

ENGLISH BOND

**Life History of the Endangered Fine-Rayed Pigtoe Pearly Mussel,
Fusconaia cuneolus (Lea, 1840), in the Clinch River, Virginia.**

by

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(ABSTRACT)

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The periods of gravidity and glochidial release, required fish hosts, and age and growth characteristics of *Fusconaia cuneolus* were studied in the upper Clinch River, Virginia during 1986 and 1987. This summer brooder is gravid from mid-May through late July, releasing most glochidia in mid-June. Gills of gravid females assume the color of enclosed conglutinates, and hues change from pink to peach as embryos mature to glochidia. As judged by diel sampling of stream drift, glochidia of the fine-rayed pigtoe are most abundant in the water column in early morning.

A total of 1,619 fish representing 39 species were electroshocked and examined from the Clinch River at Slant for glochidial attachment. Prevalence of infestation of amblymeine glochidia was highest on species of the Cyprinidae (27 to 46%), and six species were identified as possible fish hosts for the glochidia of the fine-rayed pigtoe. Amblymeine glochidia were absent on non-cyprinid fish species. Glochidia exhibited host specificity in laboratory experiments, metamorphosing on the following seven minnow and one sculpin species: fathead minnow (*Pimephales promelas*), river chub (*Nocomis micropogon*), stoneroller (*Camptostoma anomalum*), telescope shiner (*Notropis telescopus*), Tennessee shiner (*Notropis leuciodus*), white shiner (*Notropis albeolus*), whitetail shiner (*Notropis galacturus*), and mottled sculpin (*Cottus bairdi*). Tested species of sunfishes, catfishes, and darters did not serve as hosts.

Age and growth characteristics were obtained by thin-sectioning shells collected in muskrat middens at Slant and Pendleton Island, Virginia. As predicted by the von Bertalanffy equation, the fine-rayed pigtoe achieves a maximum length of roughly 90 mm and age of 35 yr in the Clinch River. Annual growth in length averaged 5 mm/yr through age 10, decreasing to a rate of roughly 2 mm/yr thereafter. Based upon an age-length key, most individuals (50%) were of intermediate

ages (13 to 16 yr). Specimens less than 10 yr old were uncommon, comprising only 22.4% of the deme at Slant. No individuals less than 6 yr old were found at Slant or Pendleton Island. Based on cohort structure at these sites, the fine-rayed pigtoe population appears to be declining in the Clinch River. A monitoring program is suggested.

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Introduction

The most diverse freshwater mussel fauna (Unionidae) occurs in North America, particularly in the Cumberland Plateau Region of the southeastern United States. This region is considered to be a major area of mussel speciation (U.S.F.W.S., 1984). Forty-five endemic or "Cumberlandian" mussel species (naiades) inhabit headwater tributaries of the Cumberland and Tennessee rivers, which flow through portions of seven states. The Clinch River in southwestern Virginia is one of these tributaries in which Cumberlandian mussels are numerous and widespread (Neves et al., 1980). The existing fauna appears rich, but species diversity and abundance have been in decline since the early 1900's (Ortmann, 1918; Ahlstedt, 1984).

Thirteen mussel species have disappeared from the Clinch River since 1918, as judged by survey records of A.E. Ortmann (1918), a pioneer in the study of the Cumberlandian fauna (Ahlstedt, 1984). Ortmann had warned that stream pollution and construction of dams for water power would have serious adverse effects on the freshwater fauna of the upper Tennessee River. According to U.S.F.W.S. (1984), dam construction in the upper Tennessee River may have been the most deleterious factor contributing to the loss and decline of Cumberlandian mussel species in the Tennessee River drainage. Siltation from poor land use practices and coal mining, and water quality degradation are additional problems for mussels in the Clinch River and the entire Tennessee River drainage. These factors result ultimately in destruction of natural aquatic habitat.

Mussel beds may be destroyed or altered directly (Scruggs, 1960), or fish species may be extirpated, indirectly affecting mussel populations. Fish serve as hosts for the parasitic larval stage in the mussel life cycle and must be present for freshwater mussels to successfully reproduce. Elimination of a host species will impair mussel reproduction and may eventually eliminate the population of mussels (Fuller, 1974). Coupled with the reduction in aquatic habitat, predation by muskrats also has a significant impact on Cumberlandian mussel species in the Tennessee River drainage (Neves and Odom, in press). An additional cause for concern is potential competition with the non-native Asiatic clam, *Corbicula fluminea*, for available resources (Kraemer, 1979).

In addition to the loss of species, numerous other mussel species have become rare. Growing awareness and concern for such losses in species diversity led to passage of the Endangered Species Act of 1973, which provided protection for threatened and endangered populations of organisms to include mollusks. Thirteen of the thirty-three mussel species currently listed as federally endangered belong to the Cumberlandian group. The fine-rayed pigtoe pearly mussel, *Fusconaia cuneolus*, is one of these species that was listed as endangered on June 14, 1976 (Federal Register 41: 24062-24067), and is the focus of this investigation. A recovery plan for *F. cuneolus* was approved by the United States Fish and Wildlife Service in 1984, the goal of which is "to restore and maintain viable populations (of this species) to a significant part of its historic range and to remove the species from the federal list of endangered and threatened species" (U.S.F.W.S., 1984). To accomplish this goal and to properly manage the species, the life cycle needs to be fully understood.

Life Histories of Freshwater Mussels

Generalities about mussel life cycles have been available since the early twentieth century. During the breeding period, sperm are expelled into the water column and are dispersed by water currents. Sperm enter the female through normal respiratory processes (siphoning) and fertilize ova within the female's suprabranchial cavity or gills. The female's gills or water tubes serve as marsupia for the development of the embryos to the mature larval glochidial stage (Pennak, 1978).

Members of the family Unionidae may be classified based on the length of time glochidia are retained in the gills of the female (Ortmann, 1911). Fertilization occurs in the spring in tachytictic species (short-term brooders), and glochidia are expelled in spring or summer. In bradytictic mussels (long-term brooders), fertilization occurs in mid to late-summer; glochidia are released the following spring and summer (U.S.F.W.S., 1984). Glochidia can either develop independently of one another within the water tubes or form a compact mass of glochidia held together in a gelatinous matrix, termed a conglutinate or ovisac (Lefevre and Curtis, 1912). Conglutinates are characteristic of short-term brooders, and their size and shape have become taxonomically important for some species (Stein, 1971). Upon release into the water column, glochidia of most species attach principally to the gills or fins of appropriate fish hosts, although a salamander (Howard, 1951) and tadpoles (Walker, 1981) are known to serve as hosts for two mussel species. The glochidium becomes encysted and metamorphoses, and the young juvenile drops from the fish to grow into an adult mussel to repeat the cycle.

The impetus for biological studies of freshwater mussels in the early 1900's was a commercial market for pearl buttons, and the white nacre of the freshwater pearly mussels of the Mississippi and Tennessee rivers provided the raw materials. In 1914, almost 650 tons of shell material were taken from the Tennessee River for the pearl button industry, and the mainstream reservoirs of this river soon became the most important source of freshwater shells in the United States (TVA, 1966). Such intensive harvesting pressure resulted in studies of reproduction and an interest in developing methods to artificially propagate commercial species. Surber (1912, 1913), Coker et al. (1921) and Lefevre and Curtis (1910, 1912) laid the groundwork for twentieth century mussel research, providing descriptions of glochidia, notes on fish hosts, and successful or unsuccessful attempts to artificially raise various mussel species in the laboratory. Van der Schalie (1938) gathered data on general reproductive patterns for 28 naiad species in the Huron River in southeast Michigan. However, detailed life history investigations of individual species were rarely conducted.

Yokley (1972) studied the life history of *Pleurobema cordatum* in the Tennessee River drainage. He examined periods of gravidity and glochidial release, and conducted artificial infestations in the laboratory to determine the fish host(s) of this mussel. Studies which followed also focused

on life histories of individual mussel species, and seemed to take an even more descriptive approach (Wood, 1974; Wiles, 1975; Smith, 1976; Stern and Felder, 1978; Trdan, 1981; Jirka, 1986; Jones et al., 1986). Zale (1980) was the first to publish a complete life history investigation of a Cumberlandian species. He studied the reproductive biology of four mussel species, and used new techniques for determining fish hosts. These techniques were repeated subsequently in life history studies of other species, including those in the Tennessee River system (Weaver, 1981; TVA, 1986; Yeager and Neves, 1986). Zale (1980) provided information on periods of female gravidity and glochidial release, fish hosts, and duration of the parasitic period for the lampsiline mussels. His investigation was unique in that he identified fish hosts using both field observations and laboratory experiments. Some workers had compiled data on wild fish naturally infested with glochidia (Surber, 1912; Tedla and Fernando, 1969; Stern and Felder, 1978; Trdan, 1981; Widlak, 1982), whereas other researchers induced glochidial infestations in the laboratory (Matteson, 1948; Yokley, 1972; Smith, 1976). Fuller (1974) provided a complete list of glochidial-fish host relationships recognized before 1972, but did not mention the methods used by researchers to identify the hosts. It has only been in the last decade that suspected fish hosts were first identified in the field followed by verification in the laboratory (Zale, 1980; Weaver, 1981; Trdan and Hoeh, 1982; Yeager and Neves, 1986; Hill, 1986).

Periods of female gravidity and subsequent release of glochidia have not been described for most naiad species. Ortmann (1921) described internal anatomy and periods of gravidity for many tachytictic species in the Tennessee River system, recording sizes and shapes of collected glochidia. Use of morphometrics has since become a popular method to identify mussel larvae (Matteson, 1948; Yokely, 1972; Wiles, 1975; Zale, 1980; Weaver, 1981; Kitchel, 1985; Jirka, 1986). Ortmann (1921) also recorded colors of the marsupia (containing developing embryos) of the various mussel species that he collected, and these colors can be diagnostic for distinguishing species which are similar in external appearance.

The reproductive cycle of a short-term brooder, *Elliptio complanata*, was thoroughly investigated by Matteson (1948), who described glochidial release and implicated certain environmental factors, such as temperature, regulating the time of glochidial "shedding". Similar information had

been reported earlier by Coker et al. (1921), and results of more recent studies concur with these early findings (Zale, 1980; Weaver, 1981; Kitchel, 1985). Periodicity of glochidial release has been determined for some short-term brooders in the Tennessee River system (Yokley, 1972; Weaver, 1981; TVA, 1986; Yeager and Neves, 1986), but most species have not been studied.

Age and Growth Characteristics

Valid information on the age and growth of freshwater mussels is scarce. In early studies, age was determined primarily by counting rings on the external surface or periostracum of the shell (Chamberlain, 1931; Scruggs, 1960; Negus, 1966). It was assumed that these rings were laid down annually during periods of slow growth (winter). However, it has been demonstrated that mussels will deposit "false" annuli when placed under stressful conditions. The erosion of shell surfaces, particularly near the umbonal region, can also result in incorrect counts of annuli. Thus, the validity of many age and growth studies is questionable (Moyer, 1984). Recent studies have addressed these problems, and new techniques were developed to distinguish true annuli from "false" rings, both internally and externally (Clark, 1980; Moyer, 1984).

Some workers have investigated the relationship between shell length and age in freshwater mussels. Haukioja and Hakala (1978, 1979) studied growth characteristics of 15 populations of *Anodonta piscinalis* and tested several growth equations for predicting length-age relationships of this species. Jirka (1986) examined the relationships between shell weight, shell length and age for four subpopulations of *Cyclonaias tuberculata* and found that growth in shell length per year is a reliable and consistent indicator of actual growth (i.e., increase in body tissue) of mussels. Other workers have reported similar findings (Negus, 1966; McCuaig and Green, 1983; Moyer, 1984).

Growth has been described for several Cumberlandian species (Zale, 1980; Weaver, 1981; Moyer, 1984; Kitchel, 1985; Yeager and Neves, 1986), and von Bertalanffy growth curves were shown to adequately relate shell growth in length to age of mussels. Thin-sectioning of shells collected in muskrat middens enabled Moyer (1984) to develop growth curves for the endangered

naiads *Fusconaia cor* (= *F. edgariana*) and *Fusconaia cuneolus*. He suggested that length-age keys could be plotted from the curves and used for future studies on endangered species.

Fine-Rayed Pigtoe

The fine-rayed pigtoe, first described as *Unio cuneolus* by Lea (1840), is a medium-sized mussel with valves subtriangular or subrhomboidal in shape. The species is identified by rounded anterior end, a straight ventral margin, a median sulcus extending into the unbonal region, and a high, moderately full beak (Figure 1). This mollusk received its common name from the presence of fine green rays on its yellowish-green periostracum. A satin-like appearance on a brown background typifies older specimens, as the fine rays usually disappear with age. A more detailed description and information on this species' distribution is included in Bogan and Parmalee (1983) and U.S.F.W.S. (1984). Ortmann (1925) described the habitat of *F. cuneolus* and stressed that substratum characteristics and current velocity were two factors most limiting to this species' occurrence. Typically, a stable substratum of coarse cobble, rubble, and gravel or sand in a fast flowing riffle depicts the habitat in which the fine-rayed pigtoe occurs. It is considered a sedentary riverine species that is well burrowed in the substratum and intolerant of lentic conditions (Ahlstedt, 1984; U.S.F.W.S., 1984).

Objectives

To re-establish and recover demes of the fine-rayed pigtoe, the complex life cycle of this mussel species must be understood. Therefore, the goal of this study was to provide information on the life history of the fine-rayed pigtoe. Specifically, research objectives were as follows:



Figure 1. Photograph of adult fine-rayed pigtoe from the Clinch River at Pendleton Island, Virginia.

1. To determine the period of gravidity, and seasonal and diel periodicity of glochidial release by *Fusconaia cuneolus* in the Clinch River at Slant, Virginia.
2. To determine the required fish host(s) of *Fusconaia cuneolus*.
3. Using length-at-age data, determine age-class structure and compute growth equations for two demes of *Fusconaia cuneolus* at Slant and Pendleton Island, Clinch River, Virginia.

Materials and Methods

Study Area

The Clinch River, a fifth-order tributary of the Tennessee River, originates in Tazewell County of southwestern Virginia and flows southwest, paralleling the main ridges of the Appalachian mountains before entering Norris Reservoir, Tennessee. From Norris Reservoir, the river flows southwesterly and joins the Tennessee River near Kingston, Tennessee. The gradient steepens upstream of Norris, to an average of 1.8 m/km from Clinch River Mile 128 (CRM 128) below Norris Dam in Tennessee, to the source of the Clinch (Masnik, 1974). This stretch of river is termed the upper Clinch, characterized by a montane aquatic fauna. Isolated from main routes of travel, this region has remained relatively unpopulated, and the remoteness has benefited the river's biota. Despite recent industrial growth in the region, the Clinch River remains as one of the least spoiled tributaries in the upper Tennessee drainage (Masnik, 1974). Fifty percent of the Clinch River watershed is still forested (Ahlstedt, 1984); second or third growth deciduous trees contribute an abundance of leaves and organic detritus to the riverine ecosystem. Exposed dolomite and limestone formations add large amounts of magnesium and calcium carbonates to the water (Masnik, 1974), providing necessary raw materials for mussel shell formation.

Clinch River Mile 223.3 (CRM 223.3), below the swinging bridge at Slant, Virginia in Scott County (latitude 36°44'12"N, longitude 82°37'12"W), was chosen as the principal study site (Figures 2 and 3). This site was approximately 325 m in length and 50 m in width; substratum was predominantly coarse gravel and cobbles with some boulders and bedrock. Depth averaged 0.5 m. In an assessment of muskrat depredation on endangered mussels in the Clinch River, Neves (1985) recorded 32 species, including *F. cuneolus*, in this riffle-and-run section of the river (Table 1). The relative abundance of the fine-rayed pigtoe, diversity of fish species, and easy access to the river were the reasons for selecting this location.

The greatest density of the fine-rayed pigtoe in the Clinch occurs upstream at Pendleton Island, CRM 226.3 (TVA, 1986; Figures 2 and 3). This island served as an additional site for the collection of glochidia from gravid females and for collection of shells of freshly dead *F. cuneolus*. Because Pendleton Island is a preserve of The Nature Conservancy, I obtained permission to sample this site for my thesis research. Sampling was limited to a shallow run approximately 25 m in width and 50 m in length. The substratum consisted of mostly sand, gravel and cobble, and depths averaged roughly 0.3 m.

Water quality at the two sites was evaluated using data obtained from EPA's water quality storage retrieval system (STORET System, Virginia State Water Control Board, Abingdon, Virginia). Water quality records from 1986 and 1987 were obtained from sampling stations 19.3 km downstream of Slant and 41.8 km upstream of Pendleton Island. It was assumed that water quality in the stretch of river between these stations would be similar. Weekly maximum and minimum water temperatures were recorded at Slant during summer 1986. In 1987, temperatures were monitored using a 90-day continuous-reading submersible thermograph (Ryan Instrument Co., Kirkland, Washington).

Table 1. Mussel species collected in muskrat middens in the Clinch River at Slant, Virginia, October 1984 - July 1985¹.

Scientific name ³	Common name	No. collected
<i>Actinonaias ligamentina</i>	mucket	145
<i>Actinonaias pectorosa</i>	pheasantshell	45
<i>Alasmidonta marginata</i>	elktoe	2
<i>Amblema plicata plicata</i>	three-ridge	9
<i>Cumberlandia monodonta</i>	spectacle case	2
<i>Cyclonaias tuberculata</i>	purple wartyback	23
<i>Dromus dromas</i> ²	dromedary mussel	1
<i>Elliptio dilatata</i>	spike	260
<i>Epioblasma brevidens</i>	cumberlandian combshell	5
<i>Epioblasma capsaeformis</i>	oyster	3
<i>Epioblasma triquetra</i>	snuffbox	1
<i>Fusconaia cuneolus</i> ²	fine-rayed pigtoe	155
<i>Fusconaia cor</i> ²	shiny pigtoe	33
<i>Fusconaia subrotunda</i>	long-solid	96
<i>Hemistena lata</i>	cracking pearlymussel	2
<i>Lampsilis fasciola</i>	wavy-rayed lampmussel	20
<i>Lampsilis ovata</i>	pocketbook	5
<i>Lasmigona costata</i>	fluted-shell	21
<i>Lemiox rimosus</i> ²	birdwing pearlymussel	1
<i>Lexingtonia dolabelloides</i>	slabside pearlymussel	3
<i>Ligumia recta</i>	black sandshell	1
<i>Medionidus conradicus</i>	Cumberland moccasinshell	5
<i>Plethobasus cyphus</i>	sheepnose	13
<i>Pleurobema oviforme</i>	Tennessee clubshell	12
<i>Potamilus alatus</i>	pink heelsplitter	1
<i>Ptychobranchnus fasciolaris</i>	kidneyshell	42
<i>Ptychobranchnus subtentum</i>	fluted kidneyshell	13
<i>Quadrula cylindrica strigillata</i>	rough rabbitsfoot	49
<i>Quadrula pustulosa pustulosa</i>	pimpleback	1
<i>Villosa nebulosa</i>	Alabama rainbow	11
<i>Villosa purpurpurea</i>	purple bean	2
<i>Villosa vanuxemensis</i>	mountain creekshell	2
Subtotal		985
<i>Corbicula fluminea</i>	Asiatic clam	4,814
Total		5,799

¹Neves (1985)

²Endangered species

³Nomenclature from Turgeon et al. (1988)

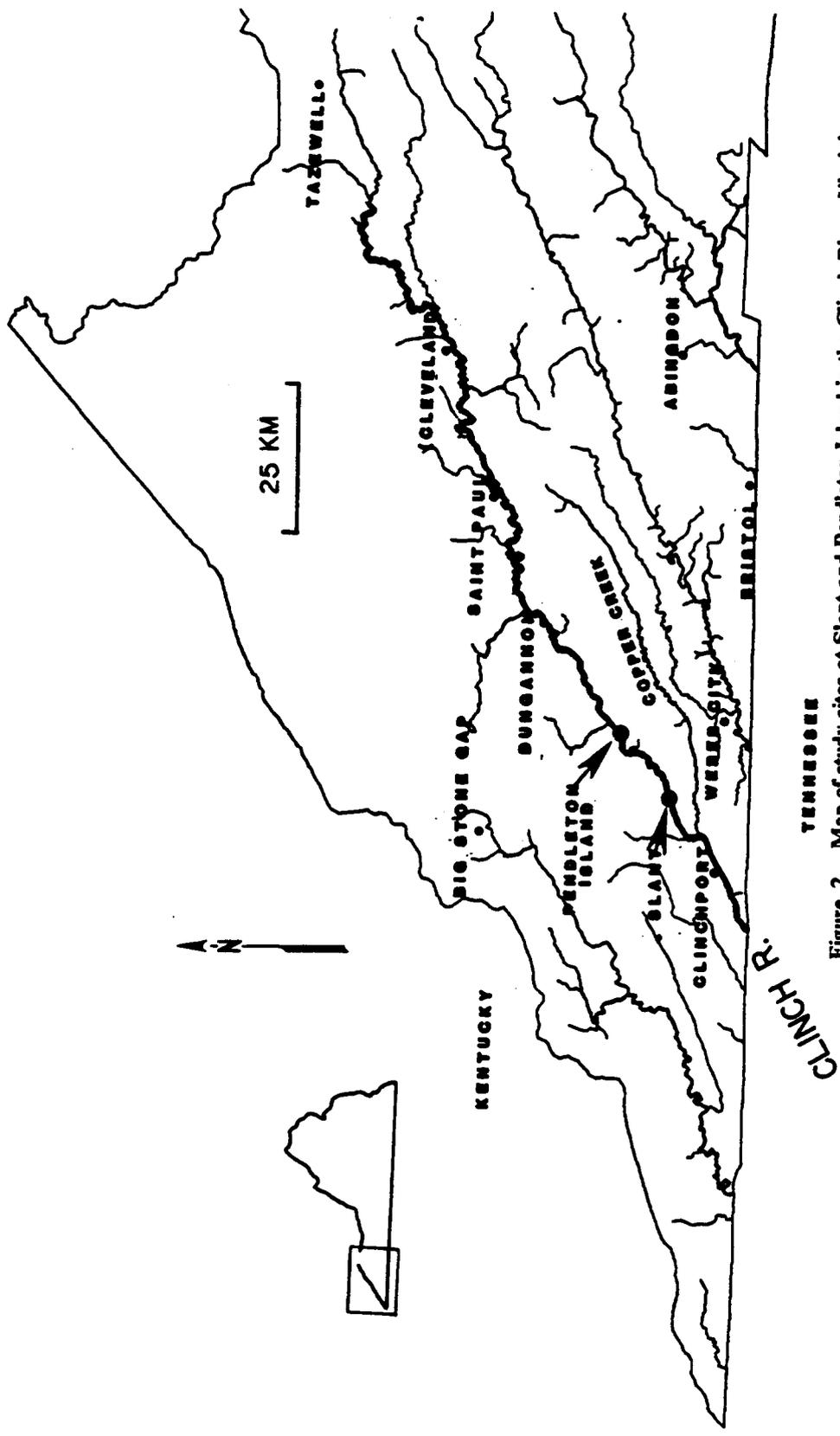


Figure 2. Map of study sites at Slant and Pendleton Island in the Clinch River, Virginia.



(A)



(B)

Figure 3. Photographs of study sites at Pendleton Island (A) and Slant (B), Clinch River, Virginia.

Reproductive Biology

Gravidity Period

The period of gravidity of *F. cuneolus* during the summers of 1986 and 1987 was determined by monitoring marsupial gills of individual females collected by handpicking and snorkeling. Collected mussels were carefully opened with modified O-ring expanders to examine their gills. Since sexual dimorphism is not exhibited in the valves of this species, gender of individuals could be determined only during the period of gravidity. Gills typically appear flattened and opaque in nongravid females and males (Kitchel, 1985). Gravid individuals of *F. cuneolus* were identified by intensely pink and swollen marsupial gills (Ortmann, 1921). In 1986, collected mussels were stockpiled in a shallow riffle area near the streambank, and covered by a 1 m² hardware cloth enclosure to eliminate risk of muskrat predation. Stockpiling was used to aggregate mussels in a known location to facilitate relocation and examination in both 1986 and 1987. In 1986, a sample of stockpiled mussels had been marked and monitored, but I failed to thoroughly differentiate males from females. In 1987, all stockpiled mussels were marked with 5 mm x 3 mm numbered plastic disc tags (Floy Tag Co., Seattle, Washington) which allowed sequential monitoring of individuals weekly. Date examined, color of marsupia, and the percentage of gravid versus nongravid females were then recorded. Males were identified by the end of the period of gravidity in 1987. These individuals then were eliminated from gravidity data for this year, and the percentage of gravid females on each sample date were adjusted accordingly. At Pendleton Island, during the peak of gravidity (determined from stockpiled females at Slant), nongravid individuals randomly collected were assumed to be males. Based on the number of gravid vs. nongravid mussels examined, an estimate of sex ratio for this species was obtained.

