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Reproductive Characteristics of a Population of the Washboard Mussel *Megalonaias nervosa* (Rafinesque 1820) in the Upper Mississippi River

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ABSTRACT

We examined monthly and age-specific gametogenic development of the washboard mussel, *Megalonaias nervosa*, from April 1986 to March 1987 in navigation Pool 10 of the upper Mississippi River. We found *M. nervosa* to be a late tachytictic breeder. Female marsupia contained eggs or glochidia primarily from August (17°C) through October (9°C). Males were mature from July through October. Most females released their glochidia in October. Only one female was gravid in November (3°C). Most mussels were sexually mature at 8 years of age and then had an estimated average size of 68 mm (shell height). Only 8% of individuals ≤ 4 years of age showed any degree of reproductive development, while > 90% of age 5 and older individuals had recognizable reproductive material present. In host specificity studies, we verified three fish species as hosts for the glochidial stage. Green sunfish (*Lepomis cyanellus*), black bullhead (*Ictalurus melas*), and channel catfish (*Ictalurus punctatus*) produced juveniles after 26-28 days at 17°C. White suckers (*Catastomus commersoni*) and yellow perch (*Perca flavescens*) retained glochidia from 23 up to 26 days, but no juveniles were produced. Glochidia remained attached to common carp (*Cyprinus carpio*) and fathead minnows (*Pimephales promelas*) ≤ 3 days. Channel catfish were retested at 12°C and produced juveniles after 56 days.

INTRODUCTION

Freshwater mussel populations have been seriously affected by habitat deterioration and commercial harvest. Historically, the upper Mississippi River contained one of the richest freshwater mussel faunas in the world (van der Schalie and van der Schalie 1950), but a well documented decline in species diversity has occurred since the 1800s (Higgins 1858, van der Schalie 1938, Coon et al. 1977, Havlik and Stansbery 1978, Fuller 1978, Thiel 1981). This decline has been variously attributed to navigation-related activities (e.g., impoundment, channelization, and traffic) and pollution. Commercial exploitation has been suggested as a primary reason for mussel decline (van der Schalie 1938, Fuller 1978). From the late 1800s to the late 1930s, millions of tons of mussels were harvested annually from the river for use in the pearl-button industry (Coker et al. 1921, van der Schalie 1938). Then, resource depletion and invention of the plastic button essentially eliminated harvest. In the 1950s, the Japanese pearl culture industry created a new demand for freshwater mussel shell material as nuclei for cultured pearls. Commercial clamming to supply this new demand has been a multi-million dollar industry for two

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decades (Sparks and Blodgett 1986, Fritz 1986) and demand continues to increase. For example, Illinois reported a 104% increase in shell harvest from the Mississippi River between 1984 and 1985 (Fritz 1986). This intense exploitation is occurring in the absence of adequate knowledge of basic biology, abundance, and recruitment potential of impacted species.

Megalonaias nervosa is the preferred commercial species of the upper Mississippi River, comprising 80% of the harvest in Illinois (Fritz 1986) and 60% in Wisconsin (Anonymous 1987). This species occurs in the Mississippi River, many of its tributaries, the Tombigbee River in Alabama, and the Nuevo Leon in Mexico (Baker 1928, Fuller 1978). However, conflicting or limited data exist on the basic reproductive strategy and reproductive development of *M. nervosa*.

Reproductive strategies of mussels are characterized as either tachytictic or bradytictic. Fertilization and release of glochidia occur during the same season (tachytictic), or glochidia are retained over winter and released during the following spring or summer (bradytictic). Utterback (1915-1916) and Coker et al. (1921) described *M. nervosa* as a bradytictic breeder. Howard (1914), Lefevre and Curtis (1910), and Bingham (1968) suggested a tachytictic strategy. Coker et al. (1921) suggested that this species may be a transitional species between the two strategies. Each of these studies described gravid periods for females but without more specific gametogenic examination. An exception was Bingham (1968) whose sample size (15) was too small to detail seasonal or age-specific patterns.

Size limits can restrict harvest in regulated mussel populations. However, the size and age at which individual *M. nervosa* become sexually mature are poorly documented. But, Coker et al. (1921) and Howard (1914) note that the smallest breeding individual they found was about 91 mm in length and 8 years old. Establishment of size-limits which permit sufficient recruitment into the population to sustain intense commercial harvest is difficult without such data. Duncan and Thiel (1983) found that old, large mussels dominated populations of *M. nervosa* in the upper Mississippi River. This observation suggests that recruitment is minimal.

