Host Fishes and Reproductive Biology of 6 Freshwater Mussel Species From Mobile Basin, U.S.A.

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Host fishes and reproductive biology of 6 freshwater mussel species from the Mobile Basin, USA.

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Abstract. Host fishes were identified for 6 species of freshwater mussels (Unionidae) from the Black Warrior River drainage, Mobile Basin, USA: Strophitus subvexus, Pleurobema furvum, Ptychobranchus greeni, Lampsilis perovalis, Medionidus acutissimus, and Villosa nebulosa. Hosts were determined as those that produced juvenile mussels from glochidial infestations in the laboratory. The following mussel - fish-host relationships were established: Strophitus subvexus with 10 species including Cyprinidae, Catostomidae, Fundulidae, Centrarchidae, and Percidae; Pleurobema furvum with Campostoma oligolepis, Cyprinella callistia, C. venusta, Semotilus atromaculatus, and Fundulus olivaceus; Ptychobranchus greeni with Etheostoma bellator, E. douglasi, Percina nigrofasciata, and Percina sp. cf. caprodes; Lampsilis perovalis with Micropterus coosae, M. punctulatus, and M. salmoides; Medionidus acutissimus with Fundulus olivaceus, Etheostoma douglasi, E. whipplei, Percina nigrofasciata, and Percina sp. cf. caprodes; and Villosa nebulosa with Lepomis megalotis, Micropterus coosae, M. punctulatus, and M. salmoides. Fundulus olivaceus served as host for 3 species and carried glochidia for long periods for 2 other species, suggesting that topminnows may serve as hosts for a wide variety of otherwise host-specialist mussel species. Host relationships for the species tested are similar to congeners. Methods of glochidial release, putative methods of host-fish attraction, and gravid periods are described for the 6 species.
Key words: Mollusca, Bivalvia, Unionidae, mussels, host fish, glochidia, life history, conglutinates.

Freshwater bivalves of the superfamily Unionacea have a highly specialized life history in which the modified veliger larvae (glochidia) undergo a brief period as obligate ectoparasites on the gills, fins, or other external parts of fish. Glochidia are brooded in the gills of female mussels until mature, then released through the siphons singly or in clusters called conglutinates (Kat 1984). If the glochidia encounter a suitable host, they encyst for a few days to several weeks, metamorphose into juvenile mussels, and then drop off the fish to assume a benthic lifestyle. Glochidia encountering an unsuitable host are rejected by the fish immune system, usually within a few days. Host specificity varies greatly among mussel species. Some species are able to parasitize a taxonomically wide variety of fish species (Trdan and Hoeh 1982), but others can use only a few, usually closely related, species (Zale and Neves 1982, Yeager and Saylor 1995).

Knowledge of fish hosts is essential in understanding patterns of distribution and abundance of mussels (Watters 1992) and in developing effective conservation programs. Lack of recruitment in some mussel populations has been attributed to unavailability of proper host fishes caused by human-induced changes in the fish community (Mathiak 1979, Smith 1985). Conservation efforts, whether centered on captive propagation and reintroduction, relocation, or habitat improvement, require comprehensive host information.

Knowledge of host fishes is lacking or incomplete for many North American mussels. In particular, there is a conspicuous dearth of information for species endemic to Atlantic and Gulf Slope drainages, including the Mobile Basin. Most species endemic to the Mobile Basin have experienced dramatic range reductions in the last 50 y (Stansbery 1976, Williams et al. 1992). Several Mobile Basin species have become extinct in this century, and 17 species are listed federally (USA) as endangered or threatened (US FWS 1994). Host fish information is needed urgently for conservation and recovery of the remaining Mobile Basin mussel fauna.