Release of Glochidia

In summer 1986, the period of glochidial release of *F. cuneolus* was determined by weekly sampling of stream drift. Three square-framed drift nets (0.045 m² frame), constructed of 5 mm

diameter copper rods and 130 μm mesh nylon netting fitted with removable cod ends, were staked into the substratum for approximately 1 h between 1000 and 1500 h on each sample date. They were spaced equidistant along a transect in the river, at the head of a riffle and downstream of a large concentration of mussels. Water depth was measured, and velocity at the mouth of each net was recorded with a pygmy gurley meter (Teledyne Gurley Co. Inc., Troy, New York) centered in the water at the mouth of the net. With these data, water volume filtered through each net was calculated, and glochidial density (no./ m^3) was determined from discharge and counts of glochidia per sample. Each drift sample was backwashed into individual containers and preserved in 10% formalin, buffered with sodium borate to prevent dissolution of glochidial valves. Rose bengal, a protein stain, was added later to samples to simplify the sorting of glochidia in the laboratory. A sieve was made by gluing a piece of 0.5 mm nylon mesh with silicon onto a 5 cm diameter section of PVC pipe. This coarse sieve filtered out large particulate matter; a 130 μm mesh sieve placed under this sieve collected glochidia and finer debris. This portion of each sample was backwashed into a jar, and examined subsequently in a gridded petri dish with a dissecting microscope (25-40X). Glochidia of *F. cuneolus* were removed from drift samples with a capillary tube and enumerated. The period of glochidial release was determined by the presence or absence of *F. cuneolus* in the drift. Peak release was considered to be the date on which maximum glochidial densities were recorded.

Glochidia of mussel species in the subfamily Ambleminae are distinguishable by their characteristic size and shape (Neves and Widlak, 1986); however, the glochidia of *F. cuneolus* are similar in appearance to the glochidia of other amblemine species, and hence some positive identification was necessary. Species of amblemine mussels at Slant, with dimensions of glochidia likely to overlap those of the fine-rayed pigtoe, were obtained and measured for use as reference material. These mussels included *Fusconaia subrotunda*, *Fusconaia cor* and *Pleurobema oviforme*. Specimens of these mussel species were transported to the laboratory in a 10 l bucket of river water and aerated with a portable "Fish Saver". In the laboratory, mussels were placed in individual 400 ml beakers without substratum. Containers were placed in a 300 l recirculating artificial stream (Frigid Units, Inc., Toledo, Ohio) at a mean temperature of 22 C, until glochidia had been expelled. If mussels

failed to release after 4 to 5 days, the beakers containing individuals were removed from the stream and maintained at room temperature. This increase in temperature and lack of flow proved to be an effective method of inducing abortions. Ten glochidia from five individuals of each species were obtained (50 glochidia per species), and valve dimensions of total length, total width, and hinge length were measured and recorded (Figure 4). A Kruskal-Wallis test was used to test for differences between valve dimensions among species, and an LSD procedure using ranks was applied to determine which species differed significantly from the others.

To examine the factor or factors potentially effecting the release of glochidia by fine-rayed pigtoes, the relationship of water temperature, water velocity, and sample dates in 1987 were analyzed using simple linear regression and multiple regression. Weekly mean water temperature, water velocity (measured at the mouth of the net from which glochidial density was determined), and sample date were treated as independent variables, and it was assumed that glochidial densities were dependent on one or more of these factors.

The diel periodicity of glochidial release was examined at Slant from 16 to 17 June 1987, determined to be the peak period of release for *F. cuneolus* in 1986. One drift net was set for 1 h at 4 h intervals, for a 24 h period. The net was set along the left ascending side of the site because the greatest densities of glochidia were collected there in 1986. Collected samples were processed as described previously for the weekly sampling. Numbers of glochidia of the fine-rayed pigtoe were counted in each sample ($n = 6$) to determine if densities in stream drift differed during a 24 h period.

Determination of Fish Hosts

Naturally Infested Stream Fishes

Fish species potentially serving as hosts for the glochidia of *F. cuneolus* were sampled weekly from a roughly 100 m section of the study area at Slant, using dip nets and a backpack electroshocking unit (Coffelt BP-1C, Coffelt Electronics Co., Eaglewood, Colorado). Sampling time was limited to approximately 2 h each week. This river section was divided into smaller subsections

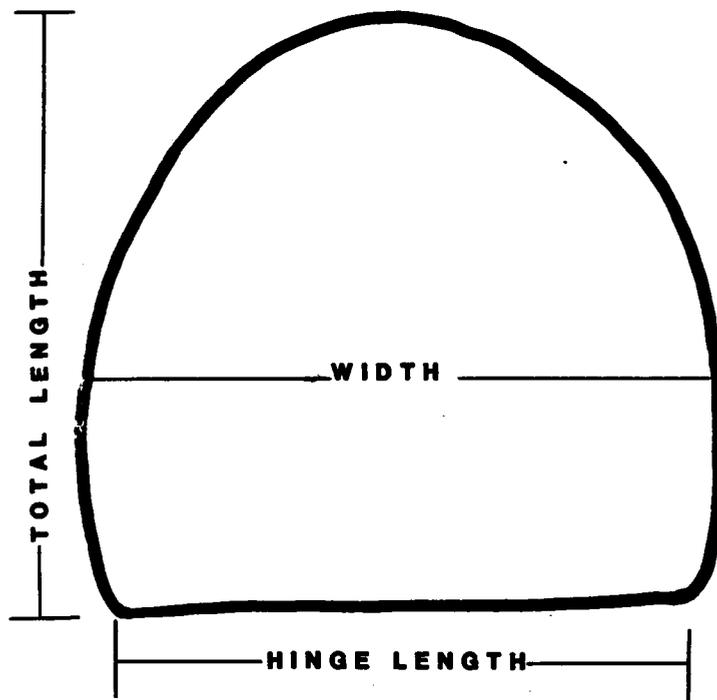


Figure 4. Length, width, and hinge length dimensions of a glochidium.

containing several habitat types; swift current with coarse gravel substratum, above a riffle with boulder-cobble substrata, perimeter of two islands with water willow (*Justicia americana*) and sandy substrata, and pool habitats along river banks with mud and silt substrata. Electroshocked fish were sorted and preserved in 10 % buffered formalin. In the laboratory, opercular flaps were removed from each fish to expose gills for examination under a dissecting microscope (24-40X). Prevalence (frequency of occurrence) and degree (number of glochidia per fish) of glochidial infestation were tabulated for each fish species. Fish encysted with amblemine glochidia were considered to be possible fish hosts for *F. cuneolus*.

Induced Infestations

Experiments were performed at an aquacultural laboratory on the campus of Virginia Polytechnic Institute and State University (VPI & SU). Municipal water, dechlorinated with a bone charcoal filtering system, was used to recirculate through the holding and test tanks. Fish metabolites were removed from this closed system as water recirculated through a submerged up-flow gravel biofilter. Water was forced up into head tanks by a 1/15 hp magnetic drive centrifugal pump (Cole-Parmer Instrument Co., Chicago, Illinois), and was oxygenated by venturi-type diffuser aerators (Figure 5).

Induced infestations of fish with glochidia were performed to verify suspected fish hosts. Cyprinid species were especially targeted because previous workers reported this family of fishes to commonly serve as hosts for other amblemine species (Weaver, 1981; Kitchel, 1985; Yeager and Neves, 1986). Glochidia for infestations were obtained from gravid mussels collected at Pendleton Island and Slant. Mussels were transported from the Clinch River to the laboratory and induced to abort glochidia as previously described. Fewer than 20 gravid *F. cuneolus* were retained at any one period, as required by Regional Endangered Species Permit Number PRT 697823. Released conglutinates were retrieved with the large end of a 10 ml pipette and were either immediately used for induced infestations or were refrigerated overnight (12 h) and used the following day. Refrigerated glochidia were allowed to acclimate to room temperature (4 to 6 h). A sample (n = 25) was tested prior to infestations by placing a salt crystal into a finger bowl containing glochidia, to

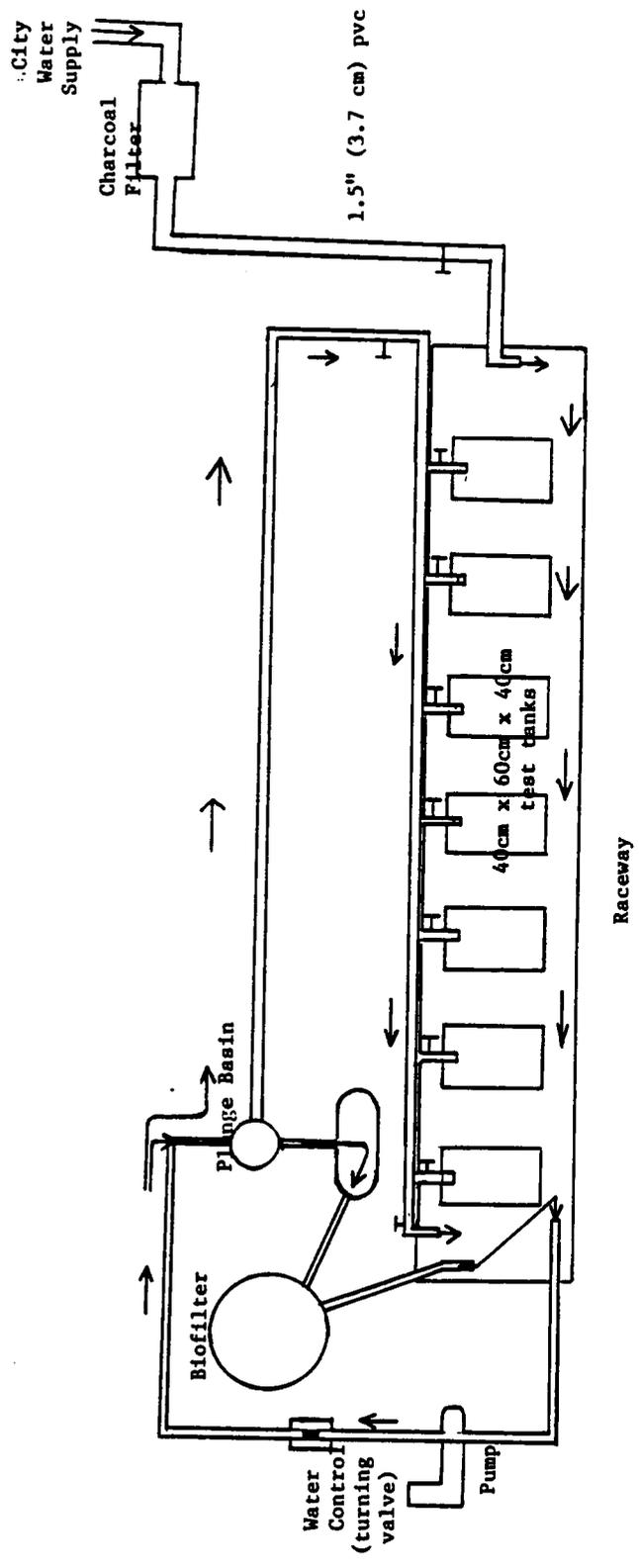


Figure 5. Recirculating charcoal biofiltration system used in fish host experiments.

ensure they were fully capable of attachment to fish. Viable glochidia rapidly opened and closed valves; a slow response indicated that additional acclimation time was needed.

Collection of Live Fish

Fish for induced infestations were collected from river reaches where mussels were rare or absent, to eliminate the possibility of collecting fish with acquired immunities from prior exposure to glochidia (Neves et al., 1985). Two sample sites were used: 1) below the Olin Mathieson plant on the North Fork Holston River (NFHRM 73.9), and 2) below the APCO electric plant on the Clinch River (CRM 264.8). A three-person field crew was typically used to collect fish effectively. Fish were electroshocked, scooped with a 10 l bucket and transferred (by pouring) into a larger (20 l) holding bucket to expedite collection and prevent direct handling of fish. A 50 l ice chest with river water served to transport fish. Aeration was provided by a portable "Fish saver"; a dilute (2%) salt solution and ice packs were added to reduce stress of fish and acclimate them to temperatures (20-26 C) in laboratory holding tanks. Use of nets was limited throughout all experimental procedures to prevent handling stress and possible transfer of disease (Weaver, 1981; Kitchel, 1985).

Several rectangular tanks (50 cm x 90 cm x 50 cm) held fish prior to experimentation. Five to 7 days typically elapsed after collecting fish to allow recovery from possible stress associated with electrofishing and transport. However, 2 fish species (bluegill, *Lepomis macrochirus*, and redbreast sunfish, *Lepomis auritus*) were tested after only 1 day of acclimation. Benzocaine (1 g ethyl p-aminobenzoate per 25 ml acetone) was added to holding tanks (5 ml solution per 8 l water) to anesthetize fish and aid identification of cyprinids. Use of a pliable container with tall sides to scoop fish replaced the use of a net. Fish were then poured into a clear, plastic container for observation. Tentative identifications of fish species were made by examining morphological features, and these identifications were later confirmed by Dr. R. Jenkins of Roanoke College in Salem, Virginia. After sorting, fish were poured into smaller individual test tanks (40 cm x 60 cm x 40 cm), with one test species per tank. Standpipes covered with 130 μ m nylon mesh were initially used to prevent the loss of glochidia. However, this small mesh size retarded water flow and readily collected debris, often causing tanks to overflow. Having previously observed that floating glochidia would settle to

the bottom of a container within roughly 30 s, the 130 μm mesh was removed and the water entering tanks was reduced to a trickle to reduce turbulence. Five mm mesh replaced the smaller mesh and was added only to prevent loss of fish through standpipes.

Infestation Procedure

Induced infestations of fish involved the use of three containers, to hold, anesthetize, and allow recovery of fish. Surgical gloves were worn while handling fish, and gloves were disinfected after handling each fish (Roberts, 1980). The procedure of infestation was as described by Weir (1977). Glochidia were separated from their conglutinal matrices by repeatedly drawing up and expelling the conglutinates and water in a finger bowl. Fish (typically 10 individuals per species) were anesthetized with benzocaine solution (time required varied from 1 to 5 min depending on species). The gill flap of an anesthetized fish was lifted with the small end of a pipette and approximately 100 glochidia were released into the right branchial chamber. Fish were then placed in containers of fresh dechlorinated water and allowed to recover. Infested specimens were returned to test tanks, one species per tank. One hour post-infestation, fish were viewed under 40X magnification to document initial attachment of glochidia on gills. Temperatures of test tanks were maintained between 21-26 C and recorded daily.

Rejection of glochidia by non-host species has been reported to occur within 3 to 5 days (Zale, 1980; Kitchel, 1985). Therefore, at least one fish per species was examined every day until the fifth day. To do so, fish were anesthetized as previously described, then placed on a wet paper towel. Right opercular flaps were carefully folded back with a disinfected probe to examine gills (40X) of each fish. If glochidia were absent on any of these days, the remaining fish were examined until glochidia were observed or until all fish had been checked. On the first day post-infestation, test tanks were siphoned and the siphonate discarded. Excess glochidia that may have been poured from the recovery bucket into the tanks along with fish following infestations were thus eliminated. A 25 mm diameter flexible hose was used to siphon the bottom of tanks. A 130 μm mesh screen, glued to a section of PVC pipe, captured sloughed glochidia and juveniles in the siphonate. Through day 5, siphonate was qualitatively examined; thereafter, tanks were siphoned every other

day and the number of juveniles was recorded. If rejection and sloughing of glochidia occurred, the siphonate was examined in three additional siphonings, and fish were sacrificed to confirm the loss of glochidia.

Siphonate was examined under 25-40X magnification in a gridded petri dish. Juveniles were recognized by their yellowish color, thickened ventral margins and often by the movement of the juvenile's foot protruding from valves. A sample of juveniles was placed in a vial containing distilled water and 3 drops of the relaxant propylene phenoxitol (Inolex Corp., Philadelphia, Pennsylvania). After 1 to 3 days, juveniles were preserved in 10% buffered formalin and later photographed. A fish species was considered a host of *F. cuneolus* if attachment, encystment, and metamorphosis to the juvenile stage resulted. Most tests that confirmed a host species in 1986 were repeated in 1987.

Validation of Annulus Formation

To confirm that growth checks on valves of the fine-rayed pigtoe are laid down annually, sixteen specimens from Slant were labeled with numbered disc tags on 10 October, 1986. Individuals were measured to the nearest 0.1mm to determine incremental growth, and notched with a triangular file at ventral margins to delineate the origin of incremental growth. Specimens were placed under a hardware mesh enclosure to prevent muskrat predation. Individuals were recovered one year from time of marking, remeasured, and examined externally for annulus formation. One specimen was sacrificed so that internal annuli could be examined using thin-sectioning techniques (described below) to validate the formation of one annulus. These procedures also allowed differences between annuli and "false" annuli formed internally and externally to be distinguished.

Determination of Age and Growth Statistics

Aging was performed by the thin-sectioning of shells, as explained by Clark (1980) and repeated by Moyer (1984) and Jirka (1986). A Buehler Isomet low-speed diamond-tipped saw (Buehler Ltd., Evanston, Illinois) was used to section shells of *F. cuneolus* collected in muskrat middens at Slant (CRM 223.3) and Pendleton Island (CRM 226.3). Five to ten valves per length group (10 mm intervals) from each study site were thin-sectioned. Initial cuts on each valve were made along the longest axis from the umbo to the ventral margin, perpendicular to external growth rings. This cut resulted in a cross section of all internal growth rings. Because of blade size, proper initial cuts could not be obtained on valves greater than 60 mm. For these specimens, a small section from the anterior portion of the valve was cut away using a hacksaw and chisel and kept for future reference. This procedure reduced valves to a size small enough to be mounted for initial cuts at the desired location. Cross-sections were roughened with 600 grit Buehler Carbimet paper strips, and glued onto 27 x 46 mm petrographic microslides using Buehler epo-kwik epoxy cement. Before gluing, the microslides were also roughened with 600 grit to improve contact with cross sections. After drying for at least 12 h, slides were vacuum-sealed onto a chuck, then screwed into the movable cutting arm of the saw. The second cut was made at a thickness of 0.25 mm, determined by Neves and Moyer (1988) to be the optimal thickness for high resolution thin-sections. This thickness was obtained by moving the saw arm toward the blade until the petrographic slide was just touching the blade, then backing 0.25 mm away from the blade. The age of each specimen was then determined by counting internal annual rings on thin sections under a compound microscope at 50X magnification. The area adjacent to the point where the annulus reached the shell margin was marked with a black felt-tipped marker. The marked thin-sections were then held facing the cross-section from which the cut was produced. False annuli were identified, and I was able to compare internal annual rings with true external annual rings. On five specimens from each study site, external annuli on the cross sections were marked; then, by overlaying this shell section onto the matching uncut valve, measurements of lengths at previous ages could be determined.

Since specimens younger than age 9 were rare, I obtained back-measured lengths of younger age groups by this procedure. For these back-measured lengths, I used only those shells which were minimally eroded, and with easily distinguishable annuli.

Total length-at-age measurements were recorded before shells were thin-sectioned. Length is the maximum anterior to posterior distance at each annulus. After aging all shells, mean lengths at each age were recorded to the nearest 0.1 mm. Mean length-at-age data obtained from shell thin-sectioning were fitted to a modified version of the von Bertalanffy equation (Gallucci and Quinn, 1979), to describe length-age relationships at Pendleton Island and at Slant:

$$L_{(t)} = (w/k)(1 - e^{-k(t-t_0)})$$

where t is a given time in years, t_0 is the theoretical time when length is zero, k is a growth constant describing the rate at which length (L) approaches the theoretical maximum length (L_{∞}), and w describes growth rate near t_0 . The parameter w is the product of k and L_{∞} , and replaces L_{∞} in the original von Bertalanffy equation. This new parameter eliminates problems associated with the interdependency of k and L_{∞} , and allows comparisons between characteristics of growth in two spatial regions because of its statistical robustness (Gallucci and Quinn, 1979).

Non-linear procedures were applied to derive estimates of w , k , and t_0 , and based on these values, estimates of L_{∞} were calculated (SAS Institute, 1982). Confidence intervals of these parameter estimates were compared for overlap to determine differences in growth of *F. cuneolus* between the two sites. The model of predicted lengths at each age derived from thin-sectioning shells then served to predict ages from total lengths of unaged specimens of *F. cuneolus*. I obtained length data from valves of *F. cuneolus* collected in muskrat middens at Slant from 1980 through 1986. Ages of 353 specimens in this collection were computed by age-length relationships, and the age-class distribution at this site was determined. Length measurements on 43 live specimens collected at Slant in 1987 were also converted to ages to determine age class structure. The two distributions were then compared (RxC test of independence using the G-test) to determine whether estimates of age-class structure differed significantly based on collection method (human vs muskrat).

Estimation of Density

To obtain an estimate of the density of *F. cuneolus* at Slant, the study area was mapped as suggested by Kovalak et al. (1986), and sampled using a 0.5 m² quadrat sampler. The 325 m stretch of river was divided into thirteen 50x25m transects. Wire rods were staked into the substratum along transect lines and flagged to delineate each rectangular plot. Obvious landmarks within each transect were noted (i.e., islands, bedrock, riffle areas), and the habitat types within each transect were subjectively classified under three categories, relative to the typical habitat in which *F. cuneolus* occurs. Areas of good (suitable), marginal, and poor habitat (specifically bedrock) were roughly measured within each transect and mapped on graph paper. After mapping the study area, fifteen 0.5 m² quadrats were taken in suitable habitat within various transects and marked on the map. This sampling procedure was then repeated in marginal habitat. Transect choice often depended on water depths and ability to sample. Otherwise, quadrats were randomly chosen. By converting grid units of the map to actual area, I estimated the number of *F. cuneolus* per 1 m² at Slant.

Results

Reproductive Biology

Gravidity Period

Gravid females of *F. cuneolus* were first collected in the Clinch River at Slant on 16 June, 1986. Four of eight specimens collected by snorkeling and examined on this date were gravid (Figure 6). Because gender of the individuals examined was not determined in 1986, the percentage of gravid females likely was underestimated. Numbers of gravid females most likely were higher in mid-June in 1986. The number of gravid individuals ($n = 12$) that were stockpiled and monitored at this site declined through 8 July, when all but one stockpiled gravid individual had released. Based on samples obtained by snorkeling at Slant after this date, it appeared that a high percentage of females remained gravid throughout the remainder of the reproductive season. Between 16 July and 6 August, 1986, the number of naturally occurring gravid individuals at Slant was high (50-100%), but sample sizes always were small ($n < 4$). However, a random sample ($n = 33$) of *F. cuneolus* taken at Pendleton Island on 25 July, 1986, also indicated that many females (61%) were still gravid. One individual was gravid at Slant on 6 August, 1986, and gravid specimens were not collected after this date in 1986.

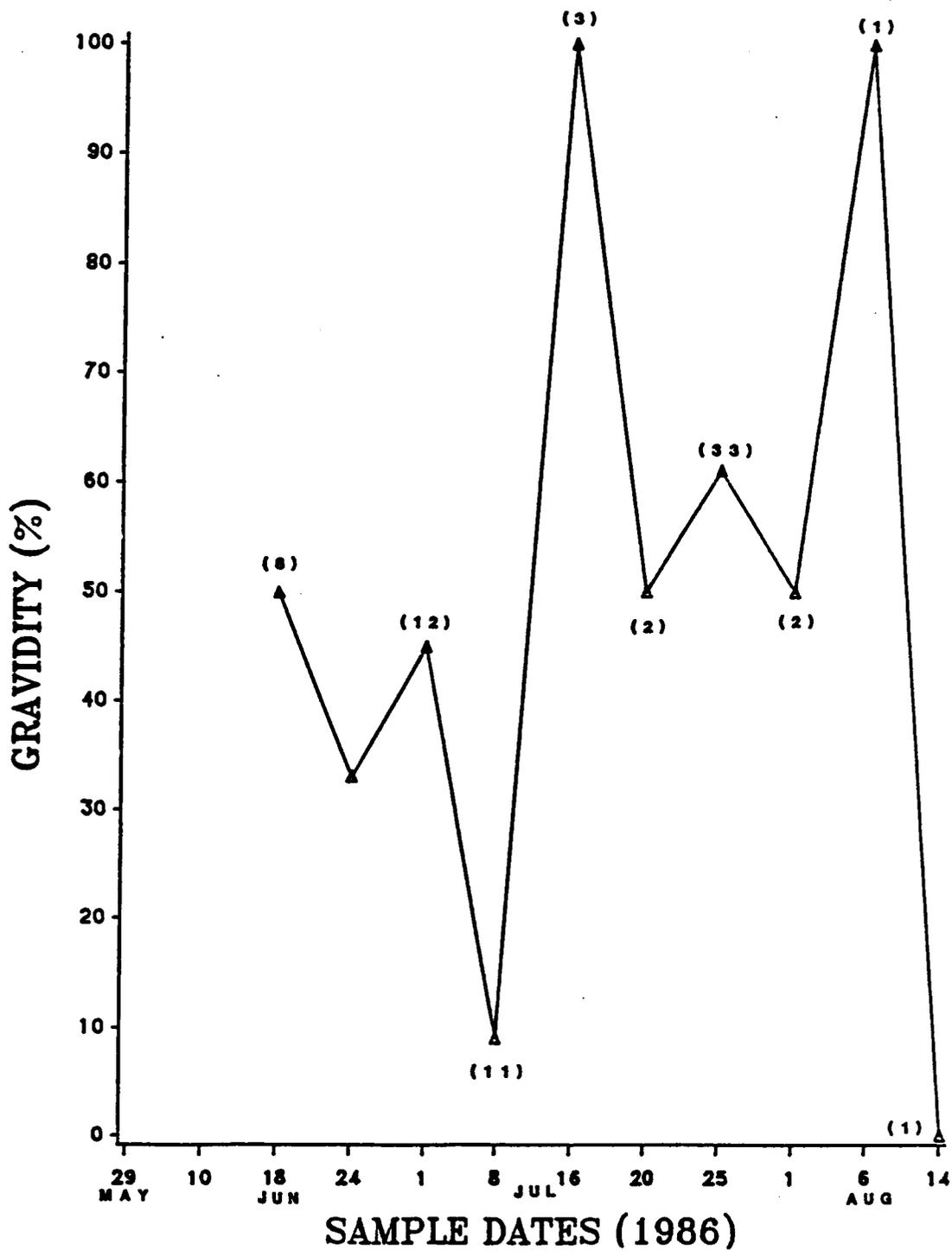


Figure 6. Frequency of gravid females in the Clinch River at Slant, 1986. Sample sizes in parentheses.

