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

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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 fin of fishes and exhibit varying degrees of host specificity. If glochidia attach to the ap propriate fish, they become encysted, metamorphose, and excyst to begin the free living benthic stage of their life cycle. Each gravid female mussel releases hundreds c thousands of glochidia annually, some of which come in contact with suitable hosts The reproductive success of mussel populations is, therefore, directly dependent on a abundance of host fishes to complete this critical stage in the life cycle.

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

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

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

MATERIALS AND METHODS

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

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

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

RESULTS

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

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

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

DISCUSSION

Zale and Neves (1982) documented the suitability of banded sculpin an 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 river within the geographical range of these mussel species. *Villosa vanuxemi* is distribute 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
Villosa vanuxemi	
Cottus baileyi	Brumley Creek, Holston River, Va.
C. bairdi	Guys Run, James River, Va.
C. cognatus	Mossy Creek, Potomac River, Va.
Villosa nebulosa	
Micropterus punctulatus	Claytor Lake, New River, Va.
M. s. salmoides	Pandapas Pond, River, Va.
M. s. floridanus	Lake Alice, St. Johns River, Fla.
M. notius	Santa Fe River, Suwannee River, Fla.
Gambusia affinis	Sweetwater Branch, St. Johns River, Fla.
Xiphophorus variatus	Sweetwater Branch, St. Johns River, Fla.
Tilapia aurea	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	
Villosa vanuxemi						
Cottus baileyi	3	2/02/82	65-81	13.3	6.5-21.0	104
C. bairdi	1	5/11/82	26-36	18.5	17.0-20.0	41
C. cognatus	7	11/03/81	27-37	17.0	15.0-19.0	257
Villosa nebulosa						
Micropterus punctulatus	7	4/12/81	15-24 +	21.4	20.5-23.5	50
M. s. salmoides	4	9/30/81	16-30	20.7	18.0-22.3	1229
M. s. floridanus	3	1/13/83	14-29	23.4	22.0-25.2	45
M. notius	6	1/13/83	11-30	23.6	22.0-26.0	243
Gambusia affinis	5	1/13/83	13-31	23.4	22.0-25.2	27
Xiphophorus variatus	5	1/13/83	-	23.6	22.0-25.2	0
Tilapia aurea	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 riv systems, as well as in the Green River in Kentucky (Burch, 1975). At least four spec each of sculpins and basses now have been shown to serve as hosts of the glochidia of *vanuxemi* and *V. nebulosa,* respectively. Our results suggest that other species of *Cot* and *Micropterus* could also be parasitized by the glochidia of these mussels. Three of t sculpin species (black, mottled and banded) co-occur with *V. vanuxemi,* but the rang of the slimy sculpin and this mussel species do not overlap. Similarly, the spotted ba and northern largemouth bass are sympatric with *V. nebulosa,* but the Flori largemouth and Suwannee bass are not. From these data, it appears that: phylogenetically similar fish species are suitable as hosts for these *Villosa* species, a (2) cohabitation of mussel and suitable host is not a prerequisite for a successful (duced) parasitic relationship.

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

The physiological mechanisms that regulate the second and third requireme (host suitability and glochidial resistance) have not been identified. Once glochidia tach to a fish, they may not be able to develop further for a variety of reasons. T question of which organism initiates incompatibility responses is unresolved. Recog tion of host suitability occurs within days of attachment, and unsuitable hosts appare ly terminate the initial infestation either: (1) by not providing the appropriate chemi cues or nutritional requirements for development, or (2) through active rejection by immune system. The first type of incompatibility has been suggested as a major fac in the natural resistance of hosts to all parasitic infestations (Barriga, 1981). With prior exposure to *Villosa nebulosa,* the two southern basses in our study were success hosts of the glochidia, whereas the exotic fishes sloughed them. These prelimin results would favor the chemical cue hypothesis for recognition of host suitabili Nutritional requirements or chemical stimuli for metamorphosis of glochidia unknown, but *in vitro* transformation of glochidia to the juvenile stage has been complished in an artificial medium that included fish blood, amino acids, vitamins, tibiotics and other ingredients (Isom and Hudson, 1982). Fish plasma (but not blc cells) was an essential component of this medium; other animal sera tested did not duce metamorphosis.

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

Early research demonstrated acquired immunity in fishes previously exposed to festations of glochidia and the importance of a blood serum component in this proc (Reuling, 1919; Arey, 1923, 1932). In a more recent series of studies, investigators amined the comparative susceptibility of salmonids to experimental and natural festations with the glochidia of *Margaritifera margaritifera* (Meyers and Millemann, 197 Karna and Millemann, 1978), described tissue reactions to these infestations (Fusti: and Millemann, 1978), and identified humoral components of the host respon (Meyers *et al.*, 1980). The relative resistance of coho salmon (*Oncorhynchus kisutch*) glochidial infestation by this mussel species was attributed to humoral factors (antiboc production) as well as to tissue response by hyperplasia (Meyers *et al.*, 1980). Howeve the presence of antibodies in vertebrates can often be shown in unsuitable hosts, b: their effectiveness against the invading parasite has rarely been demonstrated (Ke1 nedy, 1975). Results of all host suitability experiments, both *in vivo* and *in vitro,* ha\ identified chemical components of the blood serum in fishes as the key regulato1 mechanism; however, the intrinsic regulation of natural resistance in fishes—wheth by biochemical incompatibility, specific antibodies, nonspecific resistance, the compl ment system, or other biological factors—remains a subject in need of determina research.

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