We determined hosts, report observations on glochidial release and putative host-attracting behavior, and report periods of gravidity for Lampsilis perovalis, Medionidus acutissimus, Pleurobema furvum, Ptychobranchus greeni, Strophitus subvexus, and Villosa nebulosa. With the exception of S. subvexus, all are endemic to the Mobile Basin. Strophitus subvexus occurs in Gulf Slope drainages from east Texas to Florida (Williams and Butler 1994). Although Zale and Neves (1982) and Neves et al. (1985) provided host-fish data for V. nebulosa from the Tennessee River system, these populations are now referred to as V. iris (Williams et al. 1993, Yeager et al. 1994). Pleurobema furvum and P. greeni are listed as endangered, and L. perovalis and M. acutissimus are listed as threatened by the US Fish and Wildlife Service (US FWS 1994). Strophitus subvexus and V. nebulosa are considered of special concern and threatened, respectively, by the American Fisheries Society (Williams et al. 1993). These species represent a broad cross-section of unionid diversity including the subfamily Anodontinae (S. subvexus), the tribe Pleurobemini (P. furvum), and the lampsiline clade (L. perovalis, M. acutissimus, P. greeni, and V. nebulosa) (sensu Lydeard et al. 1996).

Methods

Mussels and fishes were collected mainly from tributaries of the Black Warrior River in Lawrence and Winston counties, northwestern Alabama, USA. Mussels were collected from Brushy and Flannagin creeks and the Sipsey Fork Black Warrior River. These streams are within William B. Bankhead National Forest and harbor diverse, remnant examples of the Mobile Basin upland mussel fauna. Fishes were collected from Clear and Hubbard creeks and the Sipsey Fork. Additional fishes were collected from small streams in Lafayette and Panola counties, northern Mississippi, USA. Ictalurus punctatus and Micropterus salmoides were obtained from hatchery stock.

When possible, potential host fishes were collected from streams without mussels or streams with low mussel densities in order to avoid using fish with existing glochidial infestations or acquired immunity to glochidia (Zale and Neves 1982). Some fish species could not be obtained from streams other than those with high mussel densities (e.g., Etheostoma douglasi, Percina shumardi). These species were collected in early February before natural infestations normally occur in the study streams (W.R. Haag and M.L. Warren, unpublished data) and held at room temperature (22 - 25°C) for at least 4 wk before being used in host trials to ensure that any glochidia from natural infestations had excysted.

Host fishes were determined by inducing glochidial infestations in laboratory trials and monitoring the rejection of glochidia or production of juvenile mussels (Zale and Neves 1982). For each mussel species, replicate trials were run using glochidia from 2 - 3 gravid females. Glochidia from each species were exposed to 14 - 20 fish species. Fish species were chosen to represent most families and genera and all common species present at sites inhabited by the mussels.

Study streams were searched for gravid female mussels from February to late June, 1996 and water temperature was recorded on each sample date. Gravid mussels were brought into the laboratory and placed into individual beakers at room temperature. Observations were made of the behavior of gravid females and the method of glochidial release. Lampsilis perovalis, P. furvum, and P. greeni released conglutinates within several days of exposure to room temperature. Medionidus acutissimus, S. subvexus, and V. nebulosa did not release glochidia in the laboratory. Glochidia were obtained from these species by flushing water through the gills with a hypodermic syringe. All females were returned to their stream of origin after yielding glochidia because of the threatened or endangered status of some of the species. Representative shells of each species are deposited at the Ohio State University Museum of Biological Diversity, Columbus, Ohio, USA, and the Mississippi Museum of Natural History, Jackson, Mississippi, USA. Glochidia were considered mature and able to parasitize fish if they snapped shut after exposure to synthetic aquarium salt. Immediately prior to trials, conglutinates or other aggregations of glochidia were teased apart with a fine probe and suspended in water in a small beaker.

