigtoe, particularly the identity of its glochidial host(s), is critical to recovery efforts (U.S. Fish and Wildlife Service 1991). Identification of glochidial hosts often is based on artificially infesting a suite of fish species in the laboratory to determine species capable of serving as hosts; however, some species identified as suitable hosts in the laboratory may not become infested under natural conditions because their foraging behavior or habitat use

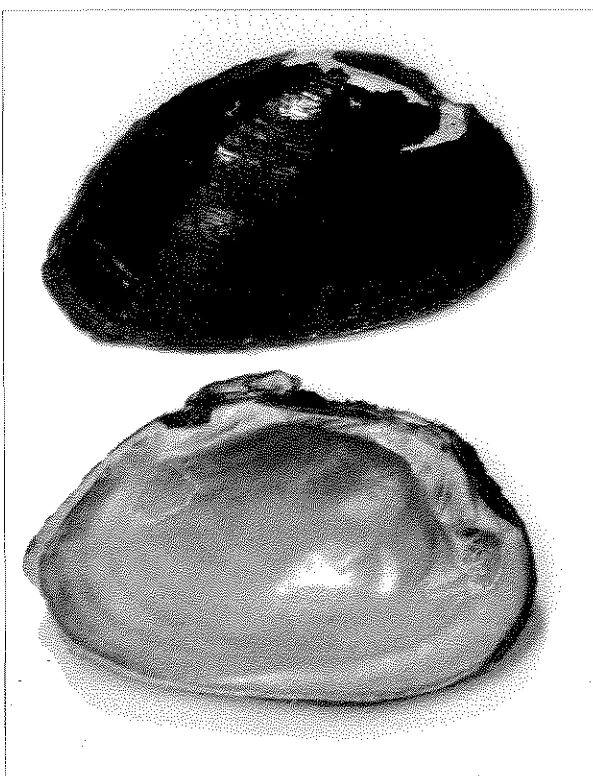

Figure 1. Adult Cumberland pigtoe, *Pleurobema gibberum*, 42 mm long.

makes it unlikely that they will encounter glochidia. An alternative method involves the collection of fish, and identifying any encysted glochidia. Although this method provides direct evidence that behavioral and distributional characteristics of the mussel and fish provide the opportunity for glochidial contact, glochidia may remain encysted on nonhost species for a prolonged period of time before being sloughed off (Gordon and Layzer 1993). Alone, neither method provides conclusive evidence of the natural host. In our study, we employed both methods to identify host fish for glochidia of the Cumberland pigtoe.

METHODS

Fish tested in the laboratory for suitability as hosts for the Cumberland pigtoe were collected by electrofishing and seining in tributaries of the Cumberland River that contained few or no mussels to avoid the possibility of an immune response to glochidia developed by previously infested fish (Arey 1923, Reuling 1919). Fish were transported to the laboratory where they were separated by species and maintained for 2 weeks in aquaria (38–208 L) prior to infestation. Mussels were collected from the Collins River by hand with the aid of a view bucket while wading. Individuals were measured to the nearest 1 mm with dial calipers, carefully pried open and examined to determine if they were gravid. In the field, we did not attempt to determine if the outer demibranchs (marsupial gills) contained only unfertilized eggs, embryos, or glochidia; we considered all individuals with inflated pink gills to be gravid. Gender could be determined only for gravid individuals because shells of the Cumberland pigtoe, like most amblemines, are not sexually dimorphic. Gravid individuals were placed in a cooler containing river water and transported to the laboratory. To monitor changes in gravidness, 13 gravid mussels and 20 nongravid individuals were placed in a wire cage within the river in July 2000, and checked weekly. Fish were exposed to glochidia in one of three ways. In 1999, aborted conglutinates were teased apart, and glochidia pipetted directly onto the gills of fish. In 2000, gravid mussels were placed into aquaria containing fish with the expectation that fish would ingest conglutinates as they were expelled. In 2001, mussels were maintained separate from fish, and aborted conglutinates were collected by pipette and fed to each species. One conglutinate from each group aborted was teased apart in a petri dish containing water, and NaCl was added to test viability of glochidia; viable glochidia snap shut when exposed to NaCl (Coker et al. 1921). Periodically, the number of glochidia contained in a conglutinate was counted. We siphoned each aquarium daily beginning 1 week after infestation. The siphonate was collected on a 100-µm-mesh filter and then examined under a cross-polarized dissecting microscope. Dimensions of juvenile mussels were measured with an ocular micrometer. In 2002, we measured glochidia from *Venustaconcha sima* (Lea, 1838) and *Villosa iris* (Lea, 1829) collected from the Collins River to compare their sizes with juvenile Cumberland pigtoes. In September 2000, cyanoacrylate was used to attach two shellfish tags to each adult mussel before returning them to the streambed (Lemarie et al. 2000), and in July and August 2001 measurements of recaptured individuals provided an estimate of growth.