Large quantities of silt accumulated on the left ascending side of the river at Slant in fall 1986, and use of the hardware mesh enclosure resulted in additional accumulation of silt and debris on stockpiled specimens. To eliminate potential problems associated with siltation, mussels were moved on 19 November, 1986, approximately 10 m from their previous location, and the mesh enclosure was discarded. Females therefore had to be relocated in May and June, 1987; most were recovered on 18 June.

In 1987, two gravid females were first collected from the Clinch River on 10 June, the date when the highest percentage of gravid females was observed at both Pendleton Island and Slant (Table 2 and Figure 7). Based on my observations in 1986 and Ortmann's (1921) records of gravidity for the fine-rayed pigtoe, it appears that the peak in gravidity for this species occurs from early to mid-June. In 1987, all nongravid adult mussels collected on 10 June were considered males. An estimated sex ratio of 3 females: 1 male ($n = 8$) was determined by snorkeling and handpicking at Pendleton Island on this date. The estimated sex ratio was questionable because it was based on such a small sample; therefore, gravidity was based on total numbers of individuals examined at Pendleton Island. It is likely that percentages are greater than reported at this site. A downward trend in the frequency of gravid females was observed at Pendleton Island and at Slant following the observed peak in gravidity (Figure 7). Numbers of gravid females at Slant sharply declined after 24 June, 1987. A higher percentage of females remained gravid later into the season at Pendleton Island in this year. Gravid specimens were last collected on 6 August 1987, at Pendleton Island, which approximated the end of the gravidity period observed in 1986.

Since the peak in gravidity in 1986 and 1987 occurred in early to mid-June, it was apparent that gravid individuals were present prior to this time. Additional observations made in 1988 revealed that females began incubating eggs between 3 and 23 May (Figure 8). Gravid females were not observed at either Pendleton Island ($n = 21$) or Slant ($n = 27$) on 3 May, 1988. By 23 May, one female ($n = 12$) examined at Slant, and nine ($n = 20$) of the individuals examined at Pendleton Island were gravid. Mid-day water temperatures recorded at Slant had increased from 16 to 21 C during this time period (Table 3). Temperatures recorded in mid-May in the Clinch River at Cleveland, Virginia (CRM 272) in 1986 and 1987 were approximately 18 C (Table 3). Thus, it appears that

Results

Table 2. Gravidity of fine-rayed pigtoes in the Clinch River at Slant and Pendleton Island, Virginia, 1987.

Date of collection	No. mussels examined	Number gravid	Percent gravid	Color of marsupia		
				pink	orange	light peach
<u>Pendleton Island</u>						
10 June	8	6	75	5	1	0
24 June	7	4	57	2	2	0
8 July	36	18	50	.1	-	-
16 July	19	4	21	0	0	4
20 July	24	5	21	4	0	1
30 July	73	4	6	-	-	-
6 August	46	2	4	0	1	1
<u>Slant²</u>						
29 May	1	0	0	0	0	0
10 June	2	2	100	0	0	2
18 June	14	11	79	6	2	3
24 June	14	9	64	4	5	2
1 July	13	3	23	1	0	1
8 July	14	1	7	1	0	0

¹Undetermined

²Gravidity determined from females only

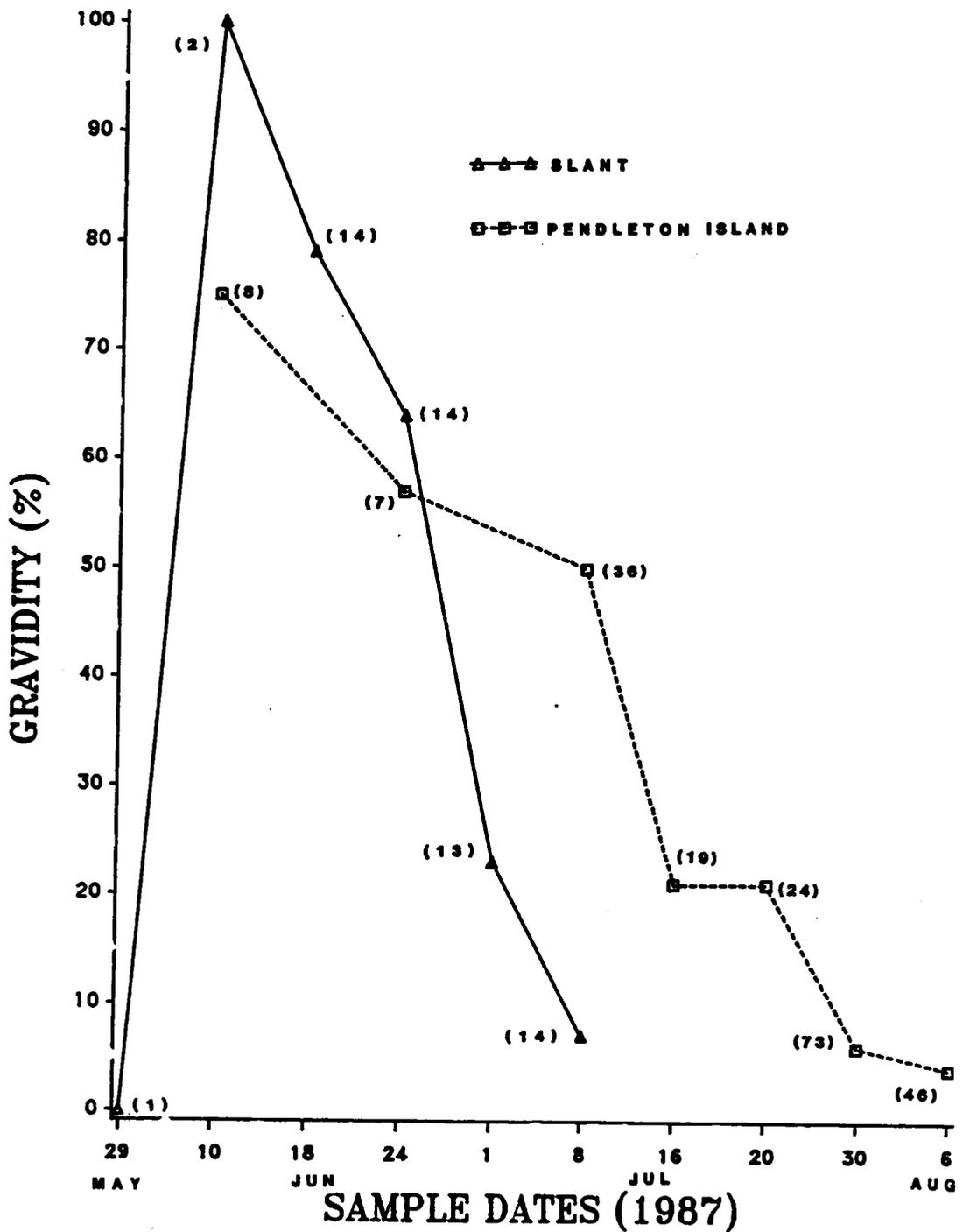


Figure 7. Frequency of gravid females in the Clinch River at Slant and Pendleton Island, 1987. Sample sizes in parentheses.

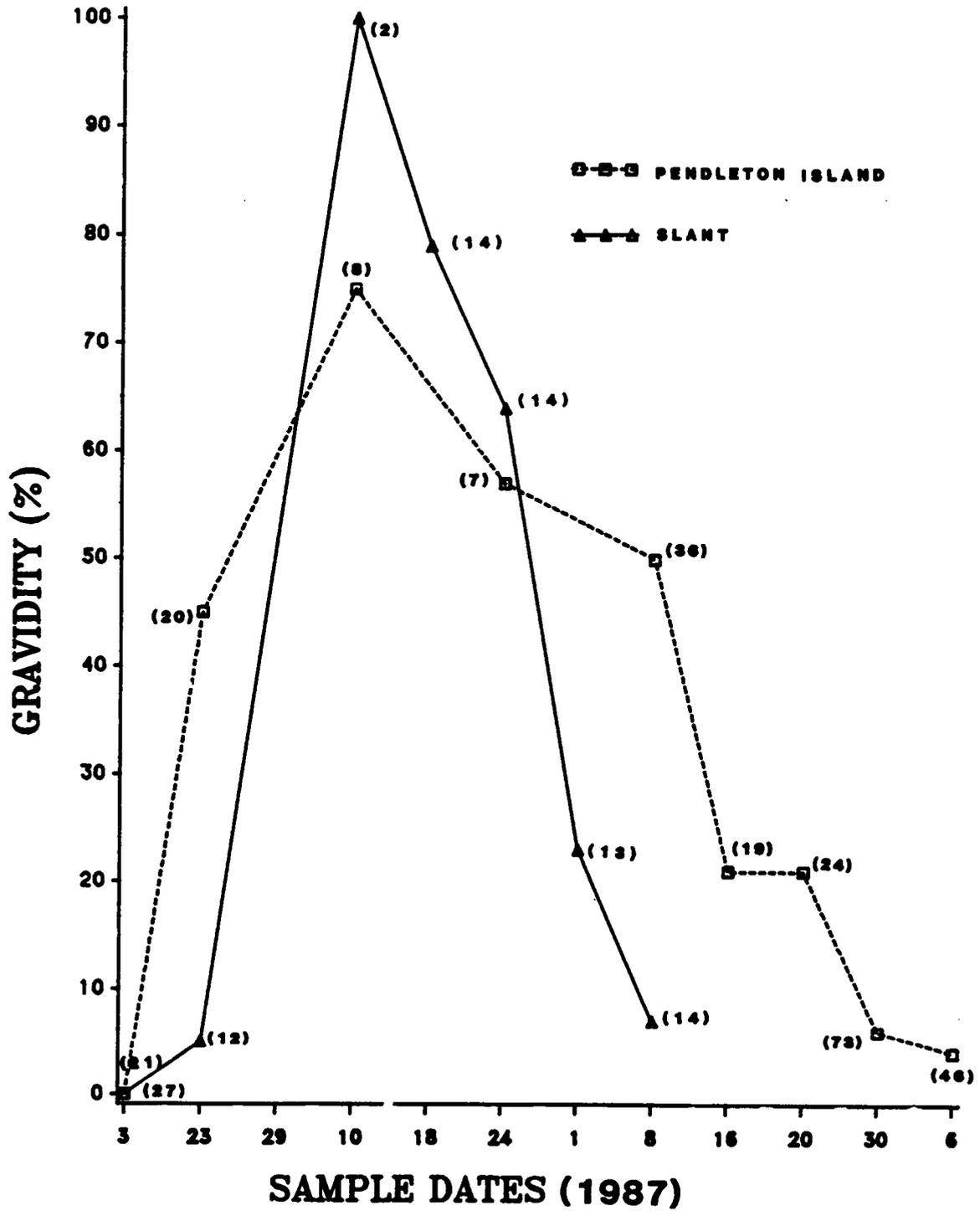


Figure 8. Frequency of gravid females in the Clinch River during summers, 1987 and 1988 combined. Sample sizes in parentheses.

Table 3. Temperatures recorded monthly at Speer's Ferry (CRM 211)¹, and mean monthly discharge in the Clinch River at Cleveland (CRM 272)², Virginia, 1986 to 1988.

Sample date	Monthly recorded temperature (C)	Temperature sampling time (h)	Mean monthly discharge (m ³ /s)
1/9/86	0.0	1100	115
2/6/86	9.0	1100	577
3/20/86	11.0	1100	284
4/3/86	17.0	1130	83
5/15/86	18.5	1145	232
6/18/86	24.0	1300	86
7/29/86	27.0	1100	57
8/14/86	25.0	1140	36
9/2/86	19.0	1130	50
10/20/86	11.0	1100	44
11/18/86	9.0	1115	128
12/11/86	8.0	1100	223
1/29/87	2.0	1115	267
2/19/87	6.5	1045	457
3/17/87	9.3	1100	396
4/23/87	17.0	1100	1041
5/11/87	18.0	1045	160
6/2/87	21.5	1050	97
7/21/87	25.3	1115	65
8/6/87	26.2	1035	25
9/8/87	21.5	1200	29
10/20/87	13.3	1240	-
11/9/87	9.3	1145	-
12/8/87	4.4	1110	-
1/19/88	3.5	1120	-
2/16/88	3.6	1050	-
3/21/88	8.4	1135	-
4/11/88	12.1	1116	-
5/3/88	16.0 ³	1200	-
5/23/88	21.0 ³	1200	-
6/27/88	24.7	1205	-
7/27/88	24.4	1155	-

¹Obtained from EPA's water quality data base (STORET), Abingdon, Virginia.

²Obtained from Virginia Water Resources Center hydrologic information storage and retrieval system (HISARS), Blacksburg, Virginia.

³Mid-day temperatures (1200h) in the Clinch River at Slant.

reproductive activities for the fine-rayed pigtoe are initiated when water temperatures range from 16 to 21 C.

As judged by data collected at Slant and Pendleton Island in 1986 and 1987, it appears that the brooding period was delayed in 1986. Numbers of gravid females appeared to reach a peak approximately one week later, and the percentage of gravid females collected in late July at Pendleton Island in 1986 (25 July, 60%), was greater than that in 1987 (20 July, 21%). Based on STORET data recorded monthly at Speer's Ferry, mid-day water temperature prior to the brooding season in March, 1986 (11.0 C) was somewhat higher than that in 1987 (9.3 C), but data were recorded at different times in April, and for most months, making it difficult to discern potential differences between years (Table 3). Temperature data at Slant were not recorded prior to early June during the study, and daily temperatures were not obtained in 1986 due to a faulty thermograph. Therefore, a direct comparison of water temperatures at this site also was difficult because of the use of different recording instruments between years. However, weekly median temperatures recorded in the Clinch River at Slant indicated that temperatures were warmer in 1986 (Table 4). Additionally, mean monthly discharge recorded in the Clinch River at Cleveland (CRM 272), in April and May, and again in June through July, 1986, was substantially lower than recorded for 1987 (Table 3).

Maturation of Conglutinates

In 1986, I observed weekly changes in marsupial coloration. Several specimens collected from Pendleton Island on 25 July, 1986 were transported to Slant and periodically monitored to determine if a pattern of coloration change occurred. After 6 days, coloration of marsupia of two of four females changed from pink to an orange color; the other two mussels released their conglutinates. No distinct pattern or trend in colors of marsupia was readily apparent from random observations made on gravid females at either Pendleton Island or Slant in 1987). However, the marsupia of all gravid females examined on 10 June at Slant, and on 4 July, 1987, at Pendleton Island contained peach-colored conglutinates. I obtained more relevant information by monitoring individual females stockpiled at Slant. The marsupial color of several individuals gradually changed from pink

Table 4. Weekly median temperatures and ranges¹ in the Clinch River at Slant, June to August, 1986 and 1987.

Sample date	Temperature range (C)	Weekly median temperatures (C)
6/16/86	-	25.4 ²
6/25/86	21.4-27.4	24.4
7/1/86	22.4-27.9	25.1
7/9/86	23.4-27.9	25.6
7/16/86	24.4-28.4	26.4
7/25/86	25.4-29.9	27.6
7/30/86	24.4-29.4	26.9
8/5/86	21.4-28.4	24.9
8/13/86	22.9-27.4	25.1
5/29/87	-	25.4 ²
6/10/87	-	23.0 ³
6/18/87	22.9-24.9	23.0
6/24/87	23.4-25.1	24.0
7/1/87	23.0-25.6	23.5
7/8/87	22.0-24.3	23.0
7/16/87	21.6-24.2	23.5
7/20/87	21.9-24.0	23.3
7/30/87	25.0-27.4	26.0
8/5/87	26.2-28.0	26.8

¹Week prior to date glochidia were sampled in drift

²Temperature recorded at 1200 h

³Median temperature for that day

to orange, and finally to light peach a color, which indicated that glochidia were mature and ready for release. In the laboratory, those individuals with light peach coloration of marsupia consistently released mature glochidia which were held together by gelatinous strings; the conglutinal matrix appeared to have partially desintegrated. Adult females released these mature glochidia within 8 to 14 days after the pink color was recorded, at a temperature range of 20 to 24 C. The marsupia of one individual remained pink in color from 18 June through 8 July, changing to orange-pink on 16 July. On this date, the individual was transported to the laboratory and induced to abort the conglutinates. Mature glochidia were light orange to peach in color and were free of vitelline membranes. Both unfertilized eggs and embryos were recognized by their pink color, but unfertilized eggs lacked vitelline membranes. Laboratory observations on conglutinates of other specimens of the fine-rayed pigtoe revealed that immature glochidia in various developmental stages were also pink in color. These glochidia were expelled in tightly organized, pink conglutinates and were enclosed within vitelline membranes. I also noted that the marsupia of females often had a "bubbly" appearance immediately following glochidial release. It appeared as if these individuals were still gravid, unless more closely examined. Gills of spent females were more inflated and less "turgid" than gills of gravid females. Four gravid females with pink marsupia were checked periodically in the laboratory. The marsupia of these individuals exhibited a peach color within 7 to 11 days, at temperatures of 20 to 23 C, suggesting that development from embryo to glochidium occurs within a period of about 2 weeks in this temperature range.

To ensure that aborted glochidia would likely be mature for fish host experiments, only females with peach-colored marsupia were transported to the laboratory. Coloration proved to be a valid indicator of the stage of larval development. In 1987, 41 gravid females were brought to the laboratory to obtain glochidia. Females released glochidia from 14 June through 11 August, 1987, at holding tank temperatures ranging from 20 to 24 C. Thirty-three mussels released conglutinates containing approximately 99% mature, viable glochidia. Eight individuals released conglutinates containing mature glochidia and 3 to 5% unfertilized eggs. Immature glochidia, with vitelline membranes intact, were released from only one female on 19 July, 1987. On 15 July, 9 of 10 mussels in the laboratory released mature glochidia simultaneously at a water temperature of 20

C. Whether this simultaneous release was induced by a sudden decline in temperature is unknown because temperature records were incomplete. The last recorded tank temperature before this date was 24 C on 10 July.

Females of *F. cuneolus* typically expelled conglomerates through the excurrent siphon, but on at least one occasion, I observed glochidia being expelled through the incurrent siphon as well. On numerous occasions, I observed females, contained in beakers, releasing mature glochidia (held together in a loose gelatinous matrix) along the entire ventral margin of their gills. Subcylindrical-shaped conglomerates of *F. cuneolus* were approximately 6 mm long and 1.5 mm wide and consisted of two layers of tightly aggregated glochidia, approximately 0.8 mm deep. One conglomerate from each of five females contained between 208 and 295 (mean + 1 s.d. = 236 + 38.1) embryos or glochidia. Fecundity, computed from the number of water tubes used for brooding embryos in one sacrificed gravid specimen (70 mm in length) and the mean number of embryos per conglomerate, was estimated to be approximately 113,000 embryos for this female.

Differentiation of Glochidia

A Kruskal-Wallis test and an LSD procedure using ranks showed that measurements of hinge length differed significantly ($p = 0.00001$) among glochidia of *F. cuneolus*, *F. subrotunda*, *F. cor* and *P. oviforme* (Table 5). Glochidia of *F. cuneolus* differed significantly in width from those of *F. cor* and *F. subrotunda* ($p = 0.0001$), but were not significantly different from *P. oviforme*. Total lengths of glochidia did not differ significantly among any species examined ($p = 0.05$).

The glochidia of *F. cuneolus* were most similar in appearance to those of *P. oviforme*, but the longer hinge length and rotund appearance of the glochidia of *F. cuneolus* enabled quick recognition of this species (Figure 9). Because the glochidia of *F. subrotunda*, *F. cor*, and *P. oviforme* were distinguishable by morphometrics, differentiation by other methods was unnecessary. However, scanning electron micrographs and description of the glochidia of *F. cuneolus* were obtained from Michael Hoggarth of Ohio State University. Valves of glochidia of the fine-rayed pigtoe are ovate

Table 5. Dimensions (mm)¹ and morphometric comparisons of glochidia of selected amblymeine species from the Clinch River, Virginia.

Glochidial ² dimension	Pleurobema oviforme	Fusconaia subrotunda	Fusconaia cor	Fusconaia cuneolus
Length	0.185 ± 0.007	0.181 ± 0.008	0.181 ± 0.006	0.181 ± 0.009
Width	0.193 ± 0.004	0.193 ± 0.005	0.146 ± 0.006	0.150 ± 0.028
Hinge length ³	0.150 ± 0.005	0.165 ± .009	0.122 ± 0.005	0.118 ± 0.005

¹Mean ± 1 s.d.

²Compared by nonparametric protected LSD. Underscore indicates no significant difference in specified dimension ($p = 0.05$).

³Dimensions differed significantly among all species ($p = 0.00001$).

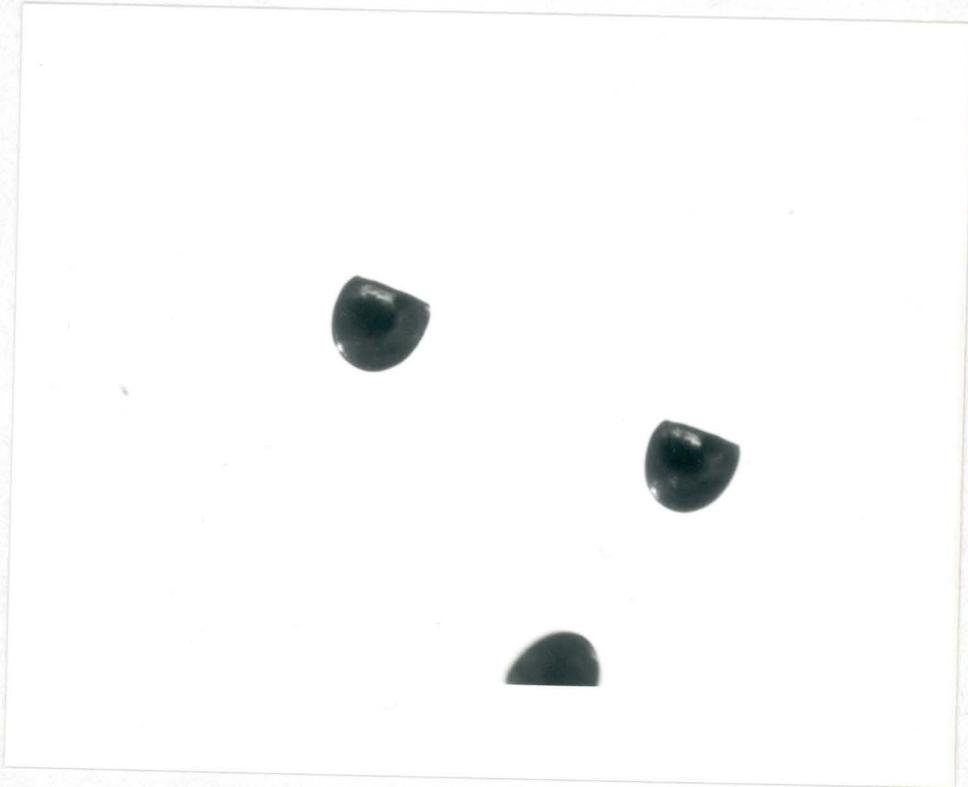


Figure 9. Photomicrograph (20X) of mature glochidia of the fine-rayed pigtoe.

subelliptical, depressed and exactly symmetrical; anterior and posterior borders are equally curved. The exterior surface of valves are finely malleated with very few "pits". The dorsal hinge is nearly straight, with a slight elevation at each dorsal-lateral margin (Figure 10). The ventral valve margin supports a flange, characterized by micropoints arranged in incomplete, vertical rows. Micropoints are pyrimidal in shape and tightly aggregated on the proximal half of the flange. The center portion of each flange has very few small micropoints; the unsculptured distal edge of the flange is narrow (Figure 11).