Potential host fishes were anaesthetized individually in tricaine methanesulfonate and inspected for existing glochidial infestations. Only 1 individual was found with a natural infestation (Cyprinella callistia with 1 glochidium of Strophitus subvexus), and this fish was not used in trials. A suspension of glochidia was applied with a pipette directly onto the right gill (0.5 - 1.5 mL) of anaesthetized fishes until > 10 glochidia could be seen attached to the outer surface of the outer gill. After recovery from anaesthetic, all individuals of a species were placed in aquaria (6 - 25 L) with aeration in a temperature-controlled environmental chamber. The first 2 trials for Lampsilis perovalis and S. subvexus were run at a mean temperature of 17°C (range 16 - 18°C). All other trials were run at a mean temperature of 20°C (range 19 - 21°C).

For most trials, a subsample of individuals of each fish species was anaesthetized 1 - 2 d after infestation to determine if rejection of glochidia had occurred. If no glochidia were found on gills of subsampled fish, all other individuals of that species were inspected, and the trial was terminated when all individuals had rejected all glochidia. If subsampled individuals carried glochidia, they were revived, returned to the aquaria, and the trial was continued. Fish species that rejected all glochidia and produced no juvenile mussels were considered non-host species. For species that varied among individuals in time to rejection, a range of days to rejection was established using the first day that clean individuals were found and the day that the last clean individual was found. For some trials, fish were not inspected soon after infestation, and the reported number of days to rejection for these trials probably overestimates the time to rejection.

After a species carried glochidia for > 10 d, we inspected material on the bottom of the aquarium approximately every 3 d to search for transformed juvenile mussels. Material was collected with a flexible hose attached to a vacuum pump, concentrated on a 100-um mesh sieve, washed into a gridded petri dish, and examined under a stereomicroscope. Juvenile mussels were distinguished from rejected glochidia by the presence of a foot and mantle and were usually crawling on the bottom of the petri dish. Juvenile mussels were counted and preserved in 70% EtOH. Inactive juveniles with a recognizable foot and mantle were counted separately because, although they had clearly undergone some degree of metamorphosis as evidenced by the presence of juvenile anatomical features, we question if these individuals represented viable offspring. From all confirmed host species, low numbers of inactive juveniles were collected several days to a week before the appearance of active juveniles, but we also collected inactive juveniles from species that later produced no active individuals. Hove and Neves (1994) described empty valves identical to those of living juveniles as premetamorphosed juveniles and similarly questioned whether or not these were indicative of a successful parasitization. If a fish species carried glochidia for several weeks but produced no juveniles, individuals were anaesthetized and inspected for encysted glochidia on an irregular basis until all fish were clean. Only fish that produced active juveniles were considered hosts. Fishes are listed in tables in phylogenetic sequence by family (Mayden et al. 1992) and alphabetically within families.

Results

Females of Medionidus acutissimus and Strophitus subvexus were found gravid with mature glochidia from 26 February to 12 March 1996 (water temperatures 8 - 13°C). Lampsilis perovalis and Villosa nebulosa were found gravid from 26 February to 2 April 1996 (water temperature 8 - 13°C). Ptychobranchus greeni were found gravid on 16 April (water temperature 13°C), and Pleurobema furvum, from 18 June to 25 June 1996 (water temperature 25°C).

These species displayed striking diversity in strategies used to infect host fishes. Glochidia of Strophitus subvexus were bound in a copious mucous matrix which, when released in the stream, may serve to tangle fish indiscriminately. Release of glochidia in a loose muscous web has been reported for at least two other anodontines, Anodonta cygnea (Wood 1974) and Anodontoides ferussacianus (Hove et al. 1995). Pleurobema furvum and Ptychobranchus greeni released glochidia in conglutinates that drift passively with the stream current, free from the gravid female, and may mimic food items of darters and minnows. Conglutinates of P. furvum were subcylindrical, flattened, and peach-to-pink colored and similar to those described for species of Pleurobema and Fusconaia (Bruenderman and Neves 1993, Hove and Neves 1994). The conglutinates of P. greeni were round and pearl-colored with 2 black eyespots and strongly resembled fertilized fish eggs. This observation is in contrast to previous descriptions of Ptychobranchus conglutinates as elongate structures resembling newly hatched larval fish (Morrison 1973, species not given) or dipteran larvae (Hartfield and Hartfield 1996, for P. greeni). Lampsilis perovalis released glochidia in a superconglutinate, a glochidial packet pigmented and shaped like a small minnow and tethered to the female, as previously described (Haag et al. 1995). Medionidus acutissimus and Villosa nebulosa displayed modified mantle margins with tentacles and flaps which may serve to attract fishes to the gravid mussel.

