

An Evaluation of Host Fish Suitability for Glochidia of *Villosa vanuxemi* and *V. nebulosa* (Pelecypoda: Unionidae)

RICHARD J. NEVES

LYNN R. WEAVER¹

Virginia Cooperative Fishery Research Unit², Department of Fisheries and Wildlife Sciences, Virginia Polytechnic Institute and State University, Blacksburg 24061

and

ALEXANDER V. ZALE

Florida Cooperative Fish and Wildlife Research Unit³, Newins-Ziegler Hall, University of Florida, Gainesville 32611

ABSTRACT: Fish species congeneric with previously identified hosts, as well as exotic fishes (*Xiphophorus variatus* and *Tilapia aurea*) and the mosquitofish (*Gambusia affinis*), were exposed to glochidia of *Villosa vanuxemi* or *V. nebulosa* to determine whether phylogenetically similar fishes can serve as hosts. Glochidia of *V. vanuxemi* metamorphosed on black (*Cottus baileyi*), mottled (*C. bairdi*) and slimy (*C. cognatus*) sculpins, and glochidia of *V. nebulosa* metamorphosed on spotted (*Micropterus punctulatus*), largemouth (*M. salmoides*) and Suwannee (*M. notius*) basses and the mosquitofish. Exotic fishes were unsuitable hosts. A review of previous *in vivo* and *in vitro* studies suggests that chemical components of the blood serum in fishes, as yet unidentified, dictate host suitability to specific glochidia.

INTRODUCTION

Glochidia of freshwater mussels (naiades) are obligate parasites on the gills or fins of fishes and exhibit varying degrees of host specificity. If glochidia attach to the appropriate fish, they become encysted, metamorphose, and excyst to begin the free-living benthic stage of their life cycle. Each gravid female mussel releases hundreds of thousands of glochidia annually, some of which come in contact with suitable hosts. The reproductive success of mussel populations is, therefore, directly dependent on an abundance of host fishes to complete this critical stage in the life cycle.

Available data on freshwater mussels of the family Unionidae reveal obvious differences in species success during the present century, and indicate that reproductive and survival strategies have favored some taxa over others. Glochidia of mussels in the subfamily Anodontinae have ventral hooks and usually parasitize the fins of host fishes (Coker *et al.*, 1921). Mussels in this subfamily have a broad ecological tolerance to different aquatic environments, demonstrate a eurytopic use of host fishes, and are widely distributed and successful in both lotic and lentic habitats (Trdan and Hoeh, 1982). As an example of nonspecificity to hosts, glochidia of the anodontine *Lasmigona compressa* have been shown to metamorphose on the guppy *Poecilia reticulata*, an exotic fish from South America (Tompa, 1979). In contrast to this apparently generalist life history capability, mussels in the subfamily Ambleminae are gill parasites with unhooked

¹ Present address: 4907 Pine Street, Wilmington, N.C. 28403.

² The Virginia Unit is jointly supported by the United States Fish & Wildlife Service, the Virginia Commission of Game and Inland Fisheries, and Virginia Polytechnic Institute and State University.

³ The Florida Unit is jointly supported by the United States Fish and Wildlife Service, the Florida Game and Fresh Water Fish Commission, and the University of Florida.

glochidia, appear to exhibit a greater degree of host fish specificity, have generally more specific habitat requirements, and have declined in abundance and distribution during this century. It appears that the known fish species that host each mussel species in this subfamily are of similar phylogeny (same genus or family) and are relatively fewer in number than in the subfamily Anodontinae (Fuller, 1974).

Burch (1975) recognized 15 species in the genus *Villosa* of North America, most of them restricted to southern river drainages and having unknown fish hosts. The high degree of host specificity for some naiades is best exemplified in a recent study by Zale and Neves (1982) of two *Villosa* species endemic to the Cumberland Plateau region, southeastern United States. Their field and laboratory research indicated that glochidia of these two species parasitized only three of the 24 fish species in Big Moccasin Creek, southwestern Virginia: *V. vanuxemi* parasitized the banded sculpin (*Cottus carolinae*) and *V. nebulosa*, the smallmouth bass (*Micropterus dolomieu*) and rock bass (*Ambloplites rupestris*). The present study was conducted to: (1) determine whether fish species congeneric with previously identified hosts could be hosts of glochidia of *Villosa vanuxemi* and *V. nebulosa*; (2) determine whether exotic fish species (*cf.*, Tompa, 1979) would host the glochidia of *V. nebulosa*, and (3) discuss possible mechanisms that may contribute to host specificity.