Release of Glochidia

Stream drift was sampled for glochidia of *F. cuneolus* from 16 June to 13 August, 1986 and from 29 May to 30 July, 1987 (Figure 12). In 1986, the highest densities of glochidia in stream drift occurred in mid-June (43.0/100 m³) and mid-July (39.7/100 m³) (Table 6). A similar trend was observed in 1987 (126.6 and 60.3/100 m³, respectively), although the second peak occurred one week before the one in 1986. The peak in the percentage of gravid mussels at Slant (100%, n = 2) in 1987 occurred one week before the peak in the release of glochidia (Figure 12).

The release of glochidia by fine-rayed pigtoes throughout the reproductive season, relative to daily median temperatures recorded at Slant in 1987, is presented in Table 7 and Figure 13. Densities of glochidia in stream drift were low on the first sampling date in 1987 (29 May, 1.7 glochidia/100m³), at the beginning of the brooding season. Mid-day water temperature (1100 h) on that day was 25.4 C. By 10 June, 1987, densities of glochidia in stream drift had increased substantially (54.7 glochidia/ 100 m³). The median temperature recorded for that day was 23.0 C. Daily water temperatures prior to 10 June were not recorded. The week prior to the date when highest densities of glochidia in stream drift (126.6 glochidia/100m³). were recorded at Slant, water temperatures ranged from 22.9 to 27.4 C. Five days later (24 June), densities of glochidia in drift were substantially lower (38.3 glochidia/100m³), even though temperatures were higher prior to this sampling date and weekly median temperatures for both dates were equivalent (23.0 C). Linear

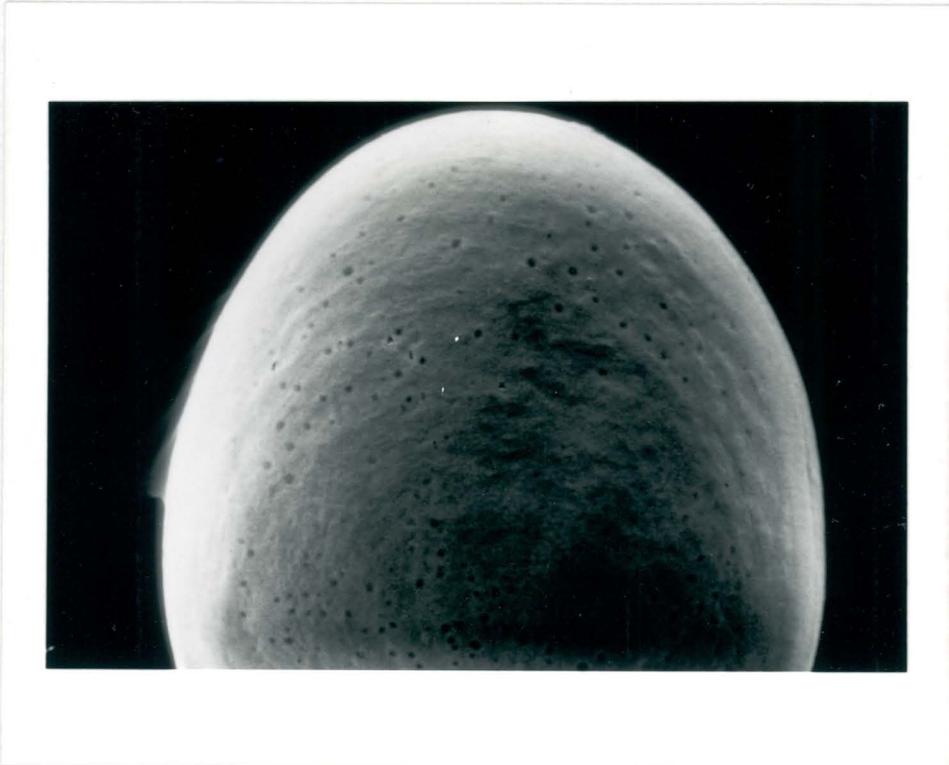


Figure 10. Scanning electron photomicrograph (500X) of the exterior surface of a glochidium of the fine-rayed pigtoe, courtesy of Michael Hoggarth, Ohio State University.

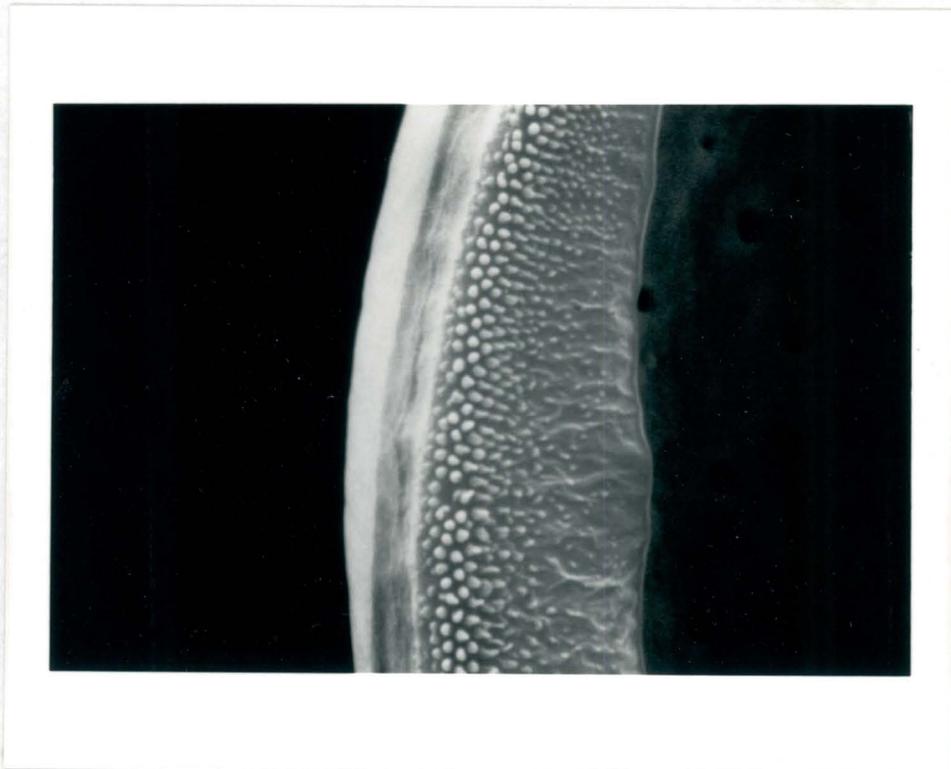


Figure 11. Scanning electron photomicrograph (5000X) of the ventral valve margin of a glochidium of the fine-rayed pigtoe, courtesy of Michael Hoggarth, Ohio State University.

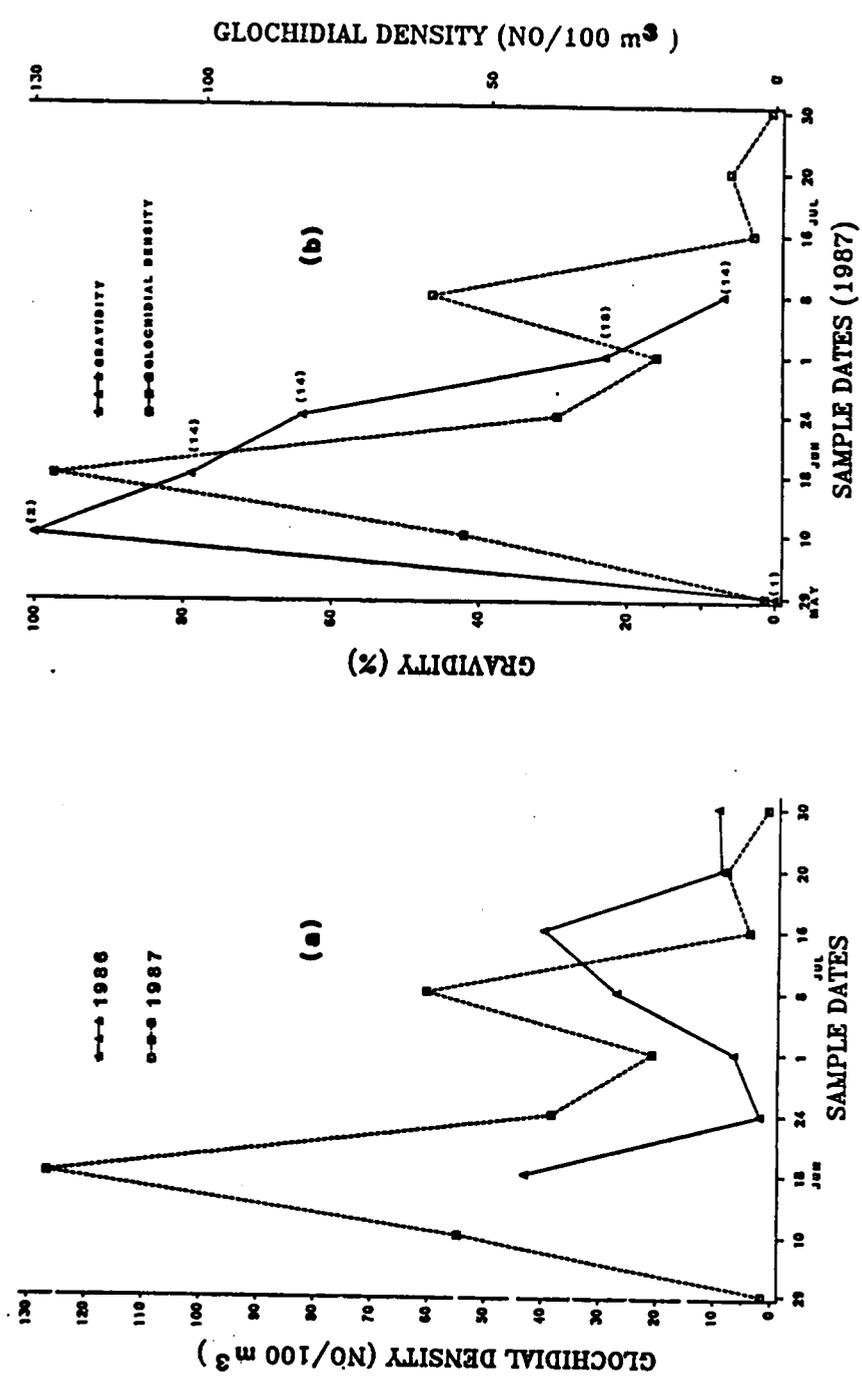


Figure 12. Densities of glochidia of the fine-rayed pigtoe in drift, 1986 and 1987 (a), and densities of glochidia vs. gravity in the Clinch River at Slant between May and August, 1987 (b).

Table 6. Densities of glochidia of the fine-rayed pigtoe in weekly drift samples, weekly median temperatures and ranges¹ in the Clinch River at Slant, June to August, 1986 and 1987.

Sample date	Temperature range (C)	Weekly median temperatures (C)	Glochidial densities (no/100m ³)
6/16/86	-	25.4 ²	43.0
6/25/86	21.4-27.4	24.4	1.9
7/1/86	22.4-27.9	25.1	6.6
7/9/86	23.4-27.9	25.6	27.0
7/16/86	24.4-28.4	26.4	39.7
7/25/86	25.4-29.9	27.6	8.9
7/30/86	24.4-29.4	26.9	9.4
8/5/86	21.4-28.4	24.9	2.9
8/13/86	22.9-27.4	25.1	3.9
5/29/87	-	25.4 ²	1.7
6/10/87	-	23.0 ³	54.7
6/18/87	22.9-24.9	23.0	126.6
6/24/87	23.4-25.1	24.0	38.3
7/1/87	23.0-25.6	23.5	20.8
7/8/87	22.0-24.3	23.0	60.3
7/16/87	21.6-24.2	23.5	3.9
7/20/87	21.9-24.0	23.3	7.9
7/30/87	25.0-27.4	26.0	0.8
8/5/87	26.2-28.0	26.8	-

¹Week prior to date glochidia were sampled in drift

²Temperature recorded at 1200 h

³Median temperature for that day

Table 7. Water temperatures and densities of glochidia in weekly drift samples at Slant, and daily mean discharge recorded at Cleveland (CRM 272) Clinch River, Virginia, late May to early August, 1987.

Sample date	Daily median temperature (C)	Temperature range (C)	Mean daily discharge (m ³ /s)	Glochidial densities (no/100 m ³)
29 May	25.4 ²		93	1.7
10 June	23.0	22.0-24.8	104	54.7
11 June	22.9	22.0-24.0	101	
12 June	23.5	22.5-24.3	82	
13 June	23.5	22.7-24.8	73	
14 June	23.5	22.8-24.0	70	
15 June	23.9	22.8-24.5	65	
16 June	23.9	23.0-24.6	61	
17 June	24.9	23.1-25.9	62	
18 June	25.1	24.0-26.5	74	126.6
19 June	24.9	23.1-25.0	61	
20 June	23.4	23.0-23.9	60	
21 June	23.9	23.0-24.8	60	
22 June	23.5	23.0-24.8	64	
23 June	24.4	23.1-25.5	61	
24 June	25.6	24.1-26.8	57	38.3
25 June	25.1	22.5-25.8	56	
26 June	23.8	22.5-24.7	126	
27 June	23.7	22.5-25.0	110	
28 June	21.8	21.8-24.8	79	
29 June	23.3	21.8-24.9	62	
30 June	24.0	22.5-25.3	53	
1 July	24.1	23.1-25.0	49	20.8
2 July	24.3	23.7-25.0	50	
3 July	24.1	21.4-25.2	58	
4 July	21.1	20.9-21.7	77	
5 July	22.0	21.0-22.2	177	
6 July	23.5	22.3-23.6	159	
7 July	23.7	23.4-24.5	101	

Table 7 (cont'd). Water temperatures and densities of glochidia in weekly drift samples at Slant, and daily mean discharge recorded at Cleveland (CRM 272), Clinch River Virginia, late May to early August, 1987.

Sample date	Daily median temperature (C)	Temperature range (C)	Daily mean discharge (m ³ /s)	Glochidial densities (no/100 m ³)
8 July	24.2	23.5-25.2	78	60.3
9 July	25.0	23.7-26.2	66	
10 July	24.3	23.6-25.2	66	
11 July	21.9	21.4-23.8	105	
12 July	21.9	21.8-22.3	112	
13 July	22.3	21.9-23.0	80	
14 July	22.3	21.5-23.0	97	
15 July	21.6	21.4-22.3	95	
16 July	21.9	21.1-23.3	70	3.9
17 July	22.6	21.2-24.3	60	
18 July	23.4	21.6-24.5	54	
19 July	24.0	22.3-25.2	48	
20 July	25.0	22.6-26.0	45	7.9
21 July	25.3	23.9-26.0	42	
22 July	26.0	24.2-26.7	39	
23 July	26.5	24.7-27.1	36	
24 July	26.7	25.4-27.9	35	
25 July	27.4	25.6-28.3	33	
26 July	27.5	25.1-28.2	31	
27 July	27.4	26.0-28.1	29	
28 July	27.0	25.9-28.5	29	
29 July	26.7	25.4-28.0	28	
30 July	26.2	25.2-26.8	27	
31 July	26.3	25.3-27.0	26	0.8
1 August	27.1	25.4-27.4	26	
2 August	27.1	25.9-28.0	24	
3 August	27.6	26.2-28.5	24	
4 August	28.0	26.8-29.0	30	
5 August	27.7	26.7-28.1	28	

¹Mid-day temperature (1100h)

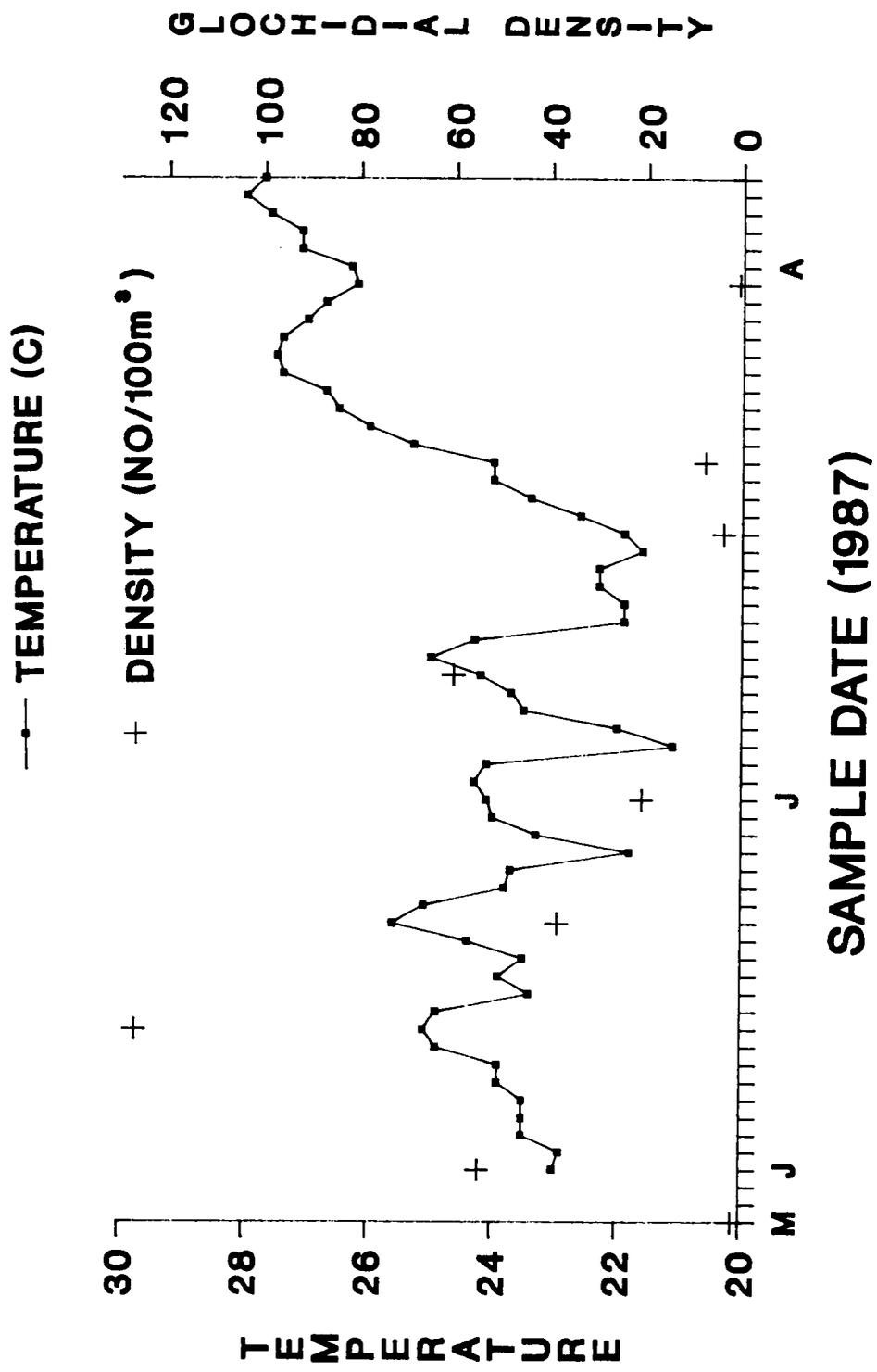


Figure 13. Densities of glochidia of the fine-rayed pigtoe in weekly drift samples and daily median temperatures in the Clinch River at Slant, late May through early August, 1987.

regression indicated that densities of the glochidia of *F. cuneolus* in stream drift in 1986 and 1987 were not significantly related to the date on which glochidia were sampled in stream drift, weekly median temperature prior to the sample date, or water velocity measured at the mouth of the drift net from which samples of glochidia were obtained ($p = 0.07$). However, multiple regression analyses of relationships between these variables revealed that in combination with sample date, weekly median temperature was positively correlated with densities of glochidia in stream drift ($p = 0.03$). No other combination of variables was significantly correlated with densities of the glochidia of *F. cuneolus* in stream drift ($p = 0.05$).

Diel sampling of stream drift from 16 to 17 July, 1987 showed that glochidia of the fine-rayed pigtoe were present in the water column during the entire 24 h period, at temperatures ranging from 21.2 to 23.0 C (Table 8). Highest densities of glochidia (118/100 m³) occurred in early morning (0700h), when water temperature was approximately 21.4 C.

Determination of Fish Hosts

From 26 June to 19 August, 1986 and from 17 June to 30 July, 1987, a total of 1,619 fish representing 39 species at Slant were electroshocked, preserved, and examined for glochidial attachment. Cyprinid species were examined exclusively in 1986, and glochidia of all species of mussels observed on these fishes were tabulated to determine the prevalence of infestation (Table 9). These data were inconclusive in indicating likely fish hosts for *F. cuneolus*, because no distinction was made between glochidia of the subfamilies of mussel species.

In 1987, 1,150 fishes representing 39 species were collected at Slant, from which gills were examined for glochidial attachment (Table 10). Thirteen (33%) of these fish species, all minnows, were infested with glochidia of the subfamily Ambleminae (Table 11). The highest prevalence of infestation of amblemine glochidia on cyprinids occurred on 17 June (17%) and 8 July (25%), dates corresponding to the highest densities of glochidia of *F. cuneolus* in stream drift (Table 12). Percentages of infested minnow species ranged from 0.4 to 45.8%. Minnow species with the highest

Table 8. Densities of glochidia of the fine-rayed pigtoe in drift samples collected at 4 h intervals at Slant, Virginia, 16 to 17 July, 1987.

	Time (h)	Temperature (C)	Water velocity (m/s)	Glochidial density (no./100 m ³)
16 July	1100	21.2	0.6	3.9
	1500	21.6	0.5	3.3
	1900	23.0	0.4	1.6
	2300	22.9	0.4	1.0
17 July	0300	22.0	0.4	1.0
	0700	21.4	0.4	118.0

Results

Table 9. Prevalence of glochidial infestations¹ on cyprinid species from the Clinch River at Slant, Virginia, 26 June to 19 August, 1986.

Common name	Scientific name	No. examined	No. infested	Percent infested
Bigeye chub	<i>Hybopsis amblops</i>	89	10	11
Mimic shiner	<i>Notropis volucellus</i>	36	17	47
Popeye shiner	<i>N. ariommus</i>	3	0	0
River chub	<i>Nocomis micropogon</i>	43	29	67
Sawfin shiner	<i>Notropis sp</i>	1	0	0
Silver shiner	<i>Notropis photogenis</i>	1	0	0
Stargazing minnow	<i>Phenacobius uranops</i>	20	0	0
Stoneroller	<i>Campostoma anomalum</i>	66	11	16
Streamline chub	<i>Hybopsis dissimilis</i>	6	0	0
Striped shiner	<i>Notropis chrysocephalus</i>	21	4	20
Telescope shiner	<i>Notropis telescopus</i>	26	5	20
Tennessee shiner	<i>Notropis leuciodus</i>	106	56	53
Warpaint shiner	<i>Notropis coccogenis</i>	10	3	30
Whitetail shiner	<i>Notropis galacturus</i>	1	1	100

¹Glochidia were not differentiated.

Table 10. Fish species collected in the Clinch River at Slant, Virginia, June through July, 1987.