Glochidia of *Strophitus subvexus* metamorphosed into juvenile mussels on 10 species of fish in 5 families, and results were equivocal for 3 other species including 1 additional family (Table 1). *Hypentelium etowanum* and *Etheostoma douglasi* produced the highest number of juveniles. All other species produced low to moderate numbers of juveniles, but this was probably because many fish died before completion of the trials. *Ictalurus punctatus* and *Micropterus coosae* carried glochidia for an extended period and produced only inactive juveniles. *Notropis asperifrons* carried glochidia for 6 d, but all fish died before termination of the trial.

Glochidia of *Pleurobema furvum* metamorphosed into juvenile mussels on 1 species of topminnow, *Fundulus olivaceus*, and 4 species of minnows: *Campostoma oligolepis*, *Cyprinella callistia*, *C. venusta*, and *Semotilus atromaculatus* (Table 2). *Campostoma oligolepis* and *F. olivaceus* produced low numbers of juveniles and *C. oligolepis* produced juveniles in less time than other host species. Three other cyprinids, *Lythrurus bellus*, *Notropis asperifrons*, and *N. stilbius*, quickly rejected glochidia.

Glochidia of *Ptychobranchus greeni* metamorphosed into juvenile mussels on 4 species of darters: *Etheostoma bellator*, *E. douglasi*, *Percina nigrofasciata*, and *Percina* sp. cf. *caprodes* (Table 3). Two other darters, *E. whipplei* and *P. shumardi*, did not serve as hosts. One individual of *E. whipplei* carried glochidia for 23 d, but all other individuals rejected glochidia in < 8 d. *Percina shumardi* carried glochidia for at least 28 d, but all individuals were clean after 53 d, and no juveniles were found. Similarly, one species of topminnow, Fundulus olivaceus, carried glochidia for an extended period but died before termination of the trial and produced no juveniles.

Glochidia of Lampsilis perovalis metamorphosed on 3 species of sunfishes (Centrarchidae): Micropterus coosae, M. punctulatus, and M. salmoides (Table 4). Two other sunfishes, Lepomis macrochirus and L. megalotis, and one topminnow (Fundulidae), Fundulus olivaceus, carried glochidia for extended periods but produced no juveniles. One specimen of pickerel (Esocidae), Esox niger, died after 4 d but carried a heavy infestation at the time of death.

Glochidia of Medionidus acutissimus metamorphosed into juvenile mussels on 1 species of topminnow, Fundulus olivaceus, and 4 species of darters: Etheostoma douglasi, E. whipplei, Percina nigrofasciata, and Percina sp. cf. caprodes (Table 5). Fundulus olivaceus produced low numbers of juveniles in less time than on other hosts.

Glochidia of Villosa nebulosa metamorphosed on 4 species of sunfishes: Lepomis megalotis, Micropterus coosae, M. punctulatus, and M. salmoides (Table 6). Lepomis megalotis produced fewer juveniles in less time than Micropterus in one trial, but rejected all glochidia in the other. Two other sunfishes, Lepomis cyanellus and L. macrochirus, carried glochidia for an extended period, but L. cyanellus produced no juveniles, and L. macrochirus, only inactive juveniles. One species of topminnow, Fundulus olivaceus, carried glochidia for 14 d but produced no juveniles.