Sixteen species of fish representing nine families may be natural hosts for the glochidia of *M. nervosa* (Fuller 1978). However, identification of these hosts has been by various methods and may not reflect the true or total host assemblage. For example, Howard (1914) defined hosts based on the degree of encystment by glochidia. Simple encystment does not guarantee metamorphosis, and it is unclear whether metamorphosed juveniles resulted. Coker et al. (1921) collected feral fish and identified glochidia. They also performed laboratory studies, but did not examine and verify all of the field-identified hosts. Wilson (1916) listed the species of feral fish upon which he found *M. nervosa* glochidia, but did not mention observing metamorphosis of juveniles.

We began our study to complement concurrent commercial harvest assessments by Heath et al. (1988). Specific objectives were to 1) describe the reproductive biology of *M. nervosa* in a selected reach of the upper Mississippi River and 2) identify some fish hosts for the parasitic glochidial stage in controlled laboratory experiments. Information on critical reproductive periods and age and size of first reproduction aids in better management of commercial harvest. Host studies can determine if host availability is a potentially critical limiting factor.

METHODS

The upper Mississippi River extends from above Minneapolis-St. Paul, Minnesota to the confluence of the Ohio River at Cairo, Illinois. A lock-and-dam system divides much of the river into a series of 27 navigation pools. Navigation Pool 10 is a 54-km reach of the river between Lock and Dam 10 at Guttenburg, Iowa and Lock and Dam 9

near Lynxville, Wisconsin. The East Channel, located at mid-pool, contains an abundance of the target species and is a site of heavy commercial clamming (Thiel 1981, Duncan and Thiel 1983).

We collected 20 *M. nervosa* each month from April 1986 to January 1987 and again in March 1987 to evaluate patterns of gametogenesis. From April to November 1986, Wisconsin Department of Natural Resources SCUBA divers randomly collected these mussels from the river substrate. Winter samples (December to March) consisted of individuals retrieved from covered wire baskets placed in the river in July 1986. *Megalonaias nervosa* collected for the study ranged in age from 3 to 34 years. The majority were between 11 and 17 years old. Individuals varied in size (length x height) from 31 mm x 21 mm to 161 mm x 111 mm. For histological studies, mussels were pegged open and then preserved in 70% ethanol. We dissected gonadal material from the viscera and processed it through a dehydration series. A portion of the material was then embedded in paraffin. We then applied standard Hematoxylin-Eosin staining procedures to serial 8-μm sections of the gonadal material (Humason 1972). Sex was identified and stage of gametogenesis recorded for 255 individuals. We assigned stage of reproductive development according to Yokely's (1972) scheme. Stage 0 describes a mussel's sex as unknown and its gonadal tissue as undifferentiated or beginning to differentiate. Stage 1 mussels have some spermatogonia or oogonia and small ovocytes with gonadal tissue well differentiated. In Stage 2, spermatids are present or developing ovocytes are moving into the lumina. At Stage 3, spermatozoa or mature ova fill the lumen. Stage 4 refers to females with marsupia filled with eggs or glochidia.

We determined the minimum age of sexual maturity by comparing the gonadal development of 74 young mussels ages 1 to 8. These young mussels where collected during the months of greatest gametogenesis (July - September), when older individuals were predominantly in Stage 2, 3, or 4 of development. Gonadal sections and marsupia were examined as described above.

The age, length, and height of 355 mussels were determined to estimate the age and size of sexually mature individuals. We measured length (maximum antero-posterior) and, at right angle to length, height (dorso-ventral) of mussels to the nearest 0.1 mm. Mussels were aged by counting annular growth rings on the shells. We averaged three blind counts from a shell to determine the age. External age estimates were compared to estimates obtained by thin-sectioning (internal method) on a subsample of 115 individuals. Sectioned shells (0.3 mm) were read with polarized light under a dissecting microscope. We validated our external aging method by regressing the external (dependent) estimate against the internal estimate (independent) for the 115 individuals. We determined that the two methods produced similar age estimates (slope=1.0, r^2=0.80, $P=\leq 0.0001$). Finally, growth characteristics were described by regressing size measurements against external age estimates.