Common name	Scientific name	No. collected
Whitetail shiner	<i>Notropis galacturus</i>	35
Warpaint shiner	<i>Notropis coccoensis</i>	23
Striped shiner	<i>Notropis chrysocephalus</i>	60
Popeye shiner	<i>Notropis ariommus</i>	31
Sawfin shiner	<i>Notropis sp.</i>	47
Mimic shiner	<i>Notropis volucellus</i>	176
Tennessee shiner	<i>Notropis leuciodus</i>	18
Spotfin shiner	<i>Notropis spilopterus</i>	13
Steelcolor shiner	<i>Notropis whipplei</i>	4
Telescope shiner	<i>Notropis telescopus</i>	11
Silver shiner	<i>Notropis photogenis</i>	7
Rosyface shiner	<i>Notropis rubellus</i>	2
Blotched chub	<i>Hybopsis insignis</i>	17
Streamline chub	<i>Hybopsis dissimilis</i>	37
Bigeye chub	<i>Hybopsis amblops</i>	284
Stoneroller	<i>Campostoma anomalum</i>	81
River chub	<i>Nocomis micropogon</i>	53
Stargazing minnow	<i>Phenacobius uranops</i>	13
Ohio logperch	<i>Percina caprodes</i>	13
Tangerine darter	<i>Percina aurantiaca</i>	6
Gilt darter	<i>Percina evides</i>	13
Dusky darter	<i>Percina sciera</i>	2
Banded darter	<i>Etheostoma zonale</i>	11
Greenside darter	<i>Etheostoma blennoides</i>	20
Redline darter	<i>Etheostoma rufilineatum</i>	26
Blueside darter	<i>Etheostoma jessiae</i>	3
Tippecanoe darter	<i>Etheostoma tippecanoe</i>	4
Tennessee snubnose darter	<i>Etheostoma simoterum</i>	5
Bluntnose minnow	<i>Pimephales notatus</i>	6
Redbreast sunfish	<i>Lepomis auritus</i>	19
Longear sunfish	<i>Lepomis megalotis</i>	21
Smallmouth bass	<i>Micropterus dolomeiui</i>	23
Rockbass	<i>Ambloplites rupestris</i>	8
Spotted bass	<i>Micropterus punctulatus</i>	3
Black redhorse	<i>Moxostoma duquesnei</i>	46
Shorthead redhorse	<i>Moxostoma macrolepidotum</i>	1
Northern hog sucker	<i>Hypentilium nigricans</i>	12
Mountain madtom	<i>Noturus eleutherus</i>	1
Gizzard shad	<i>Dorosoma cepedianum</i>	2
Total		1150

Table 11. Prevalence of glochidial infestations on cyprinid species from the Clinch River at Slant, Virginia, 17 June to 30 July, 1987.

Species	No. examined	Number infested		Percent infested	
		Amblemine	Other	Amblemine	Other
Bigeye chub	265	1	26	0.4	9.8
Mimic shiner	199	54	44	27.1	22.1
Stoneroller	88	7	5	8	5.7
Striped shiner	59	4	8	6.8	13.6
Sawfin shiner	48	22	12	45.8	25.0
Popeye shiner	46	1	2	2.2	4.3
River chub	45	14	3	31.1	6.7
Streamline chub	41	0	0	0.0	0.0
Whitetail shiner	33	13	8	39.4	24.2
Telescope shiner	31	2	1	6.7	3.2
Warpaint shiner	30	2	1	6.7	3.3
Tennessee shiner	20	6	2	30.0	10.0
Blotched chub	18	0	0	0.0	0.0
Spotfin shiner	13	4	2	30.8	15.4
Bluntnose minnow	8	1	0	12.5	0.0
Stargazing minnow	8	0	0	0.0	0.0
Silver shiner	7	0	0	0.0	0.0
Steelcolor shiner	4	0	2	0.0	5.0
Rosyface shiner	2	0	0	0.0	0.0
Total	965	131	116		

Table 12. Prevalence of infestations¹ of amblemine glochidia on cyprinids from the Clinch River at Slant, 17 June to 30 July, 1987.

Sample date	Number examined	Number infested	Percent infested
17 June	102	17	17
25 June	71	7	10
8 July	118	29	25
16 July	184	27	15
21 July	305	34	11
30 July	179	18	10
Total	959	122	

incidence of infestation were the sawfin shiner (*Notropis sp.*, 45.8%), whitetail shiner (*Notropis galacturus*, 39.4%), river chub (*Nocomis micropogon*, 31.1%), spotfin shiner (*Notropis spilopterus*, 30.8%), Tennessee shiner (*Notropis leuciodus*, 30%), and mimic shiner (*Notropis volucellus*, 27.1%). These fish species were identified as possible fish hosts for the glochidia of *F. cuneolus*. Amblemine glochidia were absent on non-cyprinid fish species (Table 13).

Glochidia identified as those of *F. cuneolus* were found encysted on the gills of four species of cyprinids; river chub, whitetail shiner, stoneroller, and spotfin shiner, on various sample dates in 1987. Of these four species, the river chub was most frequently infested both with glochidia and juveniles of the rayed pigtoe (Table 14, Figure 14). The valves of newly metamorphosed juveniles of the fine-rayed pigtoe were identical in size and shape to glochidia valves. To the untrained eye, a juvenile was difficult to differentiate from a sloughed glochidium unless movement occurred. Experience enabled me to recognize juveniles by their yellowish color and thickened ventral edges, even when valves were closed and no movement was observed. Juveniles on some hosts were easily removed from their cysts with a dissecting probe. They typically were observed on the distal portion of gills and occasionally in the gill cavities of some river chubs. Newly metamorphosed juveniles were observed on gills of naturally infested specimens of river chubs on each sample date between 8 and 30 July, 1987. Gills of spotfin shiners were also observed with juveniles of *F. cuneolus* on 21 and 30 July. Several whitetail shiners carried juveniles, but I could not determine whether they were *F. cuneolus* or *P. oviforme*.

The degree of infestation of glochidia (or juveniles) of *F. cuneolus* on individual fish specimens ranged from 1 to 26 (Table 15). Numbers of glochidia per fish were highest for the river chub (1-26 glochidia/fish) followed by the spotfin shiner (4-12), stoneroller (4-5), and whitetail shiner (1-4). The degree of infestation typically was low, and most infested fishes carried 1 to 5 glochidia per individual.

Results

Table 13. Prevalence of glochidial infestations on non-cyprinid fish species from the Clinch River at Slant, Virginia, 17 June to 30 July, 1987.

Species	No. examined	Number infested		Percent infested	
		Amblemine	Other	Amblemine	Other
Redline darter	26	0	1	0.0	3.8
Greenside darter	23	0	0	0.0	0.0
Gilt darter	12	0	0	0.0	0.0
Banded darter	12	0	0	0.0	0.0
Ohio logperch	13	0	0	0.0	0.0
Redbreast sunfish	20	0	0	0.0	0.0
Longear sunfish	21	0	0	0.0	0.0
Rockbass	15	0	0	0.0	0.0
Smallmouth bass	23	0	2	0.0	8.7
Black redhorse	46	0	0	0.0	0.0
Northern hogsucker	12	0	0	0.0	0.0

Table 13 (cont'd). Prevalence of glochidial infestations on non-cyprinid fish species from the Clinch River at Slant, Virginia, 17 June to 30 July, 1987.

Species	No. examined	Number infested		Percent infested	
		Amblemine	Other	Amblemine	Other
Blueside darter	3	0	0	0.0	0.0
Dusky darter	2	0	0	0.0	0.0
Tangerine darter	6	0	0	0.0	0.0
Tennessee snubnose darter	5	0	0	0.0	0.0
Tippecanoe darter	4	0	0	0.0	0.0
Spotted bass	3	0	1	0.0	33.0
Shorthead redhorse	1	0	0	0.0	0.0
Mountain madtom	1	0	0	0.0	0.0
Gizzard shad	2	0	0	0.0	0.0
Total	350	0	4		

Table 14. Prevalence of infestation (number examined, percent infested) of amblymeine glochidia on cyprinid species from the Clinch River at Slant, 17 June to 30 July, 1987.

	June			July			30			
	17	25	8	16	21	21	No.	%	No.	%
Bigeye chub	No. 20 % 0	No. 16 % 0	No. 26 % 0	No. 48 % 0	No. 124 % 0.8	No. 124 % 0.8	No. 31	% 0	No. 31	% 0
Mimic shiner	No. 15 % 33	No. 9 % 11	No. 39 % 49	No. 41 % 24	No. 37 % 43	No. 37 % 43	No. 58	% 5	No. 58	% 5
Stoneroller	No. 14 ¹ % 14	No. 4 % 0	No. 13 % 8	No. 18 % 22	No. 33 % 0	No. 33 % 0	No. 6	% 0	No. 6	% 0
Striped shiner	No. 11 % 0	No. 6 % 0	No. 9 % 11	No. 24 % 13	No. 5 % 0	No. 5 % 0	No. 4	% 0	No. 4	% 0
Sawfin shiner	No. 9 % 44	No. 2 % 50	No. 4 % 50	No. 12 % 33	No. 8 % 75	No. 8 % 75	No. 13	% 38	No. 13	% 38
Popeye shiner	No. 6 % 0	No. 6 % 17	No. 6 % 0	No. 4 % 0	No. 7 % 0	No. 7 % 0	No. 17	% 0	No. 17	% 0
River chub	No. 1 ¹ % 100	No. 7 ¹ % 29	No. 1 ² % 100	No. 5 ² % 80	No. 23 ²³ % 17	No. 23 ²³ % 17	No. 8 ²	% 25	No. 8 ²	% 25
Streamline chub	No. 9 % 11	No. - % -	No. 3 % 0	No. 15 % 0	No. 7 % 0	No. 7 % 0	No. 7	% 0	No. 7	% 0
Whitetail shiner	No. 8 ¹ % 38	No. 1 % 0	No. 4 % 50	No. 8 % 25	No. 3 % 33	No. 3 % 33	No. 9 ¹	% 55	No. 9 ¹	% 55
Telescope shiner	No. - % -	No. 7 % 0	No. 2 % 50	No. - % -	No. 18 % 5	No. 18 % 5	No. 4	% 0	No. 4	% 0
Warpaint shiner	No. 1 % 0	No. - % 0	No. 4 % 3	No. 0 % 8	No. 25 % 7	No. 25 % 7	No. 0	% 0	No. 0	% 0

Table 14 (cont'd). Prevalence of infestation (number examined, percent infested) of amblymeine glochidia on cyprinid species from the Clinch River at Slant, 17 June to 30 July, 1987.

	June			July			Total				
	17	25	8	16	21	30	No.	%	No.	%	
	No.	No.	No.	No.	No.	No.					
Tennessee shiner	3	5	2	-	7	3	33	20	50	29	33
Blotched chub	1	-	-	-	13	4	0	-	13	0	0
Spotfin shiner	1	5	3	-	2 ³	2 ³	0	-	2 ³	50	100
Bluntnose minnow	-	1	2	5	-	-	0	50	0	0	0
Stargazing minnow	1	1	1	3	4	3	0	0	4	0	0
Silver shiner	-	-	1	2	2	2	0	0	2	0	0
Steelcolor shiner	1	-	-	3	-	-	0	-	3	-	-
Rosyface shiner	-	1	-	-	1	-	0	-	1	0	-
Total	101	71	120	188	319	171					

¹Glochidia of the fine-rayed pigtoe observed on gills of one or more specimens

²Newly metamorphosed juveniles of the fine-rayed pigtoe observed on gills of one or more specimens

³Partially metamorphosed glochidia of the fine-rayed pigtoe observed on gills of one or more specimens

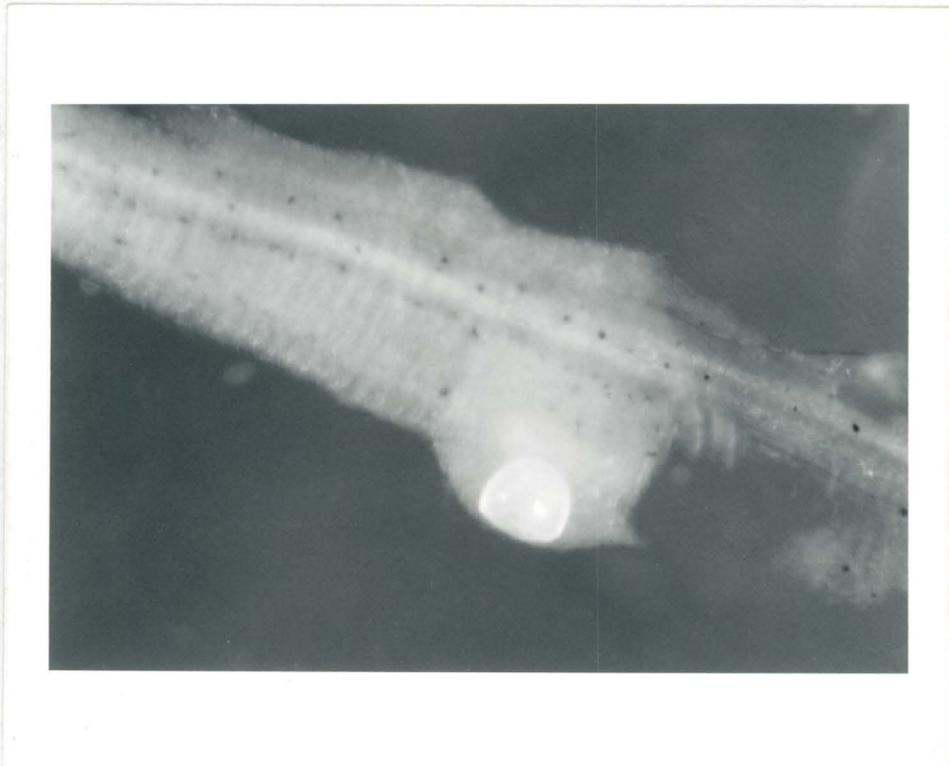


Figure 14. Photomicrograph (20X) of a glochidium of the fine-rayed pigtoe encysted on the gill filament of a river chub.

Table 15. Degree of infestation of glochidia of the fine-rayed pigtoe on fishes collected from the Clinch River at Slant, Virginia, 1987. Number in column represents number of fish examined.

Fish species	No. of glochidia per fish			
	1-5	6-10	11-20	> 20
Whitetail shiner	6	-	-	-
Stoneroller	2	1	-	-
River chub	7	3	1	1
Spotfin shiner	2	-	1	-
Total	17	4	2	1

Induced Infestations in the Laboratory

Glochidia of *F. cuneolus* were sloughed from the gills of 8 of 16 fish species that were infested in the laboratory, at temperatures between 21 and 26 C (Table 16). Seven of the eight species which did not serve as hosts were non-cyprinids, and glochidia usually were sloughed from fish within 1 to 5 days. However, the fantail darter (*Etheostoma fabellare*) retained glochidia for 9 days, and the goldfish (*Carassius auratus*) for 6 days. The goldfish was the only cyprinid tested that did not serve as a host for the fine-rayed pigtoe.

Seven species of the Cyprinidae, and one species of sculpin (*Cottus bairdi*) were confirmed as fish hosts for the glochidia of the fine-rayed pigtoe (Table 17). The period of metamorphosis ranged from 10 to 19 days at mean water temperatures between 22.5 and 25.0 C. The greatest number of juveniles (760) was obtained in trials with whitetail shiners, and most of these juveniles were collected 16 days post-infestation. Collection of high numbers of juveniles was likely a result of the number of fish infested and may not be indicative of the relative suitability of individual species as fish hosts. The number of days post-infestation when the greatest numbers of juveniles were collected in siphonate varied among species from 11 to 16 days, and based on 1987 data, appeared to be correlated with water temperatures. At mean water temperatures of roughly 23 C, most juveniles were collected 15 or 16 days post-infestation. Peak numbers of juveniles were collected from river chubs on day 11 at 24 C, and from mottled sculpins on day 13 at 25 C. Juveniles collected during experiments with mottled sculpins, 1 to 2 days after fish had been sacrificed, exhibited growth beyond the glochidial valve margins (Figure 15).

Validation of Annulus Formation

Seven specimens of *F. cuneolus* that were marked with disc tags and notched on 10 October, 1986 at Slant, were recovered on 18 June, 1987 and examined externally for annulus deposition.

Table 16. Fish species that did not serve as hosts for the glochidia of the fine-rayed pigtoe in laboratory experiments.

Infested species	No. infested	Temperature range (C)	Last day of observed attachment
Centrarchidae			
Bluegill	4	21.0-24.0	5
Redbreast sunfish	1	20.0-25.0	5
Rockbass	5	24.0-26.0	2
Cyprinidae			
Goldfish	10	24.0-25.0	6
Ictaluridae			
Channel catfish	6	24.0	1
Margined madtom	4	24.0	1
Percidae			
Fantail darter	7	24.0-26.0	9
Redline darter	10	24.0-26.0	4

Table 17. Fish species that served as hosts for the glochidia of the fine-rayed pigtoe in laboratory experiments.

Infested species	No. infested	Period of metamorphosis(d)	Peak day of excystment	Temperature range(C)	No. juveniles recovered
Cyprinidae					
Fathead minnow	10	12-16	15	21.0-26.0	76
River chub	2 ¹	11-19	11	22.0-25.5	50
Stoneroller	3	12-16	15	21.0-26.0	537
Telescope shiner	8	10-16	15	22.0-23.5	101
Tennessee shiner	9 ²	13-14	-	22.0-23.0	2
White shiner	6	10-18	16	21.0-26.0	424
Whitetail shiner	10	12-16	16	22.0-23.5	760
Cottidae					
Mottled sculpin	7	12-14	13	24.0-26.0	84

¹Experiment performed in 1986²All died from disease prior to completion of experiment

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Recovered specimens ($n = 7$) were relatively large (66 to 80 mm) and slow-growing. Growth checks near the ventral margin of these individuals were tightly spaced, and therefore I was unable to discern annulus deposition externally from the previous year. I recovered one freshly dead specimen of 77 mm in length which was taken to the laboratory and thin-sectioned to examine internal annuli. I compared the thin-section with external shell checks, but the umbo of this old specimen was badly eroded, and incremental growth near the ventral margin was negligible. I am unable to state with confidence that a growth check had formed since time of notching the previous year. Therefore, I used the conclusions of Neves and Moyer (1988) who demonstrated annual growth line formation for three subfamilies of Unionidae in the Tennessee River drainage; *Pleurobema oviforme* (Ambleminae), *Lasmigona subviridis* (Anodontinae), and *Medionidus conradicus* (Lampsilinae).

By referring to Neves and Moyer's (1988) descriptions and with experience examining thin-sections, the contrast between true and false annuli was apparent. I observed two types of false annuli; those which were generally thinner and less consistent on the surface of valves that had no counterpart internally, and those which were very dark and could not be followed to the shell margin. Most true annuli could be traced continuously from the umbo to shell margin, which was useful in confirming contiguity of internal and external growth lines. Thus, true annuli could be distinguished from false annuli on the shell periostracum.

Age and Growth

Growth Characteristics

The pattern of growth in shell length of *F. cuneolus* from Pendleton Island and Slant was clearly asymptotic (Figure 16). Mean annual growth in length at Slant averaged 5.0 mm/yr before age 6, 3.7 mm/yr between ages 6 and 16, and 2.1 mm/yr from ages 16 to 21 yr (Appendix A). Mean annual growth slowed to approximately 1 mm/yr beyond this age (Table 18). The modified version of the von Bertalanffy equation to describe growth of *F. cuneolus* at Pendleton Island and Slant is as follows:

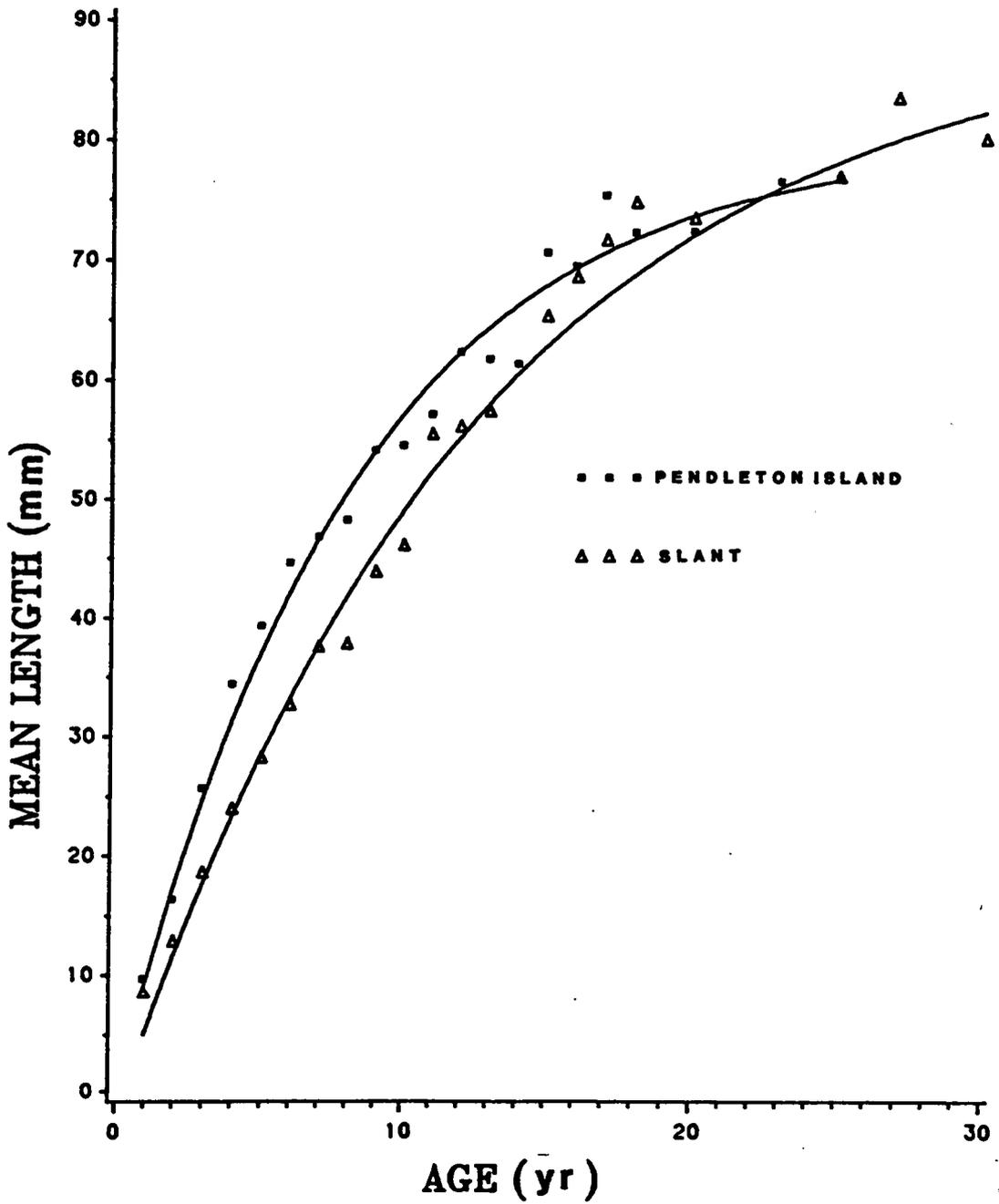


Figure 16. Growth in length of the fine-rayed pigtoe at Pendleton Island and Slant, as predicted by the von Bertalanffy growth equation.

Table 18. Mean annual growth in length (mm/yr) of the fine-rayed pigtoe in the Clinch River, Virginia.

<u>Age interval (yr)</u>	<u>Growth increment (mm)</u>	
	<u>Slant</u>	<u>Pendleton Island</u>
1-5	4.9	7.4
6-10	3.6	3.0
11-15	3.8	3.2
16-20	2.1	0.4
21-25	0.8	0.9
26-30	1.1	.

$$\text{Pendleton Island } L_{(t)} = (10.4/0.13)(1 - e^{-0.13(t-0.05)})$$

$$\text{Slant } L_{(t)} = (7.3/0.08)(1 - e^{-0.08(t-0.03)})$$

Mean growth in length of *F. cuneolus* during the first five years at Pendleton Island (7.4 mm/yr) was greater than that recorded at Slant (Appendix B). Thereafter, mean annual growth of the demes at Pendleton Island and Slant were similar, averaging 3.1 mm/yr between 6 and 15 yr of age and slowing to less than 1 mm/yr after age 16 (Table 18). However, confidence intervals around estimates of w , which is a measure of rate of increase in shell length near t_0 , indicated that growth in length was different between the two sites. Estimates of k , the rate at which shell length approaches L_∞ also differed substantially, lending support that the mean rate of increase in shell length of the fine-rayed pigtoe at Pendleton Island is more rapid than growth in length of mussels in the deme at Slant (Table 19).

Age Class Structure

Age class structure of live specimens of *F. cuneolus* from Pendleton Island and Slant is presented in Figures 17 and 18. Individuals at Pendleton Island ($n = 264$) and at Slant ($n = 43$) ranged from 6 to 32 years, and based on collections of live specimens, individuals 17 to 20 yr old were the dominant age groups at both sites. The deme at Slant consisted of fewer old specimens when compared to Pendleton Island; only one individual greater than 24 yr of age was found (Figure 18). It is unlikely that the smaller sample size at Slant accounted for this difference, since large specimens are typically found more readily than smaller individuals. Young specimens were rarely found at either site. The youngest live specimen sampled by snorkeling and handpicking was approximately 9 yr old.

Although live mussels and shells of freshly dead individuals less than age 10 were rare at both sampling sites, several 6 yr old specimens were collected in muskrat middens at Slant. The greater

Table 19. Von Bertalanffy parameters describing growth of the fine-rayed pigtoe at Pendleton Island and Slant, Clinch River, Virginia. Ninety-five percent confidence intervals are given in parentheses.