Discussion

The 6 species of mussels in this study varied in host use. Strophitus subvexus (subfamily Anodontinae) was a host generalist, using a taxonomically wide array of fish species. The other 5 species were host specialists for which host use largely followed generic or familial lines. Pleurobema furvum (tribe Pleurobemini) used minnows (Cyprinidae) and topminnows. Two species in the lampsiline clade, Lampsilis perovalis and Villosa nebulosa, used sunfishes as hosts (Centrarchidae, primarily basses, Micropterus spp.). The chain pickerel (Esox niger) may also serve as a host for L. perovalis; specimens are infected in the wild (W.R. Haag and M.L. Warren, unpublished data), but we were unable to confirm this species as host due to death of test fishes. Two other species in the lampsiline clade, Medionidus acutissimus and Ptychobranchus greeni, used darters (Percidae) and topminnows (Fundulidae).

Patterns of host-fish use for these 6 species are consistent with host information for congeners from other drainages. Strophitus undulatus, native to the Mississippi River drainage and several Atlantic Slope drainages, can use as hosts at least 11 species of fishes representing four families (Hove et al. 1997). Several other species in the subfamily Anodontinae are also host generalists (Trdan and Hoeh 1983, Watters 1994, Barnhart et al. 1997) although the subfamily does contain some specialists (Watters 1994, Michaelson and Neves 1995). The majority of hosts for Pleurobema (Pleurobemini) are minnows (Yokely 1972, Weaver et al. 1991, Hove and Neves 1994). Most species in the lampsiline clade use either sunfishes or darters as hosts. All species of Lampsilis for which hosts are identified can use sunfishes, especially basses (Micropterus spp.), although some species also can use large piscivorous percids (Perca and Stizostedion) or esocids (Esox)(Zale and Neves 1982, Waller and Holland-Bartels 1988). Other species of Medionidus and Ptychobranchus use darters as hosts (Zale and Neves 1982, Barnhart et al. 1997).

Fundulus olivaceus served as host for 3 species (Medionidus acutissimus, Pleurobema furvum, and Strophitus subvexus) and carried glochidia of 2 others (Lampsilis perovalis and Ptychobranchus greeni) for an extended period. Because F. olivaceus produced few or no juvenile mussels/fish and in less time than other hosts, it may be only a marginally suitable host for these mussels. However, other members of the order Cyprinodontiformes apparently serve as hosts for a variety of mussel species. Fundulus diaphanus is implicated as host for six genera (Watters 1994). Gambusia affinis (Poeciliidae) served as host for Villosa iris (Neves et al. 1985, as V. nebulosa), 3 species of Anodontinae (Watters 1994), and carried glochidia of Pleurobema collina for 45 d but produced no juveniles (Hove and

Neves 1994). Gambusia holbrooki serves as host for at least 9 mussel species in 6 genera in Atlantic and Gulf Slope drainages (C. O'Brien, National Biological Service, personal communication). Poecilia reticulata (as Lebistes reticulata, Poeciliidae), a cyprinidontiform not native to North America, served as host for Lasmigona compressa (Tompa 1979) and serves as host for at least 5 species in 5 genera in Atlantic and Gulf Slope drainages (C. O'Brien, National Biological Service, personal communication). Although it is well established that some mussel species are generalists in their host fish use, little is known about fishes that may be able to serve as hosts for many, unrelated mussel species. Fishes of the families Fundulidae and Poeciliidae may act as "universal hosts" for many otherwise host-specific mussel species. However, in at least some cases, these species may be only marginally suitable hosts, and in nature, the surface-feeding mode of most cyprinodontiform fishes may render them less vulnerable to infestation by host-attracting mechanisms targeted at fishes in benthic or mid-water feeding guilds.

Other fishes identified as hosts in this study gave equivocal results indicating that these species also may be only marginally suitable hosts. These species produced consistently low numbers of juveniles, gave inconsistent results among replicate trials, produced only inactive juveniles, and/or produced juveniles in less time than other hosts. The sunfish Lepomis megalotis and possibly L. macrochirus appear to be marginal hosts for Lampsilis perovalis and Villosa nebulosa; Campostoma oligolepis appears marginally suitable for Pleurobema furvum. Further work is needed to determine the significance in the wild of these marginally suitable hosts.