Host specificity of glochidia was tested in the laboratory with seven species of fish representing five families. All test fish were reared in the laboratory and had no previous history of exposure to glochidial infection. Two of the selected test species, black bullhead (*Ictalurus melas*) and channel catfish (*Ictalurus punctatus*), were previously suggested as hosts in the literature. We tested five others common to the mussel's habitat as additional potential hosts: fathead minnow (*Pimephales promelas*), common carp (*Cyprinus carpio*), white sucker (*Catastomus commersoni*), green sunfish (*Lepomis cyanellus*), and yellow perch (*Perca flavescens*). We used a water-filled hypodermic syringe to flush glochidia from the gills of a live gravid female into a watchglass. A sample of these glochidia was tested for viability by adding a few salt grains to the water. Suitably viable glochidia would react immediately with a sudden closure of valves. Twenty fish of each test species were slightly anesthetized with tricane methanosulfate (MS-222) and then exposed in groups of four to approximately 100 glochidia in a

watchglass. After infection, we held each species in separate 40-L flow-through aquaria at 17°C. This simulated early fall river temperatures when initiation of first glochidial release occurs. We examined a subsample of five fish of each species one hour after exposure and then every three days thereafter to ascertain retention of glochidia. From the second week to the end of the experiment, debris on the tank bottom was filtered every other day through a 130-um nylon mesh screen and examined with a dissecting scope for sloughed, unmetamorphosed glochidia or free-living juveniles. When juveniles were detected, we siphoned tank bottoms daily until one week after finding the last juvenile mussel. Host fish were then examined for continued infection. When we found no further glochidial attachment the experiment ended.

To determine what effect cooling water temperatures might have on the time required for glochidia to metamorphose to juveniles, we compared metamorphosis rates in the above 17°C trial for channel catfish with an additional 12°C trial on 20 channel catfish. These temperatures were within the range of ambient values experienced by females in our study area when most were gravid and glochidia were being actively released (August--21°C through October--9°C). All other procedures were as described above.

RESULTS

Individuals in the sampled population of *M. nervosa* were sexually mature by at least age 8. During the period of peak reproductive development (July through September), eight-year-olds had well differentiated, active gonadal tissue (stages 3-4), but younger specimens often did not (Figure 1). Juveniles made up over 92% of individuals four years and younger. However, juveniles represented only about 10% of individuals between age 5 and 7. There were no juveniles $\geq$8 years. Specimens in full maturity (stages 3 and 4) made up 0%, 17%, 27%, 56%, and 93% of age 4, 5, 6, 7, and 8 specimens, respectively. We found one identifiable male as early as age 4, while most females were not distinquishable under age 6.

Oogenesis and spermatogenesis followed a seasonal pattern in the 220 adult mussels (72% females, 28% males) we examined (Figure 2). From October through June, females were predominantly in Stage 1 with widely spaced ovarian alveoli containing oogonia and a few small oocytes in the alveolar walls (Figure 3). Stage 1 development also was predominant in males during this period. By July, the majority of females (84%) and males (74%) had developed to Stage 3. Swollen gonado-visceral masses were common from August to October in males with release of gametes probable. Beginning in August, ova moved into the marsupial gills (Stage 4) of females where glochidia differentiated. This change followed a declining ambient river temperature (Figure 2). Stage 4 females comprised 87% of August and 100% of September samples, but only 25% and 7% of October and November samples, respectively. September through November releases of glochidia occurred as evidenced by the change from Stage 4 to Stage 1 female development.

The age-to-length and age-to-height functions for *M. nervosa* were both significant. The age-to-length relationship was length=6+30 ln[age] ($P=\leq 0.01$, $r^2=.86$). The age-to-height relationship was height=4+44 ln[age] ($P=\leq 0.01$, $r^2=.85$).

In host specificity studies, we found glochidia of *M. nervosa* metamorphosed to free-living juveniles on three of the fish species. Hyaline threads of glochidia initially wrapped around the fins and barbels and the glochidia trailed behind all fish species tested. Glochidia metamorphosed in 26 to 28 days at 17°C on channel catfish, black bullheads, and green sunfish. Metamorphosis began in 56 days for channel catfish held at 12°C (Table 1). Number of juveniles produced per tank varied from $\leq$ 10 in green sunfish, black bullhead, and cold-water (12°C) channel catfish trials to 152 in channel catfish held

at 17°C. In all other test species, glochidia attached to over 95% of individuals but, infections did not result in metamorphosed juveniles. Infections lasted only 1 to 3 days in cyprinids, but up to 26 days in white suckers and yellow perch with hypertrophied cysts containing glochidia dropping off throughout the infection period.

