Metamorphosis of Freshwater Mussel Glochidia (Bivalvia: Unionidae) on Exotic Fishes and Amphibians

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Abstract. - This study determined that inexpensive and easily maintained amphibians and exotic fishes could act as hosts for two species of native North American unionid mussels, and bypass the need to identify 'natural' hosts when the object is to culture mussels. Two mussel species, *Lampsilis cardium* and *Utterbackia imbecillis,* were used to parasitize 48 exotic fishes or nonpiscine potential hosts. Nonpiscine hosts included amphibians and decapod crustaceans. *Lampsilis cardium* successfully metamorphosed on six species of exotic fishes, as well as on larval tiger salamanders. *Utterbackia imbecillis* successfully metamorphosed on 30 species of exotic fishes and all four amphibian species tested. No glochidia metamorphosed on crustaceans. Successful metamorphosis on amphibians indicates that mussel zoogeography may be more complicated than previously thought. Using surrogate hosts may be a valuable alternative to natural hosts in laboratory culture of mussels.

INTRODUCTION

Identification of hosts for freshwater mussels has become an important consideration in their conservation and management. While experiments with artificial media have been promising (Isom and Hudson, 1982; Keller and Zam, 1990), this approach is not successful with every species. Thus, a host is required for laboratory glochidial metamorphosis from parasitic glochidium to juvenile mussel. Unfortunately, natural hosts have not been identified for most species. Even if known, the hosts may be as endangered as their mussel parasite, or they may be difficult to maintain in captivity.

Several exotic fish species already are identified as hosts for North American mussels. The common carp, *Cyprinus carpio*, is reported as host for five mussel species (Lefevre and Curtis, 1910, 1912; Parker *et al.*, 1984). The goldfish, *Carassius auratus*, is the only known host for the rare *Cyprogenia aberti* (Chamberlain, 1934), although native hosts obviously must exist. The green swordtail, *Xiphophorus helleri*, and the guppy, *Poecilia reticulata*, also are reported as hosts for *Anodonta oregonensis* and *Lasmigona compressa*, respectively (Chamberlain and Jones, 1929; Tompa, 1979). Dechtiar (1972) found glochidia of an unidentified unionid on wild common carp in Lake Erie. Conversely, introduced mussels may use native fishes as hosts (Watters, in press, a). Despite these accounts, no deliberate study of exotic piscine hosts has been undertaken.

Although reports of glochidial metamorphosis on amphibians have been published, workers have rarely included amphibians in studies of host identification for mussels. Faussek (1901) reported that *Anodonta* glochidia metamorphosed on larval tiger salamanders (*Ambystoma tigrinum ssp.*), tadpoles of *Rana* and *Peltobates* and the Austrian cave salamander (*Proteus sp.*). Watters (in press, b) partially confirmed Faussek's results by metamorphosing the pocketbook

Lampsilis cardium on *Ambystoma tigrinum ssp.* Seshaiya (1941, 1969) and Walker (1981) demonstrated that other unionaceans metamorphosed on tadpoles. Howard (1951) reported metamorphosis of the salamander mussel *Simpsonaias ambigua* on mudpuppies (*Necturus maculosus*), and found unmetamorphosed glochidia of the washboard *Megalonaias nervosa* on them as well.

Glochidial metamorphosis on crustaceans is virtually unknown, although Walker reported metamorphosis of a hyriid mussel on freshwater decapod crustaceans in Australia (1981). Panha (1990) found unidentified glochidia on a palaemonid decapod in Thailand. Again, no deliberate study of the role of these nonpiscine potential hosts has been made.

The purpose of this study was to determine whether inexpensive and easily maintained exotic fishes, amphibians, and crustaceans could serve as hosts for native North American mussels. These could serve as surrogate hosts, and bypass the need to identify native hosts when the objective is to culture captive mussels. Two species of mussels were used: the pocketbook *Lampsilis cardium*, and the paper pondshell *Utterbackia imbecillis*. Both are long-term brooders, producing glochidia in the autumn and carrying them in the marsupial portions of the gills over winter. *Utterbackia imbecillis*, and probably most anodontines, are believed to be host generalists (Trdan and Hoeh, 1982). Although originally reported as not needing a host for metamorphosis (Howard, 1914), no study in the past fifty years has substantiated that claim. On the contrary, subsequent studies have identified 14 fish hosts (see review of Watters, 1994). There is no evidence that *Utterbackia imbecillis* develops without a host (Heard, 1975). Lampsiline glochidia generally attach to gills, whereas anodontine glochidia attach to fins. These two species were chosen as representatives of two unionid subfamilies (Lampsilinae and Anodontinae) that differ in

reproductive adaptations (Watters, in press, c) and host specificity (Trdan and Hoeh, 1982; Waller et al., 1985). They represent extremes of the unionid reproduction spectrum for these characteristics.