Most fishes identified as hosts gave consistent results among trials and produced moderate to high numbers of juveniles. Further, in most cases, host suitability for a mussel species was similar among congeneric fish species. An exception involved the use of darters as hosts by Ptychobranchus greeni. Two species each of Etheostoma and Percina produced juvenile mussels, but E. whipplei and P. shumardi failed to produce juveniles (Table 4). Percina shumardi carried glochidia for > 4 wk but produced no juveniles and may serve as a marginal host for P. greeni. Etheostoma whipplei rejected all glochidia in < 8 d in 2 trials with the exception of 1 individual that carried for 23 d. This species is clearly not a suitable host for P. greeni. In contrast, glochidia of Medionidus acutissimus metamorphosed on all species of darters tested.

One of the most important niche dimensions for freshwater mussels may be host-fish use. For host-specific mussel species, methods for infecting hosts apparently have evolved to exploit predator-prey relationships and feeding guilds of hosts. In contrast, methods of glochidial release in host-generalist mussel species may function to increase the likelihood of chance encounters with a wide variety of fishes, irrespective of feeding guild. Two extreme examples of this are illustrated by species in this study. Lampsilis perovalis, a host specialist restricted to use of basses (Micropterus spp.) and perhaps pickerel (Esox spp.), releases its glochidia in a minnow-shaped mass that may attract piscivorous fish and be too large to be ingested by most fishes but bass or pickerel (Haag et al. 1995). In contrast, glochidia of Strophitus subvexus, a host generalist, are bound in a mucous matrix which may be released as a loose web to ensnare fish indiscrimate of feeding guild or size.

Knowledge of host fishes is crucial to understanding the ecology of freshwater mussels. However, related factors such as the relationship among modes of glochidial release, natural history attributes of host-fishes, and host-fish density may have important effects on mussel reproductive success. Further study of these relationships may provide insight in explaining distribution and abundance patterns of mussels and in understanding causes for the precipitous decline of many mussel species during the past 50 years.

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Table 1. Results of host trials for _Strophitus subvexus_. Letters A-C represent replicate trials using glochidia from 3 individual mussels. An asterisk (*) denotes that all fish died before termination of the trial, and the number in parentheses represents the maximum number of days before death that fish carried glochidia. (#) indicates that only non-active juveniles were produced from trial. A dash (--) denotes that the fish species was not used in the trial.

	Number tested			Days to transformation			Mean number juveniles/fish		
Fishes	A	B	C	A	B	C	A	B	C
Campostoma oligolepis	3	4	4	*(7)	*(9)	14	0*	0*	<1
Cyprinella callistia	3	3	4	*(11)	*(9)	14	0*	0*	1
Notropis asperifrons	--	--	2	--	--	*(6)	--	--	0*
Semotilus atromaculatus	3	3	1	13*	*(10)	14-24	1*	0*	4
Hypentelium etowanum	3	3	2	13-15*	7-15*	14-28	2*	1*	25
Ictalurus punctatus	10	10	3	--	*(10)	10-14#	0	0*	0#
Fundulus olivaceus	2	3	4	15*	*(7)	*(5)	<1*	0*	0*
Lepomis megalotis	4	4	--	13-21	13-21	--	3	2	--
Micropterus coosae	--	--	1	--	--	10-14#	--	--	0#
M. salmoides	5	5	4	*(7)	*(7)	10-14	0*	0*	2

Etheostoma douglasi	5	4	--	13-15	13-28	--	1	10	--
E. whipplei	--	--	2	--	--	10	--	--	<1
Percina nigrofasciata	3	3	--	13-19	13-21	--	2	2	--

Table 2. Results of host trials for *Pleurobema furvum*. Letters A-C represent replicate trials using glochidia from 3 individual mussels. An asterisk (*) denotes that all fish died before termination of the trial. For non-host species, a dagger (+) denotes that fish were not inspected before this day and rejection likely occurred before the day indicated. A dash (--) denotes that the fish species was not used in the trial.