Table 1. Percent of fish (N=25) initially infected, duration of *Megalonaias nervosa* glochidial attachment, and juveniles produced for selected test host fish species.

Fish Species	Temperature (°C)	Infected (%)	Infection (days)	Juveniles
Black bullhead (Ictalurus melas)	17	100	28	9
Channel catfish (Ictalurus punctatus)	17	100	26	152
Channel catfish (Ictalurus punctatus)	12	100	56	10
Fathead minnow (Pimephales promelas)	17	100	3	0
Common carp (Cyprinus carpio)	17	95	1	0
White sucker (Catastomus commersoni)	17	100	26	0
Green sunfish (Lepomis cyanellus)	17	100	26	6
Yellow perch (Perca flavescens)	17	100	23	0

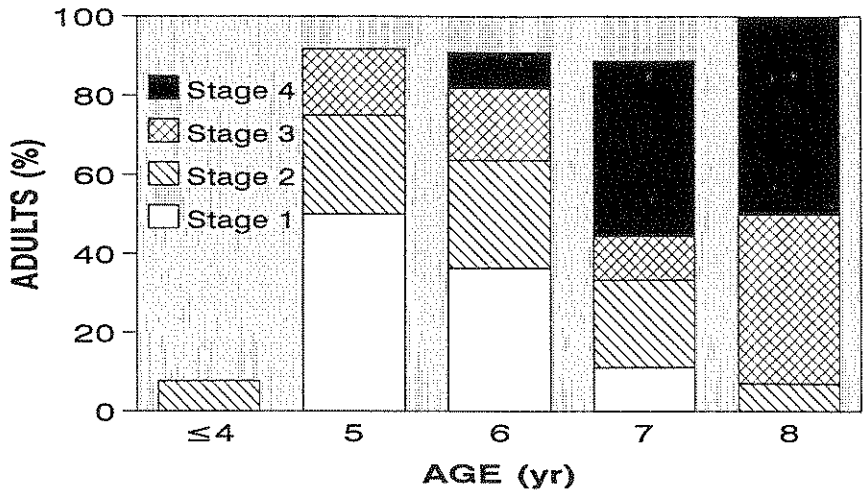

Figure 1. Developmental stages demonstrating reproductive maturity of young mussels (Age ≤4 to 8 years) during the peak reproductive season (July-September). Clear: Stage 1--some spermatogonia or oogonia present, gonadal material well differentiated; Hatched: Stage 2--spermatids present or developing ovocytes moving into lumina; Cross Hatched: Stage 3--spermatozoa or mature ova present; Black: Stage 4--female marsupia filled with eggs or glochidia.

DISCUSSION

Some disagreement has existed in the literature about the reproductive strategy employed by *M. nervosa*. Our study population clearly exhibited a short-term tachytictic