MATERIALS AND MATERIALS

Two gravid females of *Lampsilis cardium* were collected in early summer from Conneaut Creek in northeastern Ohio. Seven total gravid females of *Utterbackia imbecillis* were collected in summer from Lake Erie and Raccoon Creek, Ohio, and Lake Monticello, South Carolina. They were held in flow-through 38-L aquaria at 20-21° C.

Tiger salamander larvae, African clawed frog (*Xenopus laevis*), freshwater shrimp (*Palaemonetes sp.*), and exotic fish species were obtained commercially. These included 16 fish families, three amphibian families, and two decapod crustacean families. One to three individuals of each species were used per mussel species. Potential hosts were held in 38-L aquaria at 20-21 C, with no substrate, and were fed every two days with aquatic oligochaetes or flake fish food. Young-of-year hatchery raised largemouth bass (*Micropteris salmoides*) were used as controls, to ensure that glochidia were infective, and to give a comparable level of metamorphosis on a native piscine host. Animals were maintained according to the Ohio State University Animal Care and Use protocols.

Glochidia were removed from gravid mussels by inserting a water-filled insulin syringe into the distal portion of the marsupium and flushing glochidia from the gills. A sample of glochidia were tested for viability ~~by exposing them~~ to table salt. Live glochidia react ~~to salt~~ by rapidly closing their valves. Glochidia were suspended in a container of 20-21 C water by gentle agitation with an

airstone. ~~Glochidial densities are given in~~ Table 1. Potential hosts were placed in the container and simultaneously exposed for 1 hr. Fish were segregated by species and returned to the aquaria.

Beginning the day after exposure and continuing every other day for up to 45 days, 1 liter of water was siphoned from the bottom of the aquarium and passed through a 145-µm sieve. The debris was examined for glochidia with a stereomicroscope using the polarized light method devised for detecting zebra mussel veligers (Johnson, 1995). Metamorphosed juveniles were identified by the presence of two adductor muscles, a foot, and movement.

Percent metamorphosis was calculated from the number of metamorphosed juveniles divided by the sum of the number of nonmetamorphosed glochidia and juveniles recovered (total attached). Total attached and percent metamorphosis were each averaged for multiple individuals in the same species.

RESULTS

Lampsilis cardium successfully metamorphosed on six species of exotic fishes from the families Fundulidae, Poecilidae, and Belontiidae (=Anabantidae), as well as on larval tiger salamanders (Table 1). Metamorphosis on the salamanders was first reported in Watters (in press, b). These suitable hosts represented 13.5% of all exotic and nonpiscine species tested. Metamorphosis on none of these was comparable to or above that of the control, largemouth bass. An average of 62% of glochidia metamorphosed on controls. Successful metamorphosis on test species ranged from 3 to 22% (mean = 8.9%). An individual of *Sphaerichthys osphromenoides*, a belontiid, shed eight of 13 attached glochidia as nonmetamorphosed larvae 11 to 17 days post-exposure. This was the period when metamorphosed glochidia typically were shed

by other hosts. This phenomenon was reported in wild-caught fishes and may represent a secondary immune reaction of a suitable host (Watters and O'Dee, in press).

Utterbackia imbecillis successfully metamorphosed on 30 species (57%) of exotic or nonpiscine hosts (Table 1). Nine of these hosts produced metamorphosed juveniles at a level comparable to or above that of the ~~control~~ largemouth bass. Metamorphosis on controls averaged 43%. Successful metamorphosis on test species ranged from 7 to 83% (mean = 36%).

Three exotic host fishes previously reported in the literature, guppy, goldfish, and green swordtail, also served as hosts. However, none produced a high percentage of metamorphosis.