	Number tested			Days to transformation (hosts) or rejection (non-hosts)			Mean number juveniles/fish		
Fishes	A	B	C	A	B	C	A	B	C
Hosts									
Campostoma oligolepis	6	10	3	15	13-15	--	<1	1	0
Cyprinella callistia	8	15	5	20-25	19-25	18-21	5	3	6
C. venusta	--	2	2	--	21-25	18-27	--	12	27
Semotilus atromaculatus	2	4	2	20-28	19-27	*	26	1	*
Fundulus olivaceus	5	5	3	--	21	14	0	<1	<1
Non-hosts									
Lythrurus bellus	--	1	--	--	5	--			
Notropis asperifrons	--	12	5	--	1	1			
N. stilbius	2	5	--	7+	1	--			
Hypentelium etowanum	-	2	2	--	2	1			
Ictalurus punctatus	3	3	--	4+	2	--			

Lepomis megalotis	4	3	--	4^+	2	--
Micropterus coosae	1	1	--	4^+	2	--
M. salmoides	3	1	--	4^+	2	2
Etheostoma douglasi	1	--	--	4^+	--	--
E. whipplei	3	4	--	4^+	2-5	--
P. nigrofasciata	4	4	--	4^+	2	--
Percina sp. cf. caprodes	--	1	--	--	2	--

Table 3. Results of host trials for *Ptychobranchus greeni*. Letters A and B represent replicate trials using glochidia from 2 individual mussels. An asterisk (*) denotes that all fish died before termination of the trial. For non-host fishes, a dagger (+) denotes that fish were not inspected before this day and rejection likely occurred before the day indicated. A dash (--) denotes that the fish species was not used in the trial.

	Number tested		Days to transformation (hosts) or rejection (non-hosts)		Mean number juveniles/fish	
Fishes	A	B	A	B	A	B
Hosts						
Etheostoma bellator	1	--	30-34	--	5	--
E. douglasi	8	4	28-55	38-51	16	6
Percina nigrofasciata	6	4	26-55	32-40*	28	4*
Percina sp. cf. *caprodes*	--	1	--	48-54	--	9
Non-hosts						
Campostoma oligolepis	7	4	2-5	5+		
Cyprinella callistia	8	2	2	5+		
Semotilus atromaculatus	5	3	2-5	5+		
Hypentelium etowanum	5	--	1-2	--		

Ictalurus punctatus	--	3	--	5^{+}
Fundulus olivaceus	--	5	--	17*
Lepomis macrochirus	4	--	2-5	--
L. megalotis	2	2	2-5	7^{+}
Micropterus coosae	2	--	2-5	--
M. punctulatus	2	--	2	--
M. salmoides	--	5	--	5-6
Etheostoma whipplei	8	4	5-23	8^{+}
Percina shumardi	3	--	>28	--

Table 4. Results of host trials for *Lampsilis perovalis*. Letters A-C represent replicate trials using glochidia from 3 individual mussels. An asterisk (*) denotes that all fish died before termination of the trial. For non-host species, a dagger (+) denotes that fish were not inspected before this day and rejection likely occurred before the day indicated. A dash (--) denotes that the fish species was not tested in the trial.