Site	Parameters			L_{∞} (mm)
	w (mm/yr)	k (yr ⁻¹)	t_0	
Pendleton Island	10.4	0.13	0.05	78.4
	(9.3-11.5)	(0.11-0.16)	*	
Slant	7.3	0.08	0.30	91.5
	(7.1-7.5)	*	(0.04-0.56)	

*95% of all estimates obtainable will equal this value

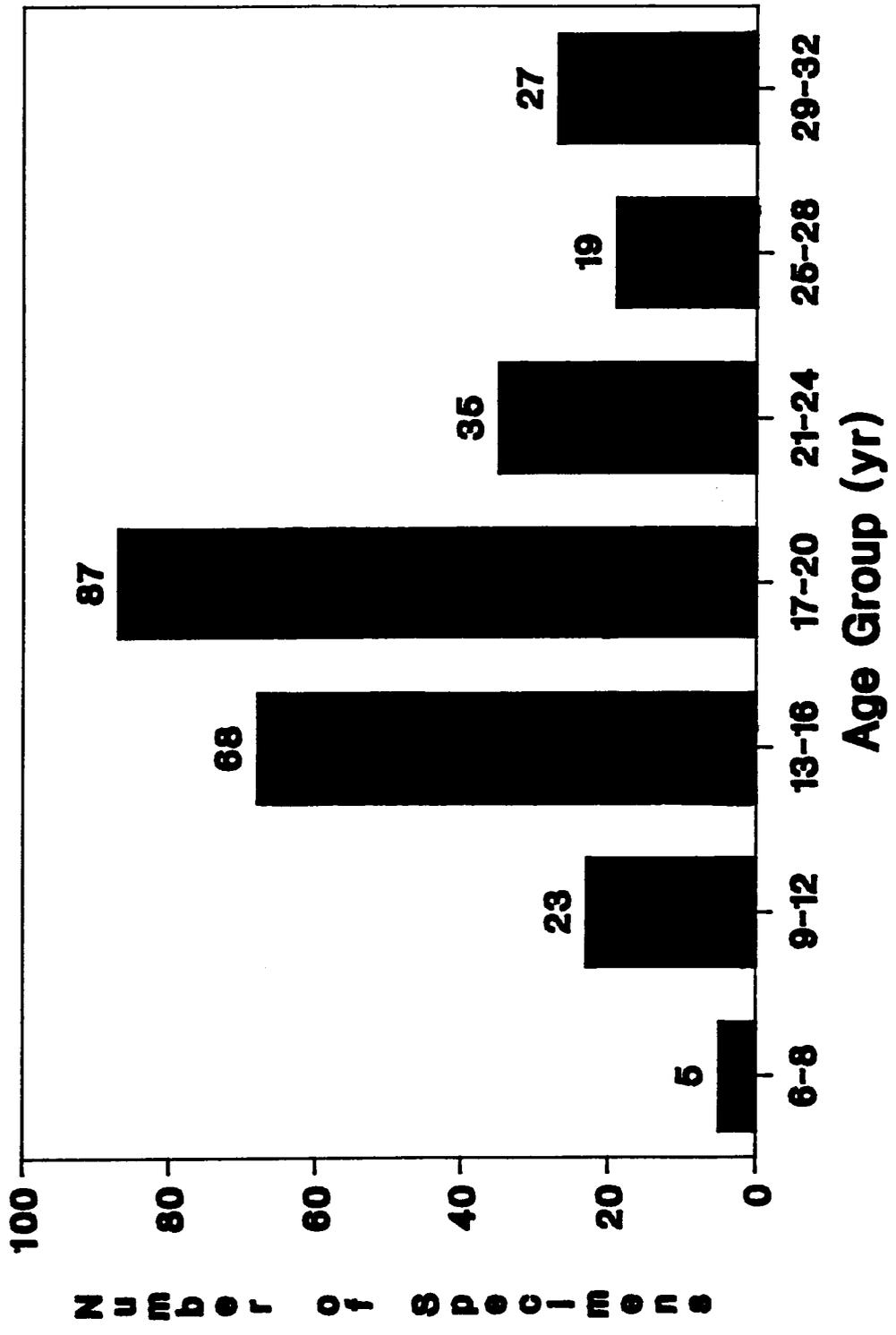


Figure 17. Age class structure of fine-rayed pigtoes collected alive in the Clinch River at Pendleton Island, 1987.

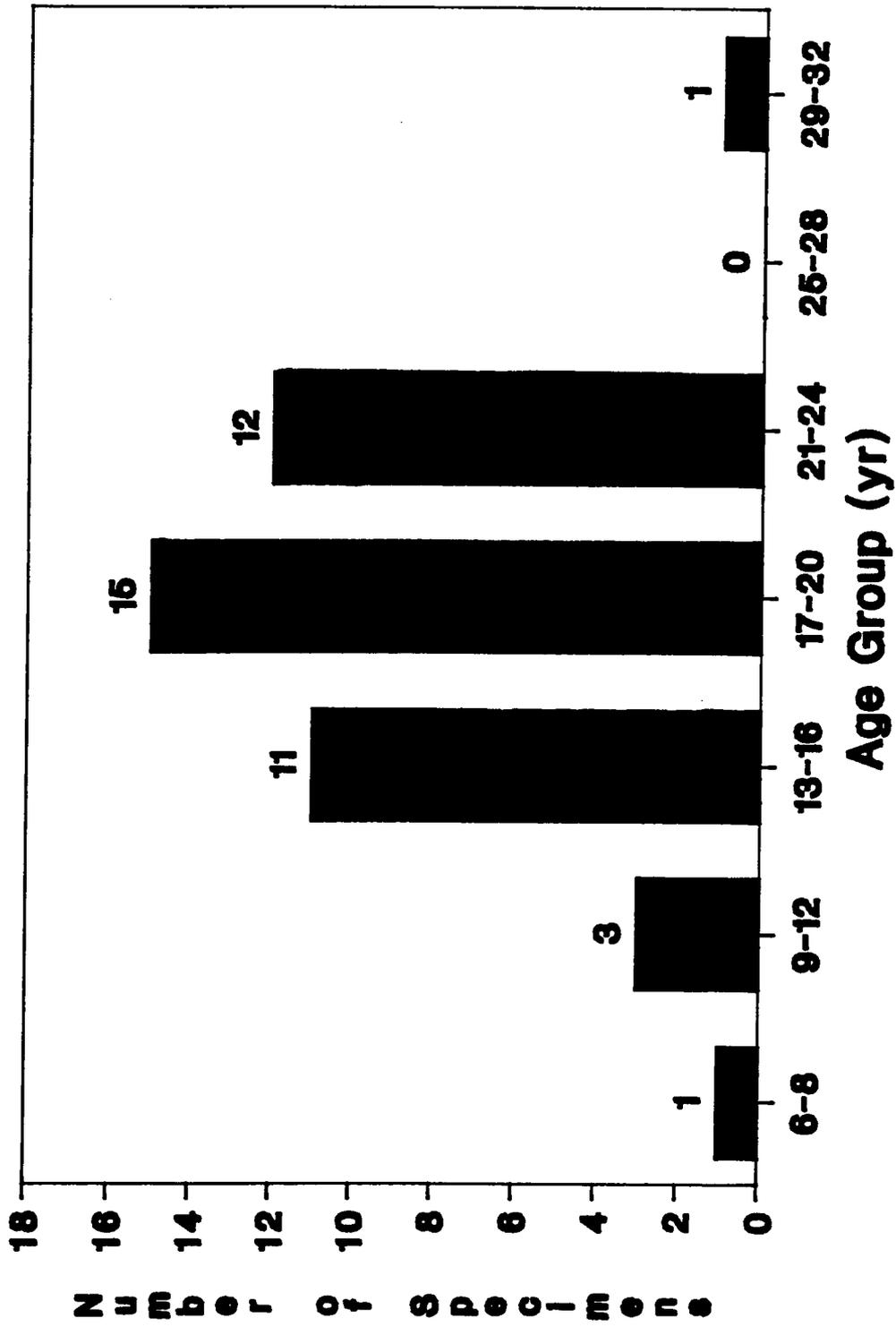


Figure 18. Age class structure of fine-rayed pigtoes collected alive in the Clinch River at Slant, 1987.

occurrence of younger specimens of the fine-rayed pigtoe in muskrat middens, when compared to live collections by handpicking at this site, suggested that sampling by the latter method may have been biased for larger adults (Figure 19). Based on my collections of live specimens, age class structure was dominated by individuals 17 to 20 yr old. However, individuals 13 to 16 yr old were the dominate age group as judged by shells collected in muskrat middens. The percentage of younger specimens (22.4%, < age 10) in muskrat middens was significantly greater than the percentage of live specimens (3.5%) of the same age group collected by snorkeling and handpicking (Table 20). Similarly, a significantly greater number of old individuals were represented in collections of live specimens (19%, ages 21-30) ($p=0.0001$).

Estimation of Density

Only one specimen of *F. cuneolus* was collected in fifteen 0.5m² quadrat samples taken within an area of suitable habitat at Slant, totaling 3,443 m². None were found within fifteen 0.5 m² quadrat samples taken within 1,391 m² of marginal habitat. Based on the implemented sampling scheme, mean density of *F. cuneolus* was estimated to be 1 adult mussel per 30 m². Extrapolating to the total area (4,834 m²) of both suitable and marginal habitat within the 325 m x 50 m study area, the number of *F. cuneolus* at Slant was roughly estimated to be 161 individuals.

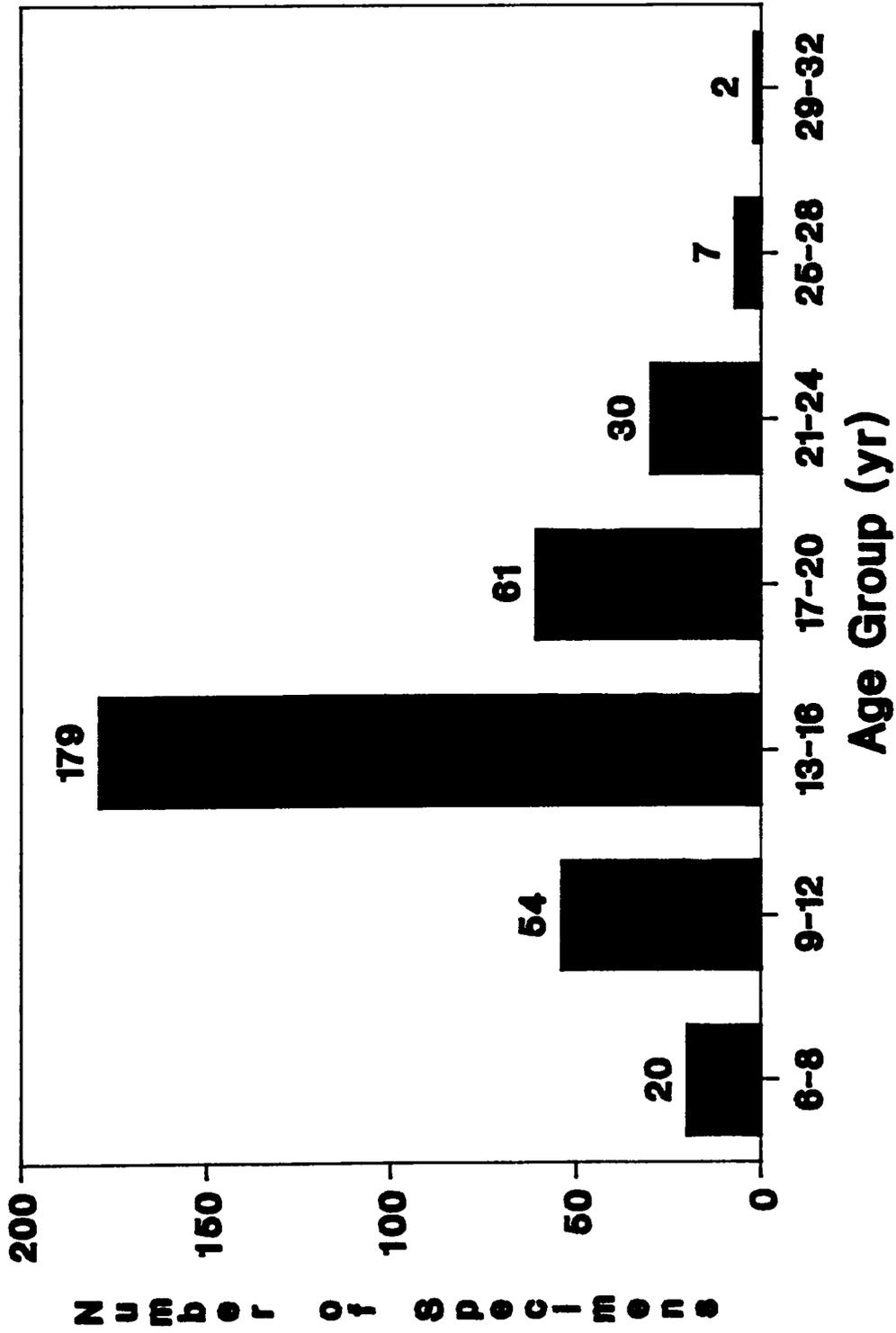


Figure 19. Age class structure of fine-rayed pigtoes collected in muskrat middens in the Clinch River at Slant, 1984-85.

Table 20. Comparison¹ of age class structure of collections of live specimens versus freshly-dead valves of the fine-rayed pigtoe in muskrat middens from the Clinch River, Virginia.

Age Class	Midden Shells		Live Specimens	
	No.	Percent	No.	Percent
< 10	79	22.4	8	3.5
11-20	263	74.5	176	77.5
21-30	11	3.1	43	19.0
Total	353	100	227	100

¹RXC test of independence using the G-test with Williams correction, $X^2 = 76.6$, 2df, significant at $p = 0.0001$.

Discussion

Reproductive Biology

Fusconaia cuneolus is a short-term brooder that is gravid from mid-May to early August in the Clinch River. Numbers of gravid females peak in early to mid-June. This is in agreement with Ortmann's (1921) records of gravidity for *F. cuneolus* in the Tennessee River drainage. He collected gravid specimens from 16 May through 13 July in the early 1900's. The chronology of the reproductive cycle of *F. cuneolus* appears similar to that of other short-term brooders in the Tennessee River drainage. Yokley (1972) observed embryos and larvae in the gills of the Ohio pigtoe, *Pleurobema cordatum*, from late April through early July, and determined that June is the peak month for glochidial maturation of this species in the Tennessee River. In the Clinch River, females of the rough rabbitsfoot, *Quadrula cylindrica strigillata*, also began incubating fertilized eggs in mid-May and were spent by late July; the percentage of gravid females peaked in late May and then gradually declined through June (Yeager and Neves, 1986). In Big Moccasin Creek in southwest Virginia, the Tennessee clubshell, *Pleurobema oviforme*, broods larvae from May through July (Weaver, 1981).

The fine-rayed pigtoe appears to begin incubating eggs when water temperatures range from 16 to 21 C in the Clinch River. Although data were not collected at the beginning of the repro-

ductive period, it appears that reproductive activities were later in 1986, when compared to 1987. The peak in gravidity, and the second peak release of glochidia in 1986, occurred about one week later than that determined for 1987. Additionally, glochidia were collected later into the season (13 August) and at greater densities (3.9 glochidia/100m³) in 1986 than those collected in 1987 (30 July, 0.8 glochidia/100m³). Temperature often has been considered to be the chief determinant of seasonal variations in the timing of reproduction by tachytictic mussel species (Coker et al., 1921). Yokley (1972) reported that brooding periods of the Ohio pigtoe differed between years, depending on river temperatures, and that a minimum of 20 C was required for the continued development of glochidia. Coker et al. (1921) stated that, in general, short-term brooders initiate reproductive activities with rising temperatures, and that spawning seasons of mussels differ slightly among years because maturation of eggs is affected by variable water temperatures. Other workers also have identified temperature as the most important exogenous factor influencing the reproductive cycle (Matteson, 1948; Smith, 1976; Kitchel, 1985).

The importance of water temperature on reproductive cycles cannot be disputed. However, there is evidence in studies of fish and other aquatic organisms that a combination of environmental stimuli may influence otherwise genetically fixed reproductive periods in temperate regions (Cushing, 1970). Seasonal change in temperature is consistently mentioned as one of the most important environmental cues triggering spawning, but additional factors, particularly photoperiod, stream discharge and food availability, may also have profound effects on reproductive activities (Harrington, 1957; Aiken, 1969; Hynes, 1970; Huh et al., 1976; Doherty et al., 1987). It is commonly accepted that a combination of both temperature and photoperiod is essential for the proper development of gonadal material and reproduction of fish, stream insects, and other aquatic organisms (Marshall, 1966; Aiken, 1969; Hynes, 1970; Huh et al., 1976). Potentially synergistic or confounding effects of two or more environmental factors on reproductive cycles of organisms are often overlooked (Bye, 1984). For example, Aiken (1969) effectively demonstrated, in laboratory experiments with crayfish, that a specific combination of photoperiod and temperature was necessary for ovarian growth and maturation, with egg-laying being triggered by increasing temperatures in spring. Based on photoperiod information for 1984, provided by Doherty et al. (1987) for the

southwestern Virginia region, reproductive activities of the fine-rayed pigtoe probably occur when daylight hours are approximately 13.5 to 14 h in length (mid-April to early August). Differences in water temperatures between years, relative to change in daylength, may account for the later spawning in 1986 and the observed variation in brooding periods of fine-rayed pigtoes. Based on STORET data, spring temperatures in 1986 were somewhat higher than those in 1987, but data were recorded inconsistently for April and most months between years. Daily temperature data at Slant were not obtained in 1986, precluding a valid comparison between years of water temperatures and degree days during the brooding period. Matteson (1948) reported that the short-term brooder, *Elliptio complanata*, reached advanced stages of gametogenesis in the fall and winter, and that mature gametes were maintained in acini until spring, when environmental conditions were favorable. Jirka (1986) reported similar results for three tachytictic species in the New River in Virginia. Many warmwater species breed successfully only in areas where temperatures remain high for a long enough period of time to allow spawning and early development (Hynes, 1970). Sastry (1979) performed radiotracer experiments with scallops (*Argopecten irradians*) and reported that nutrients stored in the digestive glands of mussels were mobilized and transferred to gonads after 1 wk at 15 C. However, at a subthreshold temperature of 5 C, uptake of nutrients into gonads was less than the uptake by any other bodily component, demonstrating the relative importance of temperature on reproductive activity. Based on mean weekly values, water temperatures at Slant appeared to be generally higher in 1986 than in 1987. Additionally, above normal temperatures, and below normal precipitation during spring and summer, 1986, were reported for southwestern Virginia and for most of the southeastern United States (Virginia Agricultural Statistics Service, 1987). It seems likely that degree days is an important determinant for spawning and other aspects of the reproductive cycle for the fine-rayed pigtoe, but experiments to control other exogenous factors are needed to document the effect of temperature on the brooding period.

Intuitively, it seems that higher temperatures in spring, 1986, would have triggered mussels to spawn earlier that year. However, stream flow and food availability also may have affected timing of reproductive activities between years. A seasonal rise in discharge and subsequent increase in nutrient levels is thought to be a signal for movement and reproductive activity for many aquatic

organisms (Hynes, 1970). Rapidly increasing discharge in upper reaches of streams is known to increase the particulate organic load downstream (Webster et al., 1979). Doherty et al. (1987) hypothesized that the Asiatic clam, *Corbicula fluminea*, became reproductively active when both suitable temperatures and food resources were available. Initiation of spawning following floods has been reported for certain mussel species (Tudorancea, 1969; Jones et al., 1986). Jones et al. (1986) determined that spawning by populations of *Cucumerunio novaehollandiae*, in New South Wales, was associated with the occurrence of floods. They speculated that the initiation of breeding was a result of a drop in water temperature. The potential influence of the rise in discharge and increased food supply was not considered. Considerable energy is required for growth and development of gonadal material in all mussels (Mackie, 1984). Energy can be derived from stored reserves and food ingested directly from the aquatic environment, but the manner in which this energy is derived varies among species of bivalves (Sastry, 1979). For some species, gonad growth and development is dependent upon direct ingestion of food during the period of gonad development, but in other species, this seasonal development is associated with the storage and utilization of reserves accumulated in the body during periods of maximum food densities (Sastry, 1979).

From January through July, rates of stream discharge recorded in the Clinch River were lower in 1986 than in 1987, for all months except February and May. Mean monthly discharge was substantially lower in April in 1986 (83 m³) than in 1987 (1041 m³). A reduction in stream flow, and hence, availability of food, may account for some of the variation in brooding periods between 1986 and 1987. However, without information regarding seasonal differences in food resources, and seasonal formation and development of gametes for the fine-rayed pigtoe, the effect of stream discharge and food availability on the reproductive cycle of this species is unknown.

I was unable to determine the length of time required for fertilized eggs to develop into mature glochidia in *F. cuneolus* because specimens could not be sacrificed for histological examination. However, I determined that it was possible to follow the development of embryos by sequentially recording colors of the marsupia of individual females. Individuals with bright pink marsupia contained early embryos, and a light peach color indicated that glochidia were fully mature, free from their embryonic membrane and ready for release. The absence of vitelline membranes is indicative

of mature stages of glochidia (Matteson, 1948; Jones et al., 1986). The observed transition in color of marsupia of *F. cuneolus* agrees with observations of Ortmann (1921), who reported that marsupia of females of *F. cuneolus* varied from pink to bright crimson and, in some cases, a pinkish-orange color. Yeager and Neves (1986) were able to determine various developmental stages of eggs of *Quadrula cylindrica* by noting the color of the marsupia of females. They found that embryos were light yellow until the gastrula stage, then assumed a light peach to reddish-brown color when development to glochidia occurred. It is likely that developmental changes in color occur in other mussel species as well, which may provide a useful means of determining when mature glochidia are present and ready for release. This knowledge is particularly important in studies of endangered species, because mature glochidia are difficult to obtain for fish host research. Additionally, use of color to identify mature glochidia may eliminate wanton sacrifice of specimens in biological studies of other mussel species.

Assuming that coloration is a valid indicator of developmental stages of glochidia within marsupial gills, development of fertilized eggs into glochidia requires approximately two weeks in female *F. cuneolus*. Lefevre and Curtis (1912) and Coker et al. (1921) stated that the time required for development of the egg into a glochidium was only a few weeks. A development time of roughly 2 weeks is similar to that reported for other summer brooders as well (Matteson, 1948; Yokley, 1972; Weaver, 1981; Kitchel, 1985).

Because early embryos are found within female marsupia shortly after spawning (Coker et al., 1921; Zale and Neves, 1982a), and brooding periods of other short-term brooders correspond closely to that of *F. cuneolus*, it appears that fine-rayed pigtoes spawn in late April and May. Weaver (1981) used histological preparations to determine that embryos develop into glochidia over a 3 to 5 week period in the short-term brooder, *P. oviforme*, following fertilization in late March through May. Spawning periods for other mussel species have been determined histologically. Jirka (1986) reported that fertilization of eggs occurred in March through July for the spike, *Elliptio dilatata*. *Q. cylindrica*, the rough rabbitsfoot, begins spawning in mid-May and continues into June (Yeager and Neves, 1986). Yokley (1972) determined that *P. cordatum* spawns in late April and early May, and Matteson (1948) reported that fertilization in *Elliptio complanatus* normally occurs

from late April to late May. It appears that both the periods of spawning and embryonic development for the fine-rayed pigtoe are similar in duration to those of other short-term brooders previously studied.

All four gills of *F. cuneolus* contained glochidia, which is characteristic of the genus *Fusconaia* (Ortmann, 1921; Kitchel, 1985). Ortmann (1921) reported that the marsupial part of the inner gill is often restricted to the middle portion, in young specimens of *F. cuneolus*, but I did not observe this difference. In the laboratory, it appeared that females of the fine-rayed pigtoe released gelatinous strings of glochidia through both incurrent and excurrent siphons and along ventral margins of gills. However, reduced water velocities may have resulted in accumulation of glochidia along the perimeter of valves, creating the impression that glochidia were released from apertures other than siphons. Short-term brooders typically release glochidia only through the excurrent siphon (Lefevre and Curtis, 1912; Matteson, 1948; Jones et al., 1986), and it would be reasonable to assume that the same is true for *F. cuneolus*. However, additional observations are necessary to ascertain alternative modes of release by the fine-rayed pigtoe.

In the laboratory, most females typically released all of their conglutinates within a period of one day, although some specimens released conglutinates over several days. I did not observe female fine-rayed pigtoes naturally releasing glochidia, but I did collect two specimens in the field with partially empty inner marsupial gills. Occurrence of the "bubbly" appearance of the gills of spent females (see p. 35) probably meant that glochidia had been released earlier that day. Jones et al. (1986) also described this condition in female mussels immediately following glochidial release. It may be that glochidia retained in the inner gills of the fine-rayed pigtoe are released first, followed by release from the outer gills. Matteson (1955) reported that unionids in streams typically expelled glochidia over a 1 to 3 day period, and Kitchel (1985) observed females of *Fusconaia cor* to release glochidia over a period of 3 to 7 days in the laboratory. It is likely that this time period is influenced by water temperatures, but as discussed previously, various environmental factors influence the reproductive cycle. Levels of dissolved oxygen may also affect the time required for both the development and expulsion of glochidia (Lefevre and Curtis, 1912; Fisher and Tevez, 1976). Without

experimentation in the laboratory to control exogenous factors, the time required to complete glochidial release is inconclusive.