MATERIALS AND METHODS

Gravid females of *Villosa vanuxemi* and *V. nebulosa* were collected in spring and autumn, 1980-1982, from Big Moccasin Creek, Russell Co., Virginia. Physical and chemical attributes of this pasture stream and a description of the mussel assemblage (seven species) were presented by Zale and Neves (1982). Specimens were transported to the laboratory on ice, and females were kept in an aerated, recirculating tank. Premature glochidial expulsion was prevented by maintaining water temperatures at roughly 10 C.

Most fishes to be experimentally exposed to glochidia were obtained by electrofishing in streams or river sections without mussels (based on recent naiad surveys) to avoid the possibility that the fish were immune as a result of previous infestations (Arey, 1923). All experiments with fishes collected in Virginia were conducted at Virginia Polytechnic Institute and State University, and trials with Florida fishes were completed at the University of Florida (Table 1). Fishes were transported to the laboratory in coolers and acclimated to experimental temperatures in Living Streams (Frigid Units, Inc., Toledo, Ohio) at least 1 week before being exposed to glochidia. Three species of sculpins, black (*Cottus baileyi*), mottled (*C. bairdi*) and slimy (*C. cognatus*), were used in trials with glochidia of *V. vanuxemi*. In exposures to glochidia of *V. nebulosa*, we tested the following species (Table 1): spotted bass (*Micropterus punctulatus*), largemouth bass (*M. s. salmoides* and *M. s. floridanus*) and Suwannee bass (*M. notius*); two exotic species, variable platyfish (*Xiphophorus variatus*) and blue tilapia [*Tilapia* (= *Sarotherodon aurea*); and mosquitofish (*Gambusia affinis*).

An abbreviated version of the procedure for laboratory infestations described by Zale and Neves (1982) follows. Fishes to be infested with glochidia were anaesthetized with tricaine methanesulfonate (MS-222) and placed in clean water to remove the chemical. Several hundred glochidia were pipetted directly onto the gill filaments. Small specimens were exposed to fewer glochidia to avoid overinfestation. After exposure, specimens of each species were revived and placed in separate 40-liter glass aquaria with aeration, filtration and temperature controls. A subsample of fish was checked 2 hr after exposure and then periodically to evaluate encystment. If an infestation was retained 5 days after exposure to glochidia, water was siphoned from the aquarium bottom at roughly daily intervals with a flexible hose (15-mm diam) into a 130-mm mesh nylon sieve. Siphoned debris was examined for juveniles in a gridded petri dish with a dissecting microscope (40X). The number of juveniles was recorded on each siphoning date, and siphoning continued for at least 3 days after the last

juveniles were found. A fish species was considered to be a suitable host for the mussel species if glochidia metamorphosed to the juvenile stage.

RESULTS

Glochidia of *Villosa vanuxemi* encysted and metamorphosed on all individuals of the three sculpin species tested (Table 2). Examination of fish several days after infestation revealed well-encysted glochidia, and no evidence of sloughing. Most of the juvenile mussels obtained from slimy and mottled sculpins excysted 26-31 days postinfestation, and the duration of metamorphosis was nearly identical. The longer period of metamorphosis (65-81 days) on black sculpins was attributed to water temperature fluctuations resulting from a faulty temperature control unit. Mean number of juveniles collected per infested fish was similar among the three species, and all fishes appeared suitable as hosts.

Three days after being infested with glochidia of *Villosa nebulosa*, specimens of the seven fish species were examined for encystment (Table 2). The basses carried well-encysted glochidia. One mosquitofish and one platyfish were sacrificed and checked microscopically; many glochidia were attached to the mosquitofish, but few remained on the platyfish. Gradual sloughing of glochidia was observed on the platyfish over a period of 8 days. Numbers of glochidia gradually declined on the tilapia as well, and all had been sloughed after 6 days.