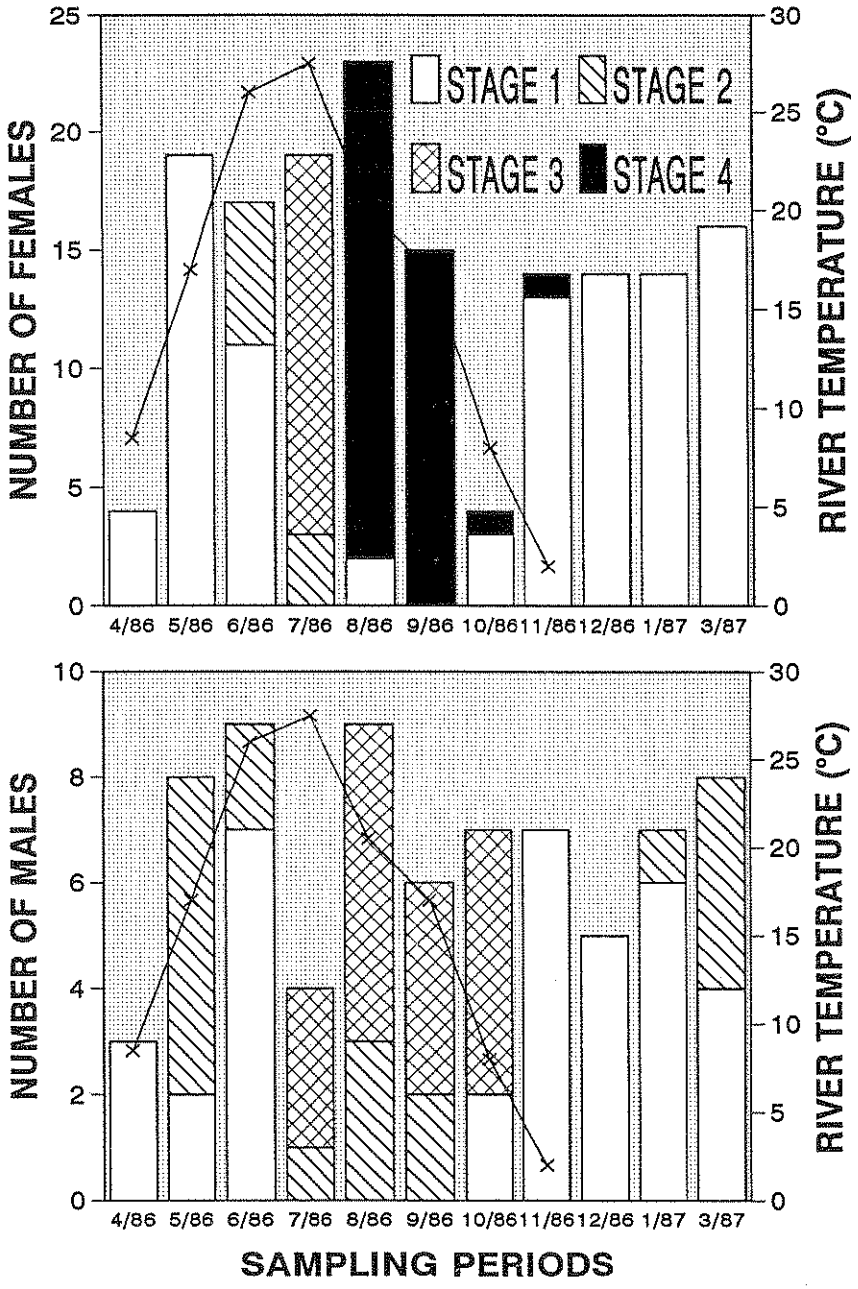

Figure 2. Seasonal reproductive development of adult females (A) and males (B). Clear: Stage 1--some spermatogonia or oogonia present, gonadal material well differentiated; Hatched: Stage 2--spermatids present or developing ovocytes moving into lumina; Cross Hatched: Stage 3--spermatozoa or mature ova present; Black: Stage 4--female marsupia filled with eggs or glochidia. River temperatures (°C) are also presented.

reproductive strategy. Fertilization and formation of glochidia occurred between July and August after river temperatures had peaked and were declining. Most glochidia likely were released between September (17°C) and November (3°C). This species' taxonomy and field observations by others (Howard 1914, Lefevre and Curtis 1910, Bingham 1968) support its classification as a short-term strategist. Coker et al. (1921), who suggest otherwise based on observations of gravid females from September through March and evidence of glochidial release from late autumn to winter, admit that they may be incorrect. It is likely that the timing of reproduction and glochidial release in this ectothermic organism is particularly sensitive to annual and latitudinal differences in river temperature patterns in late fall as suggested by Howard (1915). He reported that reproduction occurred in October in Iowa, but during later months farther south. Coker et al. (1921) and Howard (1915) agree, however, that *M. nervosa* retain their glochidia in a tachytictic manner for less time than do classic long-term breeders and that they easily