No metamorphosis was seen in the limited number of crustaceans tested here. Most subjects molted at least once during the tests, undoubtedly removing any glochidia attached to the gills or other parts of the exoskeleton.

DISCUSSION

Host use. - Of the three fish families found serving as hosts for *Lampsilis cardium*, the poecilids and fundulids have members native to North America. Several species in these families have been identified as hosts of North American mussels. In the Fundulidae, the western banded killifish, *Fundulus diaphanus diaphanus*, was identified as a host for the related fatmucket, *Lampsilis radiata luteola* (Watters, 1996) and six other species of freshwater mussels (Young, 1911; Wiles, 1975a, 1975b; Trdan and Hoeh, 1982). Golden topminnow, *Fundulus chrysotus*, was a host for the giant floater, *Pygandon grandis* (Penn, 1939). Plains killifish, *Fundulus zebrinus*, was a host for the squawfoot, *Strophitus undulatus* (Ellis and Keim, 1918). In the Poecilidae, the mosquitofish, *Gambusia affinis*, was reported as a host for three North American

mussels (D'Eliscu, 1972; Stern and Felder, 1978; Neves *et al.*, 1985). Thus, it was not surprising that other species of these fish families, from outside North America, could serve as hosts for *L. cardium*.

Explanations for the suitability of belontiids as hosts to *Lampsilis cardium* are more complicated. Belontiids are found in India, southeast Asia and Indonesia. No native representatives occur in North America. However, Asian unionids occur within the ranges of belontiids. We surmise that belontiids are suitable hosts for the two species tested, and perhaps other North American unionids, by virtue of their co-occurrence and co-evolution with Asian unionids, where they probably act as hosts. However, worldwide, unionids do not greatly overlap the ranges of the other fish families tested (Cichlidae, Callichthyidae, etc.), which predominantly come from Africa, South America, and Australia, where few or no unionids occur. But atherinids are reported as hosts for hyriid mussels (Humphrey & Simpson, 1985), cichlids are hosts for hyriid, mutelid, and mycetopodid mussels (Bonetto and Ezcurra, 1962, 1963; Kondo, 1984; Mansur and Veitenheimer-Mendes, 1979), characids as hosts for hyriids and mycetopodids (Bonetto and Ezcurra, 1962, 1963), and callichthyids are hosts for mycetopodids (Bonetto and Ezcurra, 1962). Although these fishes co-occur with and act as hosts for these freshwater mussel families, they apparently are not suitable hosts for the Unionidae.

The gyrinocheilids and silurids also are native to Indonesia where unionids occur, but were not suitable hosts for *L. cardium* in this study. It is possible that these bottom feeders ingested metamorphosed juveniles before they were sampled by us.

Of the remaining families tested for *Lampsilis cardium*, most either have no native species in North America (e.g., Cichlidae), or are represented by very few species (e.g., Characidae,

Atherinidae). Only the Cyprinidae is well-represented in North America. However, only one native cyprinid is reported as a host for a *Lampsilis*; the common shiner, *Luxilus cornutus* (Fuller, 1978).

We suggest that if a fish family in North America has suitable hosts for *Lampsilis cardium* (e.g., Poecilidae, Fundulidae), then exotic species of that family may be hosts as well. Fish families not serving as hosts in North America (e.g., Cyprinidae) do not serve as hosts when exotic members are used. Further support of this idea is the finding that mussels will metamorphose on extralimital congeners of natural hosts within North America (Neves at al., 1985).

Utterbackia imbecillis can successfully parasitize a wide range of piscine or amphibian hosts, including members of families not native to North America. Many of these may be parasitized to the same or greater degree than a native host. The broad distributions of many anodontine species undoubtedly are the result of this ~~generalist use~~ variety of hosts.

At the same temperature, *L. cardium* glochidia averaged nearly twice as long to begin metamorphosis (mean = 14 days, range 9 to 19 days) as did *U. imbecillis* glochidia (mean = 7.5 days, range 3 to 13 days). Three days to infection of *U. imbecillis* on silver tip tetra is one of the shortest glochidial parasitic durations recorded (Seshaiya, 1969).