Juvenile *Villosa nebulosa* were obtained from all bass species and the mosquitofish (Table 2). The spotted bass experiment was terminated after 24 days, because no additional juveniles were collected, but all fish still had a few glochidia attached. Juveniles were first collected from the Suwannee bass after 11 days, and all glochidia had metamorphosed and excysted by 30 days postinfestation. Juveniles were first recovered from the Florida largemouth bass after 14 days, and from the northern largemouth bass after 16 days. Except for the black sculpin experiment, mean water temperatures were similar in all experiments and periods of metamorphosis were comparable among the *Micropterus* species and the mosquitofish. The unequal number of juveniles collected per infested fish resulted from differences in the number of viable glochidia available for each experiment and in the degree of infestation.

DISCUSSION

Zale and Neves (1982) documented the suitability of banded sculpin and smallmouth bass as hosts for glochidia of *Villosa vanuxemi* and *V. nebulosa*, respectively, but acknowledged that additional host species may occur in other streams and rivers within the geographical range of these mussel species. *Villosa vanuxemi* is distributed throughout the Cumberland, Tennessee and Coosa river drainages in the Southeast,

TABLE 1. — Fish species and source of specimens infested with glochidia of *Villosa vanuxemi* and *V. nebulosa*

Mussel species and fish infested	Source of fish
<i>Villosa vanuxemi</i>	
<i>Cottus baileyi</i>	Brumley Creek, Holston River, Va.
<i>C. bairdi</i>	Guys Run, James River, Va.
<i>C. cognatus</i>	Mossy Creek, Potomac River, Va.
<i>Villosa nebulosa</i>	
<i>Micropterus punctulatus</i>	Claytor Lake, New River, Va.
<i>M. s. salmoides</i>	Pandapas Pond, River, Va.
<i>M. s. floridanus</i>	Lake Alice, St. Johns River, Fla.
<i>M. notius</i>	Santa Fe River, Suwannee River, Fla.
<i>Gambusia affinis</i>	Sweetwater Branch, St. Johns River, Fla.
<i>Xiphophorus variatus</i>	Sweetwater Branch, St. Johns River, Fla.
<i>Tilapia aurea</i>	Third-generation laboratory stock

TABLE 2. — Induced laboratory infestations of sculpins, black basses and other fishes with glochidia of *Villosa vanuxemi* and *V. nebulosa*

Mussel species and fish infested	No. of fish infested	Date of infestation	Period (days) of metamorphosis	Temperature (C)		Total no. of juveniles recovered
				Mean	Range	
<i>Villosa vanuxemi</i>						
<i>Cottus bairdi</i>	3	2/02/82	65-81	13.3	6.5-21.0	104
<i>C. bairdi</i>	1	5/11/82	26-36	18.5	17.0-20.0	41
<i>C. cognatus</i>	7	11/03/81	27-37	17.0	15.0-19.0	257
<i>Villosa nebulosa</i>						
<i>Micropterus punctulatus</i>	7	4/12/81	15-24+	21.4	20.5-23.5	50
<i>M. s. salmoides</i>	4	9/30/81	16-30	20.7	18.0-22.3	1229
<i>M. s. floridanus</i>	3	1/13/83	14-29	23.4	22.0-25.2	45
<i>M. notius</i>	6	1/13/83	11-30	23.6	22.0-26.0	243
<i>Gambusia affinis</i>	5	1/13/83	13-31	23.4	22.0-25.2	27
<i>Xiphophorus variatus</i>	5	1/13/83	-	23.6	22.0-25.2	0
<i>Tilapia aurea</i>	5	1/13/83	-	24.0	22.0-25.2	0

* Month/day/year

and *V. nebulosa* occurs in the Cumberland, Tennessee, Tombigbee and Alabama river systems, as well as in the Green River in Kentucky (Burch, 1975). At least four species each of sculpins and basses now have been shown to serve as hosts of the glochidia of *V. vanuxemi* and *V. nebulosa*, respectively. Our results suggest that other species of *Cottus* and *Micropterus* could also be parasitized by the glochidia of these mussels. Three of the sculpin species (black, mottled and banded) co-occur with *V. vanuxemi*, but the ranges of the slimy sculpin and this mussel species do not overlap. Similarly, the spotted bass and northern largemouth bass are sympatric with *V. nebulosa*, but the Florida largemouth and Suwannee bass are not. From these data, it appears that: (1) phylogenetically similar fish species are suitable as hosts for these *Villosa* species, and (2) cohabitation of mussel and suitable host is not a prerequisite for a successful (induced) parasitic relationship.