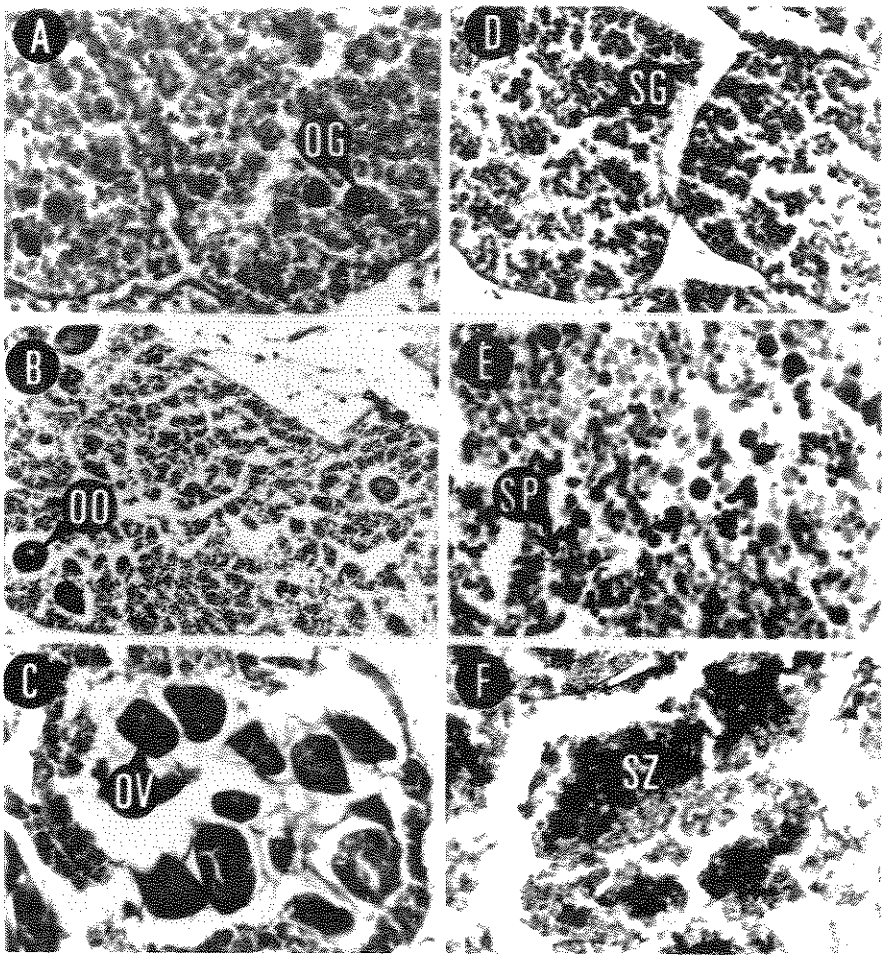

Figure 3. Histological slides of female (A-C) and male (D-F) developmental stages. A). Female stage 1 with developing oogonia (OG), B). Female stage 2 with oocyst (OO), C). Female stage 3 with developed ova (OV), D). Male stage 1 with spermatogonia (SG), E). Male stage 2 with spermatids (SP) and F). Male stage 3 with developed spermatozoa (SZ).

abort young when disturbed, another behavior of short-term strategists. Seasonal restrictions of commercial harvest activities to enhance the reproductive potential of the population might be effective if geographic and annual adjustments to regulations are made to accommodate variations in timing of reproduction and glochidial release. At present, few data for the Mississippi River population of *M. nervosa* exist to make this refinement possible.

Our seasonal and age-specific reproductive data agree with, but add significant additional detail to the reproductive information provided by Heath et al. (1988) through visual field observations on the same population. As in that study, we observed little gametogenic activity in mussels $\leq$ 4 years of age. We did find 100% activity by age 8 and could identify the sex of >90% of mussels at age 5. As in Health's study, we found females to be gravid in late summer, with few females still gravid in November (Heath: 5 out of 185 females, our study: 1 out of 19 females). In addition, we determined seasonal development in males, which cannot be done through field observation.

Fuller (1978) summarized the suspected hosts for *M. nervosa* which included species from nine families from the primitive Amiadae to the more advanced Sciaenidae. We tested representatives from five of those families, but only duplicated two of the species, black bullhead and channel catfish, both of which we confirmed as hosts. We added the green sunfish to the four species of sunfishes previously identified as hosts in the literature. Although representatives from perch and sucker families maintained glochidial attachments for several weeks, we do not view them as viable hosts because they produced no juveniles. As in other species (e.g. *Lampsilis higginsi* Waller and Holland-Bartels [1988]), host specificity is likely not consistent within taxonomic family or even genus. However, our data do confirm that *M. nervosa* employs a wide taxonomic range of fish hosts. Our study was not designed to address the relative quality of hosts, but we did find that the 17°C channel catfish trial produced 15 to 25 times more juveniles than did the trials of other verified hosts exposed to glochidia in a similar manner.

The duration of the parasitic period of *M. nervosa* was inversely related to temperature, as has been observed for other unionids (Lefevre and Curtis 1912). Although glochidia metamorphosed in 26-28 days at 17°C, it took 56 days at 12°C. Based on these data, glochidia released into the river in October (9°C) likely would start to metamorphose at about 70 days. However, temperatures in the study area rapidly declined during the fall period to 3°C in November likely causing an extension of the host period beyond 70 days. We do not know if metamorphosed juveniles drop off their hosts in late fall or are retained until spring, since hosts have not been examined in the field.

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