Numbers of attached glochidia per host species varied greatly. For example, although ~~infested~~ exposed simultaneously ~~with~~ to the same concentration of *Utterbackia imbecillis* glochidia, a *Betta splendens* harbored only five glochidia, while a *Gasteropelecus levis* bore 900. The probability of encountering glochidia as the result of differences in swimming behavior obviously will lead to differences in parasite burden, although no differences were noted between these two species. This extreme variation suggests differences in susceptibility, but the mechanisms underlying this

phenomenon are not known. Most glochidia attached to fins, but there was no obvious relationship between fin area and levels of infestation.

Some exotic fishes did not tolerate the infestation. *Gasteropelecus levis*, which bore hundreds of glochidia, usually died within a week, after shedding all glochidia. Callichthyids ('corys') and cobitids ('loaches') also had high mortality.

Phylogenetic and zoogeographic implications. - Based on host specificity, lampsilines are regarded as specialists, and anodontines as generalists (Watters, in press, c). Lampsilines have evolved morphological adaptations to more efficiently contact their hosts, including mantle displays and conglutinates. This has resulted in their specialization in certain types of hosts. Whether the exotic hosts tested in this study would be attracted to these luring devices has not been established. Anodontines have few or no such luring behaviors and morphological adaptations, but are able to parasitize successfully a wider range of host species. These represent two different co-evolutionary paths among these host/parasite relationships.

Amblemine unionids were not included in this study, and it has not been shown that they can use exotic hosts. Many amblemines produce conglutinates, packages of glochidia bound with a mucous matrix. These conglutinates often mimic specific fish food items, suggesting host specificity (Watters, in press, c). Whether exotic hosts would be attracted to these conglutinates is unknown.

Amphibians are shown to be suitable hosts for the mussels in this study. If this is a widespread phenomenon, then an important aspect of freshwater mussel zoogeography has been overlooked. Clearly efforts to identify other amphibian/mussel associations are needed.

Management implications. - In contrast to many ~~natural~~ native hosts, the exotic hosts identified here are commercially available from most tropical fish suppliers. Many are routinely bred in captivity and may be obtained in large numbers. They are easily maintained with well-known diets and breeding requirements. This is not so for many natural hosts that have been identified: darters, sculpins, dace, etc. ~~Furthermore, when the natural host is rare or endangered, it may not be feasible to collect them for host work.~~ this could be exception vs rule

Many commercially obtained surrogates are relatively parasite-free, and the probability of previous glochidial infection is ~~very small~~ low. On the other hand, wild-caught natural hosts are usually infested with numerous parasites, including glochidia. The nature of glochidial immunity in fishes is not understood, and it is not known how previous parasite loads, glochidia or otherwise, interfere with subsequent infestations. Therefore it is desirable to work with parasite-free, 'naive' fishes.

All surrogate hosts for *L. cardium* produced few juveniles when compared to the natural host, *Lasmigona* host,

~~Many of the surrogate hosts did not produce transformed juveniles in numbers comparable to~~ 'natural' hosts, ~~but others~~ whereas those for *U. imbecillus* performed as well as or better than 'natural' hosts. Surrogates are not the solution to conserving dwindling mussel populations, but they may have use where 'natural' hosts have not been identified, or are too difficult to capture and maintain. ~~Any metamorphosis of a rare mussel is better than none at all.~~

Of all groups tested, the belontiids were most suitable to both mussel species. We suggest that belontiids, at least, be included in any standard host identification study along with native fish species. Using inexpensive, easily available, parasite-free surrogate hosts may be a valuable alternative to natural hosts in some situations. Obviously, we do not advocate the release of exotic hosts to bolster native mussel populations. But in hatchery conditions, or to obtain

metamorphosed juveniles for introduction, surrogate hosts may be more cost-effective and practical.

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Table 1. - Glochidial infections of control and exotic fishes, and nonpiscine potential hosts. Origin - continent of species origin Total - mean total # all attached glochidia/host; % m/f - percent of all attached glochidia that metamorphosed; IN - density of glochidia/liter used in infection (see bottom); D - days to first metamorphosis

				Lampsilis cardium				*Utterbackia imbecillis*			
	Common Name	Scientific Name	Origin	Total	% m/f	IN	D	Total	% m/f	IN	D
Pisces											
Control											
Centrarchidae	Largemouth bass	*Micropterus salmoides