Fishes	Number tested			Days to transformation (hosts) or rejection (non-hosts)			Mean number juveniles/fish		
	A	B	C	A	B	C	A	B	C
Hosts									
Micropterus coosae	1	2	2	46-53	38-59	21-34	28	72	51
M. punctulatus	--	1	1	--	*	21-47	--	*	325
M. salmoides	6	5	5	*	*	26-49	*	*	125
Non-hosts									
Campostoma oligolepis	5	4	--	11+	13+	--			
Cyprinella callistia	6	5	--	4+	4+	--			
C. venusta	3	2	--	14+	13+	--			
Notropis asperifrons	5	4	--	3	2-3	--			
Semotilus atromaculatus	6	5	1	6+	13+	3			
Hypentelium etowanum	5	4	--	1-2	15+	--			

Ictalurus punctatus	5	5	--	18^+	17^+	--
Fundulus olivaceus	3	4	2	$4-39^+$	19^+	25^+
Ambloplites ariommus	--	--	1	--	--	7
Lepomis cyanellus	--	--	2	--	--	7
L. macrochirus	5	6	2	7-9	17-25	7
L. megalotis	5	4	3	7-20	12	7
Etheostoma douglasi	5	5	--	14^+	16^+	--
E. whipplei	2	3	--	19^+	18^+	--
Percina nigrofasciata	6	4	--	14^+	16^+	--
P. shumardi	1	1	--	19^+	16^+	--
Percina sp. cf. caprodes	2	1	--	19^+	18^+	--

Table 5. Results of host trials for *Medionidus acutissimus*. Letters A and B represent replicate trials using glochidia from 2 individual mussels. An asterisk (*) denotes that all fish died before termination of the trial, and the number in parentheses represents the maximum number of days before death that fish carried glochidia. For non-host fishes, a dagger (+) denotes that fish were not inspected before this day and rejection likely occurred before the day indicated. A dash (--) denotes that the fish species was not tested in the trial.

Fishes	Number tested		Days to transformation (hosts) or rejection (non-hosts)		Mean number juveniles/fish	
	A	B	A	B	A	B
Hosts						
Fundulus olivaceus	5	3	*(8)	12-14	*	1
Etheostoma douglasi	5	--	36-53	--	5	--
E. whipplei	7	3	29-53	25-39	30	12
Percina nigrofasciata	7	4	19-53	25-35	4	6
Percina sp. cf. *caprodes*	2	--	40-43	--	5	--

Non-hosts				
Campostoma oligolepis	4	1	4^+	1
Cyprinella callistia	4	2	4^+	1
Notropis asperifrons	2	3	4^+	1
Semotilus atromaculatus	5	--	4^+	--
Hypentelium etowanum	3	1	4^+	3
Ictalurus punctatus	3	--	4^+	--
Lepomis macrochirus	2	--	4^+	--
L. megalotis	4	2	4^+	4-8
Micropterus coosae	1	1	4^+	4
M. salmoides	5	1	4-8	3

Table 6. Results of host trials for *Villosa nebulosa*. Letters A and B represent replicate trials using glochidia from 2 individual mussels. An asterisk (*) denotes that all fish died before termination of trial. (#) indicates that only inactive juveniles were produced from the trial. A dash (--) denotes that the fish species was not used in the trial.

Fishes	Number tested		Days to transformation (hosts) or rejection (non-hosts)		Mean number juveniles/fish	
	A	B	A	B	A	B
Hosts						
Lepomis megalotis	3	7	21-23	--	2	0
Micropterus coosae	2	1	23-39	22-37	16	7
M. punctulatus	1	2	25-32	22-31*	5	6*
M. salmoides	--	3	--	22-46	--	178
Non-hosts						
Campostoma oligolepis	4	6	2-7	2-7		
Cyprinella callistia	4	--	2	--		
C. camura	--	5	--	2		
Notropis asperifrons	2	2	2	1		
Semotilus atromaculatus	3	1	3-7	2		

Hypentelium etowanum	3	--	3	--
Fundulus olivaceus	--	6	--	14
Lepomis cyanellus	--	1	--	14
L. macrochirus	3#	2	7-16	2-4
L. megalotis	3	7	--	2-14
Etheostoma bellator	1	--	3	--
E. douglasi	4	--	3-7	--
E. whipplei	4	5	7	4
Percina nigrofasciata	4	5	7	4-7
Percina sp. cf. caprodes	1	1	3	2