Explanations for the varying degrees of host-fish specificity among mussel species or the mechanisms involved in potential host recognition or rejection are highly speculative. However, the issue of host specificity and immunity is as interdependent in fish parasites as in parasites of higher vertebrates (Arme and Walkey, 1970). Three requirements are considered essential for a successful fish host-glochidia relationship: (1) contact between glochidia and host; (2) host suitability for encystment and metamorphosis of glochidia, and (3) glochidial resistance to host responses. The first of these requisites is met by a low-incidence but perennial event, resulting from the massive number of glochidia released by female mussels and their attachment to fishes through host respiratory or feeding activities (Tedla and Fernando, 1969; Dartnall and Walkey, 1979; Zale and Neves, 1982).

The physiological mechanisms that regulate the second and third requirements (host suitability and glochidial resistance) have not been identified. Once glochidia attach to a fish, they may not be able to develop further for a variety of reasons. The question of which organism initiates incompatibility responses is unresolved. Recognition of host suitability occurs within days of attachment, and unsuitable hosts apparently terminate the initial infestation either: (1) by not providing the appropriate chemical cues or nutritional requirements for development, or (2) through active rejection by the immune system. The first type of incompatibility has been suggested as a major factor in the natural resistance of hosts to all parasitic infestations (Barriga, 1981). With no prior exposure to *Villosa nebulosa*, the two southern basses in our study were successful hosts of the glochidia, whereas the exotic fishes sloughed them. These preliminary results would favor the chemical cue hypothesis for recognition of host suitability. Nutritional requirements or chemical stimuli for metamorphosis of glochidia are unknown, but *in vitro* transformation of glochidia to the juvenile stage has been accomplished in an artificial medium that included fish blood, amino acids, vitamins, antibiotics and other ingredients (Isom and Hudson, 1982). Fish plasma (but not blood cells) was an essential component of this medium; other animal sera tested did not induce metamorphosis.

Most parasites of fishes are short-lived (<1 year), but live long enough for host species to respond physiologically and acquire some degree of resistance to reinfestation (Kennedy, 1975). Fishes can produce specific agglutinins and develop protective immunity to a variety of antigens (Cushing, 1970), and humoral and cell-mediated immunity have been implicated in fish host susceptibility to glochidia. The attachment of unhooked glochidia to highly vascular gill tissue makes them readily accessible to attack by an immunocompetent host. Conversely fish fins, which serve as attachment sites for hooked glochidia, may be more isolated from the immune system, and physical seclusion is an efficient method of reducing host responses (Kennedy, 1975).

Early research demonstrated acquired immunity in fishes previously exposed to infestations of glochidia and the importance of a blood serum component in this process (Reuling, 1919; Arey, 1923, 1932). In a more recent series of studies, investigators examined the comparative susceptibility of salmonids to experimental and natural in-

festations with the glochidia of *Margaritifera margaritifera* (Meyers and Millemann, 1977; Karna and Millemann, 1978), described tissue reactions to these infestations (Fustish and Millemann, 1978), and identified humoral components of the host response (Meyers *et al.*, 1980). The relative resistance of coho salmon (*Oncorhynchus kisutch*) to glochidial infestation by this mussel species was attributed to humoral factors (antibody production) as well as to tissue response by hyperplasia (Meyers *et al.*, 1980). However, the presence of antibodies in vertebrates can often be shown in unsuitable hosts, but their effectiveness against the invading parasite has rarely been demonstrated (Kennedy, 1975). Results of all host suitability experiments, both *in vivo* and *in vitro*, have identified chemical components of the blood serum in fishes as the key regulatory mechanism; however, the intrinsic regulation of natural resistance in fishes—whether by biochemical incompatibility, specific antibodies, nonspecific resistance, the complement system, or other biological factors—remains a subject in need of determinate research.

Acknowledgments.—We thank N. Bruno, M. Hudy, W. Kelso, L. Kitchel, S. Moyer, B. Murphy, P. Pajak and J. Widlak for assisting with fish collections. Funding was provided by the Virginia Commission of Game and Inland Fisheries, and the Virginia and Florida Cooperative Research Units contributed logistic support.