My collection of specimens of fine-rayed pigtoes with partially empty inner gills raises an important question about the utility of identifying species based only on charged gills. Lefevre and Curtis (1912) also questioned the consistency of this trait, particularly in species of the genus *Quadrula*. They observed that individuals of this genus frequently aborted embryos and glochidia when removed from the river, and they speculated that this behavior probably accounted for conflicting descriptions of the marsupium in this genus. In the Clinch River, Yeager and Neves (1986) observed a high percentage (65%) of female *Q. cylindrica* with glochidia in the outer gills only. Whether variation in number of charged gills results from the manner in which glochidia are released or from abortion of conglutinates from handling is unknown. Regardless, caution should be used if species are identified solely by this character.

Female fine-rayed pigtoes with pink marsupia, which were transported to the laboratory, aborted conglutinates containing embryos in various developmental stages. Females with peach-colored marsupia consistently released conglutinates containing mature glochidia, or loose aggregates of mature glochidia held together by a gelatinous matrix. A small percentage of unfertilized eggs was always present, but this is typical for short-term brooders (Lefevre and Curtis, 1912). Yokley (1972) observed many stages of glochidial development in a single aborted conglutinate and therefore assumed that female *P. cordatum* release glochidia gradually, upon maturation. Matteson (1948) also reported multiple stages in conglutinates of *E. complanatus*, but stressed that these observations were made on prematurely aborted conglutinates. He reasoned that embryos do not necessarily develop at the same rate; mature ones are held in the water tubes until others reach that stage. I also noted that female fine-rayed pigtoes appeared to hold both embryos and glochidia until all reached the glochidial stage. This was fortuitous, in that females could be left in the river until a peach-colored marsupia was observed, ensuring that maximum numbers of glochidia would be obtained for fish host experiments.

Estimates of the fecundity of freshwater mussels range from approximately 75,000 to 3 million embryos per female, depending upon species and size of female (Coker et al., 1921). I computed the

fecundity of *F. cuneolus* using only one preserved specimen, and the estimated 113,000 embryos per female was nearly identical to the estimated mean of 114,246 glochidia from individual females of *Q. cylindrica* (Yeager and Neves, 1986). Despite this agreement in estimates of fecundity, the computed value for the fine-rayed pigtoe should be considered a rough estimate at best. Fecundity may increase with length, weight, or age; considerable individual variation exists within a species; and annual differences may occur as a result of environmental effects, particularly the quality and quantity of diet (Blaxter, 1969).

My limited sampling at Pendleton Island in 1987 appeared to indicate that females outnumbered males. The sex ratio for the fine-rayed pigtoe was estimated to be 1 male for every 3 females. Sex ratios have been estimated previously for some short-term brooders in the Tennessee River drainage. Yokley (1972) and Yeager and Neves (1986) determined through histological studies that samples from populations of both *P. cordatum* and *Q. cylindrica* contained roughly equal sex ratios of males and females. In the North Fork Holston River in Virginia, female and male shiny pigtoes, *Fusconaia cor*, also occurred in equal proportions (Kitchel, 1985). Because sexual dimorphism is not evident in the shells of *F. cuneolus*, and because it is not feasible to sacrifice specimens for histological examination, an accurate sex ratio for *F. cuneolus* was not obtainable. Estimates were based upon the total number ($n = 8$) of gravid versus nongravid specimens randomly collected at Pendleton Island during the peak of gravidity, 10 June, 1987. Similarly, only two stockpiled females at Slant ($n = 2$) were used to determine the peak in gravidity. Additionally, sampling bias must be considered. It has been reported that post-spawning males of some freshwater mussel species will reburrow themselves, whereas gravid females will remain more exposed in the substrata prior to the release of glochidia (Ahlstedt, 1984). This may explain the greater number of female fine-rayed pigtoes in my estimated sex ratio. Because of this potential bias and the small sample sizes obtained for estimation, the 3:1 sex ratio is tenuous.

Differentiation of Glochidia

Dimensions of glochidia of *F. cuneolus* from the Clinch River were slightly larger than the length and height (0.16 mm) reported by Ortmann (1921) for *F. cuneolus* in the upper Tennessee River drainage. Kitchel (1985) also reported differences between measurements of glochidia of *F. cor* from the North Fork Holston River, Virginia, compared to those reported by Ortmann (1921). These discrepancies may be attributed to intraspecific variation between demes examined by each worker. However, it is more likely that the advancement in scientific instrumentation since the early 1900's enabled more accurate and precise measurements of glochidia in my study and that of Kitchel (1985).

The glochidia of the fine-rayed pigtoe were readily distinguishable, based solely on size and shape, from those of most species in stream drift at Slant. Although I performed statistical analyses to document differences in dimensions of glochidia among *F. cor*, *F. subrotunda* and *F. cuneolus*, I could readily differentiate between these species based on the significantly shorter hinge lengths and narrower widths of both *F. cor* and *F. subrotunda*. The general size and shape of the glochidia of *P. oviforme* were most similar to those of *F. cuneolus*, and differentiation between these two species was difficult initially. Intra-specific variation was evident, but not problematic, since dorsal hinge lengths of glochidia of the fine-rayed pigtoe were consistently greater than those of *P. oviforme*. This dimension seemed to be a reliable distinguishing characteristic. Jirka (1986) also used dorsal hinge lengths to differentiate glochidia. In the New River, Virginia, glochidia of *Actinonaias carinata* (*ligamentina*) were distinguishable from *Elliptio dilatata* based on their longer dorsal hinge. However, the use of a single dimension for identification of glochidia is not always sufficient. Kitchel (1985) was unable to distinguish glochidia of *F. cor* from those of *Lexingtonia dollabelloides* in the North Fork Holston River, based solely on one glochidial dimension. In some cases, considerable overlap in ranges of glochidial dimensions may preclude the use of morphometrics as a means of differentiation between mussel species. Scanning electron microscopy is an effective alternative method of differentiation, but it is a time consuming and costly technique (Calloway and Turner,

1978; Hoggarth, 1987). Concave valves also gave the glochidia of the fine-rayed pigtoe a more rotund appearance than those of *P. oviforme*. Thus, I felt that I could distinguish glochidia of these two species in drift samples without having to repeatedly measure specimens. Because of differences in the abundance of these species at Slant, the probability of collecting glochidia of *P. oviforme* in drift samples at Slant was low. Kitchel (1985) demonstrated that densities of glochidia of three mussel species in the North Fork Holston River approximated percentages of adults of each species in that mussel assemblage. As judged by the species composition of valves collected in muskrat middens in 1984 and 1985, *P. oviforme* comprised only 1% of the mussel assemblage at Slant. This is likely to be a maximum estimate of its abundance at this site, because this species is in a size range on which muskrats preferentially feed (Neves, 1985).

Release of Glochidia

The period of glochidial release for *F. cuneolus*, June through early August, is similar to that reported for other amblymeine species (Surber, 1912; Coker et al., 1921; Matteson, 1948; Yokley, 1972; Wiles, 1975; Yeager and Neves, 1986). Temperature has been mentioned most often as an important, if not chief factor affecting the release of glochidia (Lefevre and Curtis, 1912; Coker et al., 1921; Matteson, 1948; Yokley, 1972; Weaver, 1981; Kitchel, 1985). Yokley (1972) noted that glochidia of *P. cordatum* were most abundant in June each year, three to four weeks after the water temperature reached 21 C. Kitchel (1985) stated that glochidia of *F. cor* were most abundant in the water column in mid-July, after water temperatures reached 26 C, and that peaks were correlated with maximum water temperatures of 29 C. She reported maximum densities of glochidia of *Pleurobema oviforme* and *Lexingtonia dollabelloides* during mid-July in the same river. In Big Moccasin Creek, Virginia, however, Weaver (1981) reported a peak in release of glochidia for *P. oviforme* in June, when mean water temperature was approximately 24 C.

Previous studies seem to suggest that glochidia are released after water temperatures reach a threshold level, but this hypothesis has not been tested. Hynes (1970) discounted the develop-

mental threshold theory for stream insects, stating that change of day length, increased temperatures, and also increased discharge establishes emergence times for these organisms. My analysis documented that densities of glochidia of the fine-rayed pigtoe in the water column increase with increasing temperatures, but only when an additional variable (sample date) was included in a multivariate analysis. Temperature alone was not significantly correlated with glochidial release. This suggests that an interaction of more than one environmental factor affects the release of glochidia. Zale and Neves (1982a) reported that *Medionidus conradicus*, a long-term brooder, released glochidia throughout the year under a variety of environmental conditions, and were absent only in extremely cold weather (< 5 C). Khoo (1964) demonstrated that stoneflies could be stimulated to emerge with rising temperatures, but would also emerge from water at winter temperatures if exposed to long days. In my study, it is possible that the addition of the variable "sample date" was equivalent to the effect of photoperiod, and coupled with photoperiod, affected the release of glochidia of the fine-rayed pigtoe. However, photoperiod is probably most important relative to the entire annual cycle and the initiation of spawning. As discussed previously, daylight hours are probably maintained between 13.5 and 14 h during the entire reproductive period, and would vary little during that time.

It seems more reasonable that development of embryos and the release of glochidia would be determined more by cumulative temperature, or the number of degree days during the brooding season (Blaxter, 1969). In 1987, fine-rayed pigtoes appeared to begin brooding eggs when water temperatures were between 16 and 21 C. Temperatures did not drop below this level at any time during the brooding season. Temperatures below threshold would slow development, but if above this level, embryos should continue to develop and be released after a sufficient number of degree days (Blaxter, 1969). Perhaps this is the reason for the lack of a significant relationship between temperature and release of glochidia by fine-rayed pigtoes at Slant. As soon as temperatures are favorable, spawning and brooding occur, and continue unless temperatures fall below threshold. Doherty et al. (1987) stated that this may be true for *C. fluminea* in the New River in Virginia. They reported three pulses in release of larvae by this species, two of which coincided with either the spring rise or fall decline in water temperature; however, a third peak occurred in mid-summer

when temperatures were relatively stable. *C. fluminea* became reproductively active when water temperatures were between 15 and 26.1 C. They concluded that a minimal temperature may be necessary for the initiation of reproductive events, but stated that there was insufficient evidence to support the hypothesis that temperature induced reproduction or spawning during the reproductive season. I recorded high densities of glochidia of fine-rayed pigtoes in mid-June and again in July in both 1986 and 1987. Kitchel (1985) also reported several peaks in release of glochidia by *F. cor* in the North Fork Holston River. It may be that female fine-rayed pigtoes are fertilized at various times in the spring, and therefore, glochidia develop and are released at different times during the brooding season.

Determination of Fish Hosts

The glochidia of *F. cuneolus* appear host specific, primarily to fish species in the family Cyprinidae. The absence of amblymine glochidia on gills of field specimens of noncyprinid species, and the sloughing of glochidia of *F. cuneolus* from most noncyprinid species in the laboratory, support that conclusion. Specificity of the glochidia of short-term brooders for cyprinids has been demonstrated in other laboratory and field studies. Glochidia of the short-term brooder *Q. cylindrica strigillata* metamorphosed on only three cyprinids of 34 fish species tested in the laboratory (Yeager and Neves, 1986). Weaver (1981) provided both laboratory and field evidence that the river chub and whitetail shiner were primary fish hosts for *P. oviforme* in Big Moccasin Creek, Virginia. Glochidia of short-term brooding mussels were attached only to gills of minnows collected in this stream. Neves and Widlak (1988) reported that amblymine glochidia were attached only to 12 species of the Cyprinidae in the North Fork Holston River, Virginia.

As judged by earlier studies and my results, generalizations can be made on fish host specificity of short-term brooders at Slant. Amblymine glochidia were encysted only in the gill lamellae of thirteen cyprinid species. Although a number of these species were relatively common, the prevalence of infestation was low. Neves and Widlak (1988) also reported that the relative

abundance of twelve minnow species hosting amblyminine glochidia was not significantly correlated with prevalence of infestation on each, providing evidence for fish host specificity of short-term brooders in the North Fork Holston River, Virginia. They stated that incidences of infestation on telescope shiners and stonerollers were low, even though these species were relatively abundant. These two species were relatively abundant at Slant, but they also were rarely infested. The bigeye chub was the most commonly collected species ($n = 265$) at Slant in 1987, yet only one fish was observed with encysted amblyminine glochidia. Results of field collections implied specificity of the glochidia of *F. cuneolus* for species of the Cyprinidae.

Three of the six minnow species which had the highest prevalence of infestation of amblyminine glochidia at Slant; river chub, whitetail shiner, and Tennessee shiner, were verified as fish hosts in the laboratory. Glochidia of *F. cuneolus* were not observed on captured specimens of Tennessee shiners, but only 20 specimens were examined. A high percentage of spotfin shiners were also infested with amblyminine glochidia, and juveniles of *F. cuneolus* were found on gills of one field-collected specimen. However, I did not verify that the spotfin shiner is a host for the fine-rayed pigtoe, because this fish species was not collected at the sites chosen to obtain fish for infestations in the laboratory. Several other fish were determined capable of serving as hosts for the glochidia of *F. cuneolus* in the laboratory. However, these species were either absent in field-collections (fathead minnow), were not naturally infested with glochidia of *F. cuneolus* (telescope shiner), or supposedly are absent in the Clinch River (white shiner and mottled sculpin; Robert E. Jenkins, pers. comm., Roanoke College, Salem, Virginia) and from other rivers in which the fine-rayed pigtoe occurs (Appendix D). The river chub, whitetail shiner, and stoneroller served as hosts when tested in the laboratory, and field-captured specimens of each were either encysted with the glochidia, or in the case of the river chub, had newly metamorphosed juveniles of *F. cuneolus*. Wiles (1975) suggested that the prevalence of infestations on fish was correlated with the abundance of glochidia in the water column, and in the North Fork Holston River, peak infestations of amblyminine glochidia on cyprinids occurred shortly after maximum densities of glochidia in stream drift (Neves and Widlak, 1988). Trdan (1981) reported similar results in the release of glochidia of *Lampsilis radiata siliquoidea* and infestations of largemouth bass (*Micropterus salmoides*). The

trend in prevalence of infestations for river chubs, whitetail shiners, and stonerollers was similar to the pattern of occurrence of glochidia of *F. cuneolus* in stream drift. Additionally, peak infestations of amblymeine glochidia on these fish species occurred simultaneously with peak densities of glochidia of *F. cuneolus* in drift.

Prevalence of infestation at Slant was highest for the river chub; the greatest number of river chubs ($n = 12$) were infested with glochidia of the fine-rayed pigtoe, and juveniles of this mussel species were observed on six field-collected specimens of river chubs. These data support my conclusion that the river chub may serve as primary fish host for the fine-rayed pigtoe at Slant. Based on their high prevalence of infestation and verification in the laboratory, I suspect that the whitetail and Tennessee shiner are equally important for the successful reproduction of *F. cuneolus* at Slant. The spotfin shiner was not tested in the laboratory to confirm its suitability as host, but field observations indicate that it likely serves as a host for *F. cuneolus*. Although not commonly collected at Slant ($n = 13$), the percentage of infested individuals was relatively high (30.8%). Three specimens were observed with glochidia of *F. cuneolus*, and two of these fish had partially metamorphosed juveniles whose dimensions of hinge length, shape and size were comparable to those of the fine-rayed pigtoe. Additionally, Neves et. al (1980) collected both spotfin shiners and *F. cuneolus* at the same sites in the headwaters of the upper Clinch. Evidence to support that river chubs, whitetail shiners, Tennessee shiners, and most likely spotfin shiners, are important hosts for the fine-rayed pigtoe in the Clinch and in other tributaries of the Tennessee River drainage, is the co-occurrence of *F. cuneolus* and these fishes within its present range (Appendix D). Mendelson (1975) found niche overlap among *Notropis* species to be common, stating that this genus tends to form large aggregates of multispecific groups. Trdan and Hoeh (1982) discussed how using a large array of host fishes can be advantageous in uncertain environments, and Kat (1984) stated that narrow specificity can have negative consequences for a parasite, often restricting its geographic range, and especially costly if unsuitable hosts are encountered frequently. *F. cuneolus* apparently is dependent upon a number of host species for successful reproduction, and it is likely that other *Notropis* species also serve as hosts for the fine-rayed pigtoe in the Clinch and other rivers in the Tennessee River drainage.

Unlike most of the species determined to serve as hosts for the fine-rayed pigtoe, the fathead minnow does not appear to be a common member of the Tennessee River drainage (Appendix D). This fish species may not typically serve as host for the fine-rayed pigtoe. However, the bluntnose minnow, *Pimephales notatus*, was sampled in most rivers within both the present and historic range of this mussel species. Additionally, I collected bluntnose minnows at Slant; fathead minnows were absent from all samples. Neves et al. (1985) determined that fish species congeneric with previously identified hosts can also serve as hosts. Because glochidia of the fine-rayed pigtoe metamorphosed on fathead minnows in the laboratory and because the bluntnose and fathead minnows are closely related, I believe that the bluntnose minnow also may serve as host for the fine-rayed pigtoe.

In addition to prevalence of infestation, the degree to which fish are parasitized (or number of glochidia on parasitized fish) has been used in field studies to implicate fish species as hosts for various mussel species (Surber, 1912; Coker et al, 1921; Howard and Anson, 1922; Tedla and Fernando, 1969; Zale and Neves, 1982b; Weaver, 1981; Kitchel, 1985). Fish species with few encysted glochidia may only be an incidental host, and may not be suitable for the attached glochidia to complete metamorphosis (Surber, 1912; Arey, 1932). Conversely, high densities of glochidia on a species likely indicates a true host. However, an average of only 1 to 10 glochidia per infested fish has been reported most often (Coker et al., 1921; Trdan, 1981), but appears adequate to sustain most mussel assemblages (Neves and Widlak, 1988). Numbers of glochidia on fish therefore are not always good indicators of likely host species. As reported for other species, 1 to 5 glochidia per fish was typical for the four fish species (stoneroller, whitetail shiner, river chub, and spotfin shiner) on which glochidia or juveniles of *F. cuneolus* were observed at Slant. The greatest number ($n = 26$) of glochidia of the fine-rayed pigtoe occurred on river chubs, but as judged by the discussion above, this higher degree of infestation may not be significant. The river chub occurs higher in the water column than the stoneroller and commonly feeds on drifting insects (Jenkins and Burkhead, in press). Sight-feeding fish species may become infested by feeding on conglomerates released into the water column, which may result in a relatively high degree of infestation. In the North Fork Holston River, Virginia, infestations were fewer in benthic fish having subterminal

mouths (greenside and snubnose darters, stonerollers) than on closely related species with terminal mouths (Neves and Widlak, 1988). It appears that infestations occur primarily via ingestion of drifting glochidia for small drift-feeding species. The stoneroller is a grazing minnow, feeding primarily by scraping aufwuchs off rocks and other submerged objects. Glochidia of the fine-rayed pigtoe may come in contact with this fish species via respiratory movements, which may result in lower numbers of glochidia on gills of this species (Howard and Anson, 1922). Because the mode of infestation of glochidia on fish is unknown, stonerollers and river chubs are equally likely to serve as hosts for this species. I concluded that the river chub was the primary fish host for the fine-rayed pigtoe at Slant based on a combination of results and observations. Degree of infestation on fish may provide additional information, but the utility of using this method alone for determining fish hosts is questionable.

Induced Infestations in the Laboratory

Fish host specificity was demonstrated for *F. cuneolus* through induced infestations in the laboratory. Glochidia of the fine-rayed pigtoe were sloughed from sunfishes, catfishes, darters and goldfish. The goldfish, an exotic species, was the only species of cyprinid on which glochidia did not metamorphose. Neves et al. (1985) reported that exotic fishes were unsuitable hosts for glochidia of *Villosa nebulosa*. *In vitro* and *in vivo* studies have suggested that certain unidentified chemical components of the blood serum in fishes determine fish host suitability (Meyers et al., 1980; Isom and Hudson, 1982). It appears that the goldfish did not provide the appropriate chemical cues or nutritional requirements for the development of glochidia of *F. cuneolus*.

The mottled sculpin was the only non-cyprinid determined to be a suitable host for the glochidia of *F. cuneolus*. Sculpin species were not collected at Slant and therefore, I do not know whether the mottled sculpin is a host for the fine-rayed pigtoe at this site. Greenberg et al. (1987) reported that banded sculpins, *Cottus carolinae*, in the Little River in Tennessee, resided under rocks during the day and were on top of rocks at night when they were most active. This species

was infrequently collected in Big Moccasin Creek, Virginia, in summer months, but they were abundant in fall and spring (Zale and Neves, 1982a). Sculpin species are ubiquitous in the Tennessee River drainage (Masnik, 1974; Feeman, 1987; Angermeir et al., 1988; TVA, 1988). It is likely that sculpins occur at Slant, but they were not collected during my study. Masnik (1974) sampled mottled sculpins from two tributaries in the upper Clinch, but reported that this species was rare in this river. Jenkins (pers. comm., Roanoke College, Salem, Virginia) believes that the mottled sculpin does not occur in the Clinch, and attributed previous records to misidentifications by researchers. Masnik (1974) also discussed the difficulty involved in identifying certain sculpin species, particularly differentiating *Cottus carolinae*, the banded sculpin, from subspecies of the mottled sculpin, *Cottus bairdi*, in the Clinch River. This confusion and disagreement among taxonomists over identification of the mottled sculpin creates uncertainty that it is a natural fish host for the fine-rayed pigtoe. From the data available, it appears that the mottled sculpin does not occur at Slant. However, the banded sculpin is a very common native sculpin throughout the entire Clinch River, and is closely related to the mottled sculpin.

As discussed previously, fish species congeneric with previously identified hosts also can serve as hosts (Neves et al., 1985). Considering this fact, I believe that the banded sculpin can serve as a host for the fine-rayed pigtoe. Weaver (1981) demonstrated that the banded sculpin served as host for *Pleurobema oviforme* in laboratory infestations. Her results support speculation that this fish species also is a suitable host for the fine-rayed pigtoe, because *P. oviforme* and *F. cuneolus* use the same cyprinids (river chub, whitetail shiner, stoneroller). Examinations of sculpins for glochidia in rivers indicate that this genus is not typically used by short-term brooders. Weaver (1981) collected 112 specimens of banded sculpins in Big Moccasin Creek (where *P. oviforme* occurs), but none were infested with glochidia. Neves and Widlak (1988) reported limited usage of both darters and sculpins by the mussel assemblage (including both long and short-term brooders) in the upper North Fork Holston River, Virginia. *F. cuneolus* shares similar riffle habitat with sculpins, and a host-parasite relationship could have evolved. Even so, it is most likely that sculpins would be used to a limited extent. In the upper North Fork Holston River, Virginia, infestations on sculpins were higher in November, when temperatures decreased and activity of these fish increased (Neves and

Widlak, 1988). Zale and Neves (1982a) determined that glochidial release by *Villosa vanuxemensis* from fall to spring in Big Moccasin Creek, Virginia, coincided with the presence of its fish host, the banded sculpin. However, this fish species was rarely collected in summer in this stream. Other workers also have classified this fish species as a winter spawner (Masnik, 1974; Jenkins and Burkhead, in press). As judged by other studies, sculpins would not be available in the summer months during the period when fine-rayed pigtoes release glochidia. Therefore, it seems unlikely that this species is commonly used as a fish host by *F. cuneolus*.

Factors known to influence metamorphosis or duration of metamorphosis of a glochidium on a fish host are water temperature, fish host and mussel species, and the condition or health of the host (Lefevre and Curtis, 1912; Howard and Anson, 1922; Weaver, 1981). The time when glochidia are obtained from females has also been shown to affect duration of the attachment period, but this factor is more applicable to long-term brooding species (Tedla and Fernando, 1969; Zale and Neves, 1982b). Glochidia of *F. cuneolus* completed metamorphosis on most fish hosts in approximately two weeks at mean water temperatures between 22.5 and 25.0 C. Variation in the length of attachment appeared to be correlated with water temperatures. This time period is similar to periods of metamorphosis reported for other short-term brooders. Glochidia of *P. cordatum* metamorphosed on rosefin shiners (*Notropis ardens*) in 14 to 18 days at 21.0 C (Yokley, 1972). Weaver (1981) obtained juveniles of *P. oviforme* from stonerollers in 13 to 16 days at water temperatures between 20.5 and 24.0 C. She showed that this time period was shortened when water temperatures were elevated. The correlation between development time and water temperature is well documented; increased water temperatures will shorten the period of metamorphosis, while decreased temperatures will lengthen it (Lefevre and Curtis, 1912; Matteson, 1948; Weaver, 1981; Zale and Neves, 1982a).