We assessed fish populations for natural glochidial infestations at our primary study site on the Collins River on two occasions in 2001. Fish were collected by electrofishing and seining; we continued sampling until no additional species were collected. Fish were immediately fixed in 10 % formalin. In the laboratory, fish were soaked in water for 1 day, and then examined under a dissecting microscope for glochidial infestations. Gills were excised and transferred to 0.05 molar KOH for ≥2 minutes prior to examination. The KOH clears the gills and enhances the visibility of encysted glochidia (pers. comm., M.C. Barnhart, Springfield, MO). On a third occasion in 2001, we collected cyprinids from our study site, transported them alive, and maintained them in monospecific groups in 208 L aquaria in the laboratory. The bottoms of the aquaria were siphoned daily and siphonate was treated as described above.

RESULTS

Mussel populations

In addition to the Cumberland pigtoe, we collected a single wavyrayed lampmussel (*Lampsilis fasciola* Rafinesque, 1820) and numerous individuals of *Venustaconcha sima*, and occasional specimens of *Villosa iris*. Nearly all Cumberland pigtoes observed were completely imbedded in the streambed with the posterior margins of their shells flush with the substrate surface. Although most individuals were found in sand, a few were collected from a mixture of sand, gravel and small cobble. Length frequency distributions of the Cumberland pigtoe were similar between years (Smirnov test $P > 0.05$); each year 88% of the individuals were 42 to 50 mm long (Fig. 2). All gravid individuals ranged from 42 to 54 mm in length. Growth was negligible between years; mean increase in length for 33 recaptured individuals was 0.42 mm, and 45 % of the individuals had no measurable growth between years. All recaptured individuals retained both tags.

Cumberland pigtoes became gravid by the end of June (Table 1). Despite warmer water temperatures in July and August 2000 (24–27 °C) compared to 2001 (19–23 °C), gravid individuals were found later in August 2000. The inflated marsupial gills of gravid individuals were pinkish-red in color, as opposed to the more flaccid white gills of nongravid mussels. Individuals aborted pink conglutinates (8–10 mm long and about 1.5 mm in diameter) during transport or within 1 week. Examination of eight conglutinates in 1999, indicated they contained few glochidia (3–16). Similarly, conglutinates examined in subsequent years consisted mainly of unfertilized eggs and contained few or no glochidia. One conglutinate containing 10 glochidia and numerous unfertilized eggs also had about 40 glochidia surrounding and attached to it by larval threads. Vitelline membranes contained each glochidium within conglutinates but were not present when glochidia were attached by larval threads to the conglutinate. Weekly examination of mussels confined to the basket in the Collins River indicated no change in the coloration or appearance of the marsupial gills until conglutinates had been released. Following release of conglutinates, the marsupial gills appeared somewhat distended but translucent.

Glochidial hosts

In July 2001, we collected and microscopically examined 177 fish of 12 species (Table 2). Encysted glochidia were most prevalent on telescope shiners (*Notropis telescopus* (Cope, 1868)). Although ≥ 30 % of the telescope shiners were infested with glochidia, infestation intensities were low (mean 2 glochidia per infested fish; range 1 to 7). Three of

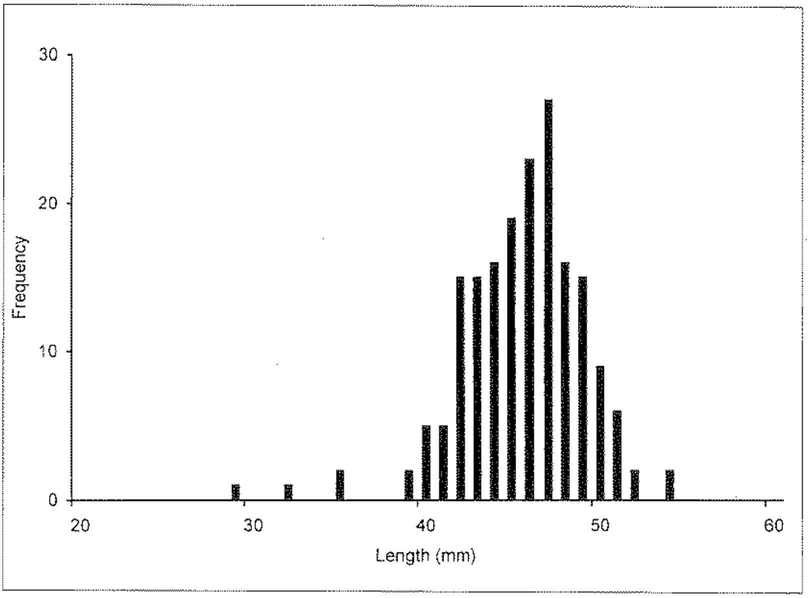

Figure 2. Length frequency distribution of Cumberland pigtoes collected from the Collins River in 2000 and 2001.

20 rock bass (*Ambloplites rupestris* (Rafinesque, 1817)) examined had a single encysted glochidium.

In all, 18 species of fish were exposed to glochidia or conglutinates of the Cumberland pigtoe in the laboratory (Table 3). Metamorphosis

Table 1. Dates and numbers of *Pleurobema gibberum* collected from the Collins River, and percent gravid (in parentheses).

	Month and day							
	June			July		August		September
Year	10	17–18	28	13–14	20–27	3–10	17–21	15–20
1999	—	—	—	—	42 (19)	27 (4)	—	—
2000	—	—	—	65 (20)	14 (29)	3 (0)	35 (9)	10 (0)
2001	—	—	—	26 (23)	49 (22)	39 (37)	23 (0)	—
2002	8 (0)	21 (0)	25 (16)	—	—	—	—	—

Table 2. Numbers and species of fish collected from the Collins River in 2001, and percent infested with glochidia.

	Number examined and (percent infested)	
Species	July 16	July 25
Ambloplites rupestris (Rafinesque, 1817)	18 (17%)	2
Campostoma anomalum (Rafinesque, 1820)	14	8
Cottus carolinae (Gill, 1861)	5	3
Cyprinella galactura (Cope, 1868)	6	5
Etheostoma etneiri Bourchard, 1977	1	1
Etheostoma sanguifluum (Cope, 1870)	8	8
Etheostoma virgatum (Jordan, 1880)	5	7
Lepomis machrochirus Rafinesque, 1819	1	—
Micropterus punctulatus (Rafinesque, 1819)	1	—
Notropis sp. (sawfin shiner)	7	11
Notropis telescopus (Cope, 1868)	31 (39%)	20 (30%)
Notropis volucellus (Cope, 1865)	12	3

Table 3. Numbers and species of fish exposed in the laboratory to glochidial of *Pleurobema gibberum*. Number of juveniles recovered in parentheses.

		Year and Number		
Species	Common name	1999	2000	2001
Ambloplites rupestris (Rafinesque, 1817)	rock bass	1	—	2
Campostoma anomalum (Rafinesque, 1820)	central stoneroller	2	2	—
Cottus carolinae (Gill, 1861)	banded Sculpin	—	—	3
Cyprinella galactura (Cope, 1868)	whitetail shiner	—	2	1
Etheostoma blenniodes Rafinesque, 1819	greenside darter	—	—	2
Etheostoma caeruleum Storer, 1845	rainbow darter	—	—	25
Etheostoma flabellare Rafinesque, 1819	faintail darter	3	—	—
Fundulus catenatus (Storer, 1846)	northern studfish	—	2	2
Hypentilium nigricans (Lesueur, 1817)	northern hog sucker	—	—	5
Lepomis machrochirus Rafinesque, 1819	bluegill	—	3	—
Luxilus chrysocephales Rafinesque, 1820	striped Shiner	—	5	14 (1)
Lythrurus ardens (Cope, 1868)	rosefin shiner	—	10	—
Nocomis micropogon (Cope, 1865)	river chub	—	2	—
Notropis telescopus (Cope, 1868)	telescope shiner	—	—	15 (16)
Percina caprodes (Rafinesque, 1820)	logperch	1	—	—
Pimephales notatus (Rafinesque, 1820)	bluntnose minnow	—	3	—
Pimephales promelas Rafinesque, 1820	fathead minnow	—	5	—
Rhinichthys atratulus (Hermann, 1804)	blacknose dace	2	2	—

occurred 8–10 days postinfestation on telescope shiners and striped shiners (*Luxilus chrysocephales* Rafinesque, 1820) at a mean water temperature of 21 ± 1 °C. Other species tested sloughed all glochidia within 5 days of infestation. On August 7, 2001, we collected 53 telescope shiners and 10 whitetail shiners (*Cyprinella galactura* (Cope, 1868)) from the Collins River, transported them to the laboratory and maintained them in monospecific groups in 208 L aquaria. We recovered 12 juveniles from the aquarium containing telescope shiners within 1– 6 days after the fish were collected. No juveniles were found in the aquarium containing whitetail shiners. Shell dimensions of juvenile mussels that excysted from naturally infested telescope shiners collected from the Collins River were similar ($P > 0.05$) to those of juvenile Cumberland pigtoes that metamorphosed on telescope shiners artificially infested with glochidia in the laboratory (Table 4). One or more shell dimensions clearly distinguishes juvenile Cumberland pigtoes from other species present (Table 5). The triangular glochidia of

Table 4. Mean shell dimensions of juvenile Cumberland pigtoes transformed on laboratory-infested telescope shiners, and juvenile mussels that excysted from natural infestations on telescope shiners collected from the Collins River and held in laboratory.

Dimension	Mean ± 1 SE (µm) Laboratory (N = 8)	Wild fish (N = 7)
Length	213 ± 7	223 ± 6
Height	203 ± 3	203 ± 4
Hinge length	153 ± 3	158 ± 5

Table 5. Dimensions of juvenile Cumberland pigtoes and glochidia of mussel species reported from the Caney Fork River system upstream of Great Falls. Unknown status indicates species has not been reported from the Collins River.

Subfamily Species	Status in Collins River	Mean glochidial dimensions (µm) Length	Height	Hinge length	Source
Anodontinae					
Alasmidonta atropurpurea (Rafinesque, 1831)	Extirpated	—	—	—	
Alasmidonta marginata Say, 1818	Unknown	339	365	233	Hoggarth (1999)
Alasmidonta viridis (Rafinesque, 1820)	Unknown	307	250	251	Hoggarth (1999)
Lasmigona sp. (Barrens heelsplitter)	Extant	—	—	—	
Pegias fabula (Lea, 1838)	Extirpated	386	322	206	Hoggarth (1999)
Ambleminae					
Pleurobema gibberum (Lea, 1838)	Extant	213	203	153	Juveniles, this study
Lampsilinae					
Lampsilis fasciola Rafinesque, 1820	Extant	238	293	111	Zale and Neves (1982a)
Medionidus conradicus (Lea, 1834)	Extirpated	218	274	93	Zale and Neves (1982a)
Venustaconcha sima (Lea, 1838)	Extant	244	319	132	This study
Villosa iris (Lea, 1829)	Extant	205	273	108	This study

anodontine species occurring in the upper Caney Fork River system are considerably longer and higher than juvenile Cumberland pigtoes; glochidia of lampsiline species are higher and have shorter hinges than Cumberland pigtoes.

DISCUSSION

Cumberland pigtoes were difficult to locate because the shells of most individuals did not protrude above the substrate surface. Although we searched for mussels slowly, with minimal disturbance of the substrate and water surface, many individuals seemingly detected our approach and ceased siphoning. We found these individuals only after we remained motionless for several minutes, at which time they resumed siphoning. Because we used only visual searches, the absence of Cumberland pigtoes < 29 mm long in our collections most likely reflects sampling bias rather than a lack of recruitment.

Most species in the subfamily Ambleminae are short-term brooders (Gordon and Layzer 1989). Cumberland pigtoes released all conglutinates by early to mid August each year. In most years, we were unable to estimate spawning time and the first appearance of gravid individuals, because weather and water conditions prior to mid-July were frequently unsuitable for sampling. In 2002, however, Cumberland pigtoes did not become gravid until late June. Other Cumberlandian amblemines spawn in May and are gravid through July or early August (Bruenderman and Neves 1993, Yeager and Neves 1986).

Identification of encysted glochidia or recently excysted juveniles can be difficult depending upon the diversity and composition of a mussel assemblage; however, the shape and dimensions of juvenile Cumberland pigtoes differed substantially from those of other species occurring in the area. A review of reported hosts (Watters 1994), coupled with our unpublished data on hosts of *Pegias fabula* and *Venustaconcha sima* indicate that no other mussel species occurring upstream of Great Falls is known to use a cyprinid as a host. Telescope shiners are the primary, if not the only host for glochidia of the Cumberland pigtoe in the Collins River. The high frequency of occurrence of glochidia on telescope shiners collected from the Collins River, and glochidial metamorphosis on naturally and artificially infested fish in laboratory trials confirm telescope shiners as a natural host for the Cumberland pigtoe. The single glochidium that metamorphosed on 1 of 19 striped shiners exposed to glochidia suggests that this species is a marginal host for the Cumberland pigtoe. Furthermore, we did not collect any striped shiners from the Collins River at our study site even though they are present in the drainage (Layman et al. 1993). Glochidia encysted on rock bass were damaged as we removed them from the gills;

consequently, we could not measure and identify them. Nonetheless, it is unlikely that the three glochidia on rock bass were Cumberland pigtoe glochidia, or if they were, we believe they would have eventually sloughed off. In 1999, approximately 150 Cumberland pigtoe glochidia were pipetted directly onto the gills of a rock bass and all were sloughed off within 2 days. In subsequent trials, all species except rock bass readily attacked and consumed conglutinates introduced to aquaria. The glochidia encysted on rock bass were most likely *Villosa iris* because rock bass are one of their hosts, and *V. iris* release glochidia between April and August (Zale and Neves 1982a, b). Although rock bass can serve as a host for at least nine species, only one of these (*Amblema plicata* (Say, 1817)) is an amblemine (Watters 1994). In laboratory trials, fish had access to the bottoms of aquaria. Consequently, it is possible that some species consumed all newly metamorphosed juveniles, and were not identified as a suitable host. However, encysted glochidia were found only on telescope shiners and rock bass collected from the Collins River. The identification of telescope shiners as the primary host, and striped shiners as a marginal host for the Cumberland pigtoe is consistent with other Cumberlandian amblemines using primarily cyprinids as hosts, and none use a centrarchid (Bruenderman and Neves 1993, Neves and Widlak 1988, Weaver et al. 1991, Yeager and Neves 1986, Yeager and Saylor 1995).

Although fecundity of many freshwater mussel species is high, some species simply broadcast glochidia, which results in relatively few contacting suitable hosts (Jansen and Hanson 1991, Young and Williams 1984). However, some species of mussels have evolved mechanisms that presumably increase the probability of glochidia contacting suitable hosts. For instance, the fluted kidneyshell (*Ptychobranchus subtentum* (Say, 1925)) expels intricate ovisacs that mimic larval aquatic insects and contain hundreds of glochidia (Luo 1993). Presumably, the benthic insectivorous fishes that serve as hosts for the fluted kidneyshell readily ingest these ovisacs and become infested with glochidia released inside the buccal cavity. When conglutinates of the creeper (*Strophitus undulatus* (Say, 1817)) are expelled, they begin a writhing motion that presumably mimics live prey items to attract hosts (Watters 2002). Despite the potential for high infestation intensities on fish ingesting or attacking a mussel "lure" low infestations of glochidia may result. Haag and Warren (1999) found only four to eight glochidia on fish they observed attacking either a mantle flap mimicking a minnow or a superconglutinate containing thousands of glochidia. Surprisingly, telescope shiners collected from the Collins River had similar infestation intensities (1 to 7 glochidia per fish), even though conglutinates of the Cumberland pigtoe contained few glochidia. Apparently the number of glochidia in a conglutinate is not related (or only at a very coarse level) to the number of glochidia that successfully attach to a host fish.

Initially, we believed that the occurrence of few glochidia in each conglutinate indicated either a low fertilization rate or differential development; however, conglutinates released by mussels caged for 2 weeks in the Collins River also contained few glochidia. The consistent occurrence of few glochidia in conglutinates during three reproductive seasons suggests that the prevalence of unfertilized eggs in conglutinates of the Cumberland pigtoe is not an anomaly. Lefevre and Curtis (1912) indicated that the predominance of unfertilized eggs is characteristic of some species and should be expected. Barnhart (1997a, b) also reported high proportions of unfertilized eggs in certain species and advanced the idea that this condition was an unrecognized reproductive strategy; he suggested that the unfertilized eggs provide structural integrity, color, and opacity to the conglutinates, thereby increasing the probability that host fish will feed upon them. The predominance of unfertilized eggs in conglutinates of the Cumberland pigtoe, the high incidence of glochidia encysted on wild telescope shiners, and our observation of telescope shiners voraciously consuming conglutinates support the hypothesis that a high proportion of unfertilized eggs in the conglutinates of some species is adaptive.

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