LITERATURE CITED

- AREY, L. B. 1923. Observations on an acquired immunity to a metazoan parasite. *J. Exp. Zool.*, **38**:377-381.
- . 1932. A microscopical study of glochidial immunity. *J. Morphol.*, **53**:367-379.
- ARME, C. AND M. WALKER. 1970. The physiology of fish parasites, p. 79-101. In: A. E. R. Taylor and R. Muller (eds.). *Aspects of fish parasitology. Symp. Br. Soc. Parasitol.*, vol. 8.
- BARRIGA, O. O. 1981. The immunology of parasitic infections. University Park Press, Baltimore, Md. 354 p.
- BURCH, J. B. 1975. Freshwater unionacean clams (Mollusca: Pelecypoda) of North America. Malacol. Publ., Hamburg, Mich. 204 p.
- CÖKER, R. E., A. F. SHIRA, H. W. CLARK AND A. D. HOWARD. 1921. Natural history and propagation of fresh-water mussels. *Bull. U.S. Bur. Fish.*, **37**:76-181.
- CUSHING, J. E. 1970. Immunology of fish, p. 465-500. In: W. S. Hoar and D. J. Randall (eds.). *Fish physiology*, vol. IV. Academic Press, New York.
- DARTNALL, H. J. G. AND M. WALKER. 1979. The distribution of glochidia of the swan mussel, *Anodonta cygnea* (Mollusca) on the three-spined stickleback *Gasterosteus aculeatus* (Pisces). *J. Zool. Proc. Zool. Soc. Lond.*, **18**:31-37.
- FULLER, S. L. H. 1974. Clams and mussels (Mollusca: Bivalvia), p. 215-273. In: C. W. Hart, Jr. and S. L. H. Fuller (eds.). *Pollution ecology of freshwater invertebrates*. Academic Press, New York.
- FUSTISH, C. A. AND R. E. MILLEMAN. 1978. Glochidiosis of salmonid fishes. II. Comparison of tissue response of coho and chinook salmon to experimental infection with *Margaritifera margaritifera* (L.) (Pelecypoda: Margaritanidae). *J. Parasitol.*, **64**:155-157.
- ISOM, B. G. AND R. G. HUDSON. 1982. In vitro culture of parasitic freshwater mussel glochidia. *Nautilus*, **96**:147-151.
- KARNA, D. W. AND R. E. MILLEMAN. 1978. Glochidiosis of salmonid fishes. III. Comparative susceptibility to natural infection with *Margaritifera margaritifera* (L.) (Pelecypoda: Margaritanidae) and associated histopathology. *J. Parasitol.*, **64**:528-537.
- KENNEDY, C. R. 1975. Ecological animal parasitology. Wiley and Sons, New York. 163 p.
- MEYERS, T. R. AND R. E. MILLEMAN. 1977. Glochidiosis of salmonid fishes. I. Comparative susceptibility to experimental infection with *Margaritifera margaritifera* (L.) (Pelecypoda: Margaritanidae). *J. Parasitol.*, **63**:728-733.
- , ——— AND C. A. FUSTISH. 1980. Glochidiosis of salmonid fishes. IV. Humoral and tissue responses of coho and chinook salmon to experimental infection with *Margaritifera margaritifera* (L.) (Pelecypoda: Margaritanidae). *Ibid.*, **66**:274-281.
- REULING, F. H. 1919. Acquired immunity to an animal parasite. *J. Infect. Dis.*, **24**:337-346.
- TEDLA, S. AND C. H. FERNANDO. 1969. Observations on the glochidia of *Lampsilis radiata* (Gmelin) infesting yellow perch, *Perca flavescens* (Mitchill) in the Bay of Quinte, Lake Ontario. *Can. J. Zool.*, **47**:705-712.

- TOMPA, A. S. 1979. Life-cycle completion of the freshwater clam *Lasmigona compressa* (Bivalvia: Unionidae) in an experimental host, *Lebistes reticulatus*. *Veliger*, **22**:188-190.
- TRDAN, R. J. AND W. R. HOEH. 1982. Eurytopic host use by two congeneric species of freshwater mussel (Pelecypoda:Unionidae:Anodonta). *Am. Midl. Nat.*, **108**:381-388.
- ZALE, A. V. AND R. J. NEVES. 1982. Fish hosts of four species of lampsiline mussels (Mollusca: Unionidae) in Big Moccasin Creek, Virginia. *Can. J. Zool.*, **60**:2535-2542.

SUBMITTED 5 SEPTEMBER 1983

ACCEPTED 28 NOVEMBER 1983