Diseased fish hosts may affect the success of glochidia metamorphosing to juveniles. I collected only two juveniles from infestations with diseased Tennessee shiners; sloughed glochidia were most commonly collected before all fish died from fungal infections. Howard and Anson (1922) and Weaver (1981) also reported that fish succumbing to fungal infections sloughed encysted glochidia.

However, whether glochidia were sensitive to the condition of the host or presence of the fungus is unknown.

Age and Growth

Evaluation of Thin-Sectioning Technique

It typically was difficult to distinguish annuli in sectioned valves of *F. cuneolus*, but thin-sections with high resolution enabled better accuracy in aging. Moyer (1984) discussed problems involved in aging this species because of indistinct annuli, stating that of five freshwater mussel species analyzed using thin-sectioning, the fine-rayed pigtoe was the most difficult to age. Because *F. cuneolus* is a slow-growing, heavy-shelled species, 1.0 to 1.5 h was required to prepare each specimen, not including time needed for epoxy to dry (12 h). High-quality thin-sections required even more time to prepare, and several cuts were necessary to obtain thin-sections from valves greater than 60 mm. Despite these shortcomings, thin-sectioning enabled me to differentiate "false" from true annuli, thus lending confidence to determined ages. Without having used the thin-sectioning technique, ages would most likely have been underestimated due to erosion of umbos and because of the difficulty in counting closely deposited growth checks near the margin of shells of older specimens. Age and growth statistics obtained for *F. cuneolus* at Pendleton Island (CRM 226.3) were very similar to those reported by Moyer (1984) for *F. cuneolus* at CRM 219, providing further evidence that thin-sectioning is both an accurate and precise aging technique. Utilization of thin-sectioning to age mussels provides data for valid comparisons of age and growth between demes of the same species, and between different species of freshwater mussels. Neves and Moyer (1988) suggested that a recently modified acetate peel technique may provide a more efficient alternative to aging freshwater bivalves, but this technique has not been thoroughly tested (Ropes, 1987). Results of my analysis indicate that advantages of thin-sectioning outweigh its shortcomings. After considerable practice, I felt confident that specimens of *F. cuneolus* were aged accurately, re-

sulting in valid assessments of age-class structure and growth statistics of this species at Pendleton Island and Slant.

Age-Length Relationships

The growth pattern depicted by the modified version of the von Bertalanffy equation for *F. cuneolus* at Pendleton Island was very similar to the pattern previously reported for this species (Moyer, 1984). The von Bertalanffy equation, as determined by Moyer (1984) for *F. cuneolus* at CRM 219, was as follows:

$$L_{(t)} = 82.5 \text{ mm } (1 - e^{-0.13(t-1.04)}).$$

If my equation describing growth of *F. cuneolus* at Pendleton Island is converted to the unmodified von Bertalanffy equation, it is comparable:

$$L_{(t)} = 78.4 \text{ mm } (1 - e^{-0.13(t-0.05)}).$$

The asymptotic lengths and estimates of k for both equations are very similar, but estimates of t_0 are quite different. This discrepancy is most likely a result of different estimates of mean lengths at age 1. Eroded umbos often make it difficult or impractical to distinguish the first annulus, and estimated lengths at age 1 are often "best guesses". Moyer (1984) fit two other asymptotic equations, the Gompertz and logistic curves, to length-at-age data of *F. cuneolus* at CRM 219 and determined that the logistic equation best described growth in length. However, it was not statistically different from either the Gompertz or the von Bertalanffy equations. The suitability of each equation was measured by the mean length residual mean square error (RMSE), reported as 12.45 for the logistic and 21.76 for the von Bertalanffy equation. In my analysis, the majority of observed variability in growth was explained by the growth model, and thus the von Bertalanffy equation seemed to satisfactorily describe growth in length of *F. cuneolus* at both Pendleton Island (RSME = 5.69) and at Slant (RMSE = 12.93). As emphasized by Haukioja and Hakala (1979), the best fit to length-at-age data may be given by different equations for different populations, but if a good description of measurements is all that is desired, it matters little which equation is used.

Mean lengths of some cohorts of mussels were typically underestimated by the von Bertalanffy model, but were relatively accurate (within 1 to 3 mm) when sample sizes (n) were

greater than 5. Kitchel (1985) also reported that the von Bertalanffy equation accurately predicted lengths of the endangered shiny pigtoe, *F. cor*, only if sample sizes were large. I was unable to obtain sufficient valves of both old and young *F. cuneolus* for my analysis, and therefore predicted mean lengths at these ages may be more accurate than observed values. This may be particularly true for the oldest ages (> 20 yr), for which length-at-age data were obtained from thin-sections of only one or two specimens.

Haukioja and Hakala (1979) stated that computing a growth equation may not necessarily add anything essential as compared with a vector of lengths of mussels at different ages. However, considering the difficulty in obtaining a representative sample of both young and old specimens, as discussed above, the growth models formulated for the demes at Pendleton Island and Slant may be more useful for monitoring the age-class structure of *F. cuneolus* at these sites than a simple vector of lengths. The growth equations developed for the fine-rayed pigtoe at Pendleton Island and Slant appear to be relatively accurate. Plotted curves potentially can be used to estimate ages without wanton sacrifice of live specimens. Age class structure thus can be constructed, and from periodic collection of these data, recruitment of fine-rayed pigtoes at each site can be monitored. My growth equations and those obtained by Moyer (1984) for the fine-rayed pigtoe were in agreement. Therefore, growth of this species at other sites in the Clinch River is likely similar, and should approximate growth at Pendleton Island and Slant.

Estimates of t_0 , the theoretical time when length is zero, were not substantially different between demes of *F. cuneolus* at Pendleton Island and Slant. Estimated theoretical maximum lengths (L_{∞}) and estimated values of k (the rate at which length approaches L_{∞}) were different between sites, but these parameters are not suitable for comparisons of growth among different populations (Gallucci and Quinn, 1979; Haukioja and Hakala, 1979; Jirka, 1986). Alternatively, the parameter w , a measure of growth rate near t_0 with respect to length, has been shown to be an adequate parameter with which to compare growth (Gallucci and Quinn, 1977; Jirka, 1986). Using this criterion, differences between growth rates of *F. cuneolus* at Slant and Pendleton Island were evident. Specimens of *F. cuneolus* at Pendleton Island exhibited a faster rate of growth than those at Slant. Bivalve growth rates are known to vary with environmental conditions, such as temperature, oxy-

gen, and food supply, and the quality of various other water and habitat characteristics (Seed, 1980; Weatherley and Gill, 1987). Although records of water quality parameters were obtained from sites both upstream and downstream of Pendleton Island and Slant (see Appendix C), lack of water quality and habitat measurements at these sites prevents an assessment of observed differences in growth. However, several hypotheses can be formulated from observed differences in the physical characteristics at each site.

The Pendleton Island site is characterized by a large island, and a series of channels and smaller islands, on which can be found a diversity of tree species including hemlock (*Tsuga sp.*), sycamore (*Planatus occidentalis*), and ashleaf maple (*Acer negundo*). *Rhododendron sp.* and other herbaceous and woody cove species of plants were observed along streambanks. Protected by The Nature Conservancy, the riparian zone of the islands remains generally unaltered, often forming canopies along channel widths. In comparison, the land adjacent to the river reach at Slant is owned locally, and the riparian zone on one side of the river has been reduced to a single row of trees, for agriculture and for easy access to the river for recreation. The quality of the riparian zones likely influence the amount of sediment entering the different river reaches. The abundance of trees and plants along the channel at Pendleton Island would decrease the sediment load into the river from adjacent land run-off. The sand, gravel and cobble substrata at Pendleton Island were firm and evidently suitable for mussels along the entire channel width, because mussels (including the fine-rayed pigtoe) were found in all areas of this habitat, including areas immediately adjacent to the shoreline. During summer rains at Slant, I observed mud and silt entering the stream at high velocities from man-made ditches, greatly increasing turbidity. Mud and silt along streambanks were typically observed at this site. Rhoads and Pannella (1970) performed field investigations to study potential reasons for differences in growth between two populations of *Mercenaria mercenaria* and reported that both substratum and depth of water will influence bivalve growth. They determined that specimens living in sand exhibited a greater rate of growth than those living in mud, and that growth was more inhibited in deep compared to shallow water. A change from shallow water was accompanied by decreased flow and light penetration; temperature and food supply were also affected. Ellis (1936) determined that light penetration is limited by silt, which dulls the sensitivity

of mussels phototactic responses and reduces the production of mussel foodstuffs (Fuller, 1974). Suspended silt also has been shown to cause mussels to remain closed twice as long as they would normally, and as a result, growth is inhibited (Ellis, 1936). Increased mud and silt at Slant may account partially for decreased growth in fine-rayed pigtoes at Slant, but siltation problems seem to be restricted to the edges of the stream. Siltation may not directly account for slower growth of fine-rayed pigtoes, but its presence reduces available habitat for mussels which require sand and gravel substratum. I calculated that 70% of the stream bottom at Slant was unsuitable for the fine-rayed pigtoe. This area likely was overestimated, but nevertheless, good habitat is more limited at Slant than it is at Pendleton Island. Bedrock was common at Slant but rare at Pendleton Island, and it is possible that differences exist in sources of nutrients for mussels at these sites. Decreased productivity and a lower quality food base at Slant may have contributed to slower growth of fine-rayed pigtoes at this site.

Seed (1980) reviewed the literature on the ecology of bivalves prior to 1975, and discussed various factors potentially affecting growth in these organisms. Temperature and light often were reported as important variables, but discrepancies existed in conditions that were favorable for growth. Inconsistencies in results of these studies implicated the difficulty in controlling for exogenous factors in the natural environment; it appeared that factors other than temperature and light may be more important for growth, most likely food supply (Wilson, 1971). Food supply is probably the most important single factor in determining growth rate in organisms (Seed, 1980). Growth rates of fish are known to respond sensitively to differences in food supply (Weatherley and Gill, 1987). For example, Jirka (1986) examined four subpopulations of the purple wartyback, *Cyclonaias tuberculata*, in the New River below Bluestone Dam, and reported that mussels exhibited decreased growth with increased distance from the dam. Differences in growth were attributed to the quantity and quality of food available for mussels at the different sites. Temperature was considered to be an unlikely reason for observed differences, because it was relatively similar among sites. Mean water depth and channel depth were narrower at Pendleton Island when compared to these characteristics at Slant. Greater mean water velocities probably exist at the Pendleton Island site, resulting in more food per unit time and more oxygen available to filter-feeders at this site.

Additionally, a smaller percentage of suitable habitat would confine mussels to a smaller area at Slant when compared to Pendleton Island. It has been suggested by some workers that filter feeders may partition the habitat for feeding (Green, 1971; Heard, 1977; Kraemer, 1979). Thus, limited habitat at Slant may increase the potential for competition for food between individuals requiring similar substratum. Whether it is more the quality or quantity of food that affects growth is unknown, but food seems to be the most likely environmental factor to explain differences in growth of fine-rayed pigtoes between sites. Other hypotheses could explain growth differences between the two demes of the fine-rayed pigtoe at Pendleton Island and Slant, but without quantitative data on both water quality and habitat variables, reasons for these differences remain largely conjecture.

Age Characteristics

The range in ages (6-32) of specimens of *F. cuneolus* at Pendleton and Slant was in close agreement with the range of ages (4-35) reported for *F. cuneolus* at CRM 219 (Moyer, 1984). The age class structure of live specimens at both Pendleton Island and Slant consisted mostly of older individuals (17 -20 yr), a phenomenon which has been reported for populations of other mussel species, to include five species in streams in southwestern Virginia, *Pleurobema cordatum* in the Tennessee River, and eight unionid species in the Mississippi River (Yokley, 1972; Coon et al., 1977; Moyer, 1984). It is common for freshwater mussel populations to appear dominated by older adult mussels. However, Moyer (1984) attributed the lack of individuals in the juvenile or young adult age groups to biased sampling techniques. He reported that specimens less than 3 years of age were not found, and that 3 to 6 year-old specimens were rarely sampled using handpicking and waterscoping techniques. In comparison, more young mussels were collected by Zale (1980) using a quadrat sampler at the same site sampled by Moyer (1984). Other workers similarly have warned that age group estimates were biased towards older age classes because of sampling techniques (Chamberlain, 1931; Coon et al., 1977).

In my analysis, sampling by handpicking was biased for larger individuals at Slant. This bias is corroborated by the greater percentage of younger individuals typically collected and consumed by muskrats. In an attempt to remedy this problem with sampling bias, Moyer (1984) suggested the

use of quadrat sampling to obtain unbiased samples for determining mussel age class structure. However, this quantitative sampling technique is often inadequate to collect rare or endangered mussels (Neves et al., 1980; Kovalak et al., 1986; Neves and Odom, in press). An alternative in this case might be to collect specimens from muskrat middens, if available, for analysis of age-class structure. Neves and Odom (in press) reported that median lengths of mussels in muskrat middens collected from the North Fork Holston and Clinch rivers were typically smaller than those collected in quadrats. Considering the high predation rate on mussels by muskrats at Slant, and that fine-rayed pigtoes were consumed with the greatest frequency of all mussels at this site (Neves and Odom, in press), using valves of *F. cuneolus* from muskrat middens for growth analyses likely resulted in a more accurate assessment of the true age-class structure of this species at Slant. This assessment may not be free of bias, since muskrats were found to select against very small species and also against the largest of the large-size species (Neves and Odom, in press). Younger specimens (0 to 5 yr), if present, likely would not be collected and consumed.

The rare occurrence of young specimens of fine-rayed pigtoes at Pendleton Island and Slant is cause for concern. My results indicated that sampling by snorkeling may be biased for older specimens, but the rare occurrence of mussels less than age 10 in muskrat middens, in a large sample ($n = 264$) collected over a period of 1 yr, indicates that recruitment at Slant is poor. The preference by muskrats for medium-size mussel species potentially results in under-representation of young specimens in the age class structure at these sites. However, in the age analysis for the fine-rayed pigtoe, the largest specimens observed would still be a relatively medium-sized individual, and should therefore minimize this bias. As judged by freshly-dead specimens collected in muskrat middens for the age and growth analysis, recruitment also appears to be low at Pendleton Island. This could have more important ramifications than at Slant, considering that the best breeding population of the fine-rayed pigtoe in the Clinch River may occur at this site (Ahlstedt, 1984). The impact of muskrats on the deme of fine-rayed pigtoes at Slant is significant. It is estimated that more than 1,000 fine-rayed pigtoes from the Clinch River are consumed each year by muskrats (Neves and Odom, in press). Based on age-length relationships developed in this study for the fine-rayed pigtoe at Slant, the age 11 to 14 cohorts (56 to 65 mm) are consumed with greatest frequency at this

site. The decline in numbers of this age group, coupled with the rare occurrence of individuals less than age 10, reduces the reproductive potential and places the deme of fine-rayed pigtoes at Slant in danger of decline and eventual extirpation. Densities of fine-rayed pigtoes at Pendleton Island are greater than at Slant, but limited evidence of recruitment indicates that this and other demes of the fine-rayed pigtoe in the Clinch River may be declining.

Age-class structure of the fine-rayed pigtoe demes at Pendleton Island and Slant should be closely monitored. The age-length keys provided for *F. cuneolus* at Pendleton and Slant can be used readily to assess both age-class structure and recruitment at these sites (Appendices A and B). Total-length measurements of live specimens should be taken at each site using methods previously described; individuals subsequently can be returned to the substratum. To obtain the most accurate assessment of true age-class structure, this procedure should be repeated using valves of freshly-dead specimens collected along streambanks in muskrat middens. Ages of fine-rayed pigtoes in samples thus can be estimated by comparing lengths of specimens collected at Pendleton Island and Slant with those provided in respective keys. Relatively accurate estimates of ages for specimens should be obtained using observed mean lengths in these keys. However, for specimens > 40 mm or > 70 mm, estimates might be more accurate by using predicted mean lengths. This suggested procedure seems to be an effective method for monitoring age-class structure and recruitment of the fine-rayed pigtoe at Pendleton Island and Slant, and could be performed annually with relatively minimal effort.

Estimation of Density

Considering the densities of glochidia of *F. cuneolus* collected in samples of stream drift, and also the number of freshly dead specimens observed in muskrat middens, the projected number of fine-rayed pigtoes at Slant was a gross underestimate. I believe that this underestimate most likely occurred because of an underestimate of available habitat for mussels at Slant. An underestimated area of suitable and marginal habitat would result in a lower projected number of individuals pres-

ent at Slant. If the derived density estimate ($1/30 \text{ m}^2$) of fine-rayed pigtoes is extrapolated to the total area, the estimated density probably approaches the actual abundance. Predation by muskrats has had a significant impact on the abundance of this species at Slant (Neves and Odom, in press), but most likely the failure to find this species in quadrats was a function of insufficient sampling. Density estimates of endangered species requires a more intensive sampling scheme, as discussed by Kovalak et al. (1986). Their suggestions for improved density estimates for rare mussels were applied in this study, but apparently were insufficient. A better estimate may have been obtained if a larger quadrat sampler had been used, and sample sizes had been increased. Sampling by depletion, in which several passes are made over an area, may be an alternative method of sampling for rare mussels. The density and estimated number of live fine-rayed pigtoes at Slant is considerably greater than my results would indicate, but my sampling effort and methods were inadequate to estimate these values.

Summary

1. *F. cuneolus* is a short-term brooder, gravid from mid-May through early August in the Clinch River at Slant and Pendleton Island in Virginia.

2. Gills of gravid females assumed the color of enclosed conglomerates; hues changed from pink to light peach as embryos matured. A light peach color indicated that glochidia were mature and ready for release.

3. Glochidia were released by fine-rayed pigtoes from early June to early August at Slant. Highest densities in stream drift occurred in mid-June.

4. Glochidia of fine-rayed pigtoes were present in the water column throughout a 24 h sampling period, but were most abundant in early morning (0700 h).

5. Sawfin shiners, whitetail shiners, river chubs, spotfin shiners, Tennessee shiners, and mimic shiners were identified as possible fish hosts for the fine-rayed pigtoe, as judged by the high prevalence of infestation of amblemine glochidia on these fish species at Slant.

6. Naturally encysted glochidia of *F. cuneolus* were observed on river chubs, whitetail shiners, stonerollers, and spotfin shiners; prevalence of infestation was highest on the river chub at Slant. Degree of infestation ranged from 1 to 26 glochidia, but a range of 1 to 5 glochidia of the fine-rayed pigtoe per fish was typical.

7. In the laboratory, glochidia of the fine-rayed pigtoe metamorphosed on fathead minnows, river chubs, stonerollers, telescope shiners, Tennessee shiners, white shiners, whitetail shiners, and

mottled sculpins. Three species of sunfish, two darter species, and the goldfish did not serve as hosts.

8. Specimens of *F. cuneolus* collected at Pendleton and Slant ranged from 6 to 32 yr old; as judged by freshly-dead specimens collected in muskrat middens, the dominant age group appeared to be 13 to 16 yr old.

9. As predicted by the von Bertalanffy equation, the fine-rayed pigtoe achieves a maximum length of approximately 90 mm and an age of 35 yr in the upper Clinch River.

10. Mean annual growth-in-length of *F. cuneolus* at Pendleton Island and Slant is relatively rapid during the first 5 to 6 yr of age (5 to 7 mm/yr), but declines to 3 to 4 mm/yr between 6 and 15 yr. The fine-rayed pigtoe approaches its asymptotic lengths near age 21, increasing approximately 1 mm/yr thereafter.

11. The rare occurrence of young specimens (< 10 yr), and the high predation rate by muskrats on individuals in the age 11 to 14 cohorts, indicates that the reproductive potential of fine-rayed pigtoes at Slant has been reduced. This deme appears to be in decline. Recruitment also appears to be low at Pendleton Island, which may indicate that this deme is also declining in abundance. Growth curves provided in this document can serve as tools to monitor age-class structure and recruitment of the fine-rayed pigtoe at these and other sites in the Clinch River.

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Appendices

Appendix A. Sample size, and observed and predicted lengths of the fine-rayed pigtoe in the Clinch River at Slant, Virginia.

<u>Age</u>	<u>N</u>	<u>Observed mean length</u>	<u>± 1 s.d.</u>	<u>Predicted mean length</u>
1	10	8.6	1.7	5.0
2	10	12.9	1.9	11.6
3	9	18.7	4.2	24.8
4	10	24.0	4.5	23.3
5	10	28.3	3.8	28.5
6	11	32.7	4.4	33.2
7	11	37.6	5.9	37.7
8	10	37.8	2.6	41.8
9	2	43.9	1.9	45.5
10	8	46.1	7.6	49.0
11	2	55.5	8.7	52.2
12	8	56.1	5.0	55.2
13	6	57.4	7.8	58.0
15	5	65.3	5.0	62.8
16	3	68.6	6.8	65.0
17	1	71.7	-	67.0
18	1	74.8	-	68.8
20	1	73.5	3.8	72.1
23	1	65.9	-	76.1
25	1	77.0	-	78.2
27	1	83.6	-	80.1
30	1	80.2	-	82.4

Appendix B. Sample size, and observed and predicted lengths of the fine-rayed pigtoe in the Clinch River at Pendleton Island, Virginia.

<u>Age</u>	<u>N</u>	<u>Observed mean length</u>	<u>± 1 s.d.</u>	<u>Predicted mean length</u>
1	9	9.8	0.7	9.0
2	9	16.5	2.2	17.4
3	9	25.8	4.2	24.8
4	9	34.5	2.5	31.4
5	9	39.4	3.2	37.1
6	11	44.7	4.1	42.2
7	10	46.9	3.4	46.7
8	13	48.3	4.4	50.6
9	2	54.2	6.2	54.1
10	4	54.6	3.9	57.2
11	2	57.2	5.2	60.0
12	1	62.4	-	62.3
13	2	61.8	10.0	64.4
14	1	61.4	-	66.3
15	2	70.7	0.5	68.0
16	3	69.6	5.0	69.4
17	1	75.5	-	70.7
18	4	72.4	6.2	71.8
20	2	72.5	6.7	73.7
23	1	76.7	-	75.7
25	2	77.0	4.5	76.7

Appendix C. Mean¹ water quality parameters at CRM 211.0 and CRM 249.6 obtained from EPA's STORET retrieval system, January to December, 1986 and 1987.

	1986		1987	
	CRM 211.0 x	CRM 249.6 s.d.	CRM 211.0 x	CRM 249.6 s.d.
D.O. (mg/l)	9.6	1.7	10.1	2.2
pH	7.8	0.3	8.2	0.2
Total hardness (mg/l)	125.6	50.0	159.3	13.9
Conductivity (µmhos)	277.4	52.3	-	-
Mercury (mg/l)	0.2	0.1	0.3	0.07
Zinc (µg/l)	58.5	52.9	660.0	79.2
Copper (µg/l)	20.4 ^a	-	20.2 ^b	-
Nitrogen (total,mg/l)	0.1	0.03	0.1	0.0
			348.0	44.8
			0.2	0.1
			102.0	2.8
			24.9 ^a	-
			0.1	0.0
			9.0	1.7
			8.1	0.4
			52.2	45.5
			16.9 ^b	-
			0.1	0.0

¹ Mean ± 1 s.d.

^a sediment, mg/kg dry wt.

^b total, µg/l

Appendix D. Checklist of fish hosts of the fine-rayed pigtoe and their occurrence¹ within the present and historical range of this species²

Fish Species	Clinch	Powell	N. Fork Holston	Elk	Paint Rock	
River chub	+	+	+	+	-	
Stoneroller	+	+	+	+	+	
Fathead minnow	-	+	-	-	-	
Bluntnose minnow	+	+	-	+	+	
Telescope shiner	+	+	+	+	+	
Tennessee shiner	+	+	+	-	+	
Whitetail shiner	+	+	+	+	+	
Banded sculpin	+	+	+	+	+	
Spotfin shiner	+	+	+	+	+	
Fish Species	Sequatchie	Copper Cr.	Nolichucky ³	Buffalo ³	Bear Cr. ³	Emory R. ³
River chub	+	+	+	+	-	-
Stoneroller	+	+	+	+	+	+
Fathead minnow	-	-	-	-	-	-
Bluntnose minnow	+	+	-	+	+	+
Telescope shiner	-	+	+	+	-	+
Tennessee shiner	-	+	+	+	-	+
Whitetail shiner	+	+	-	+	-	+
Banded sculpin	+	+	+	+	-	-
Spotfin shiner	+	+	+	+	+	+

¹Based on collections by Freeman (1987); Angermeier et al. (1988); and TVA (1988)

²Based on U.S.F.W.S., 1984

³Rivers in which the fine-rayed pigtoe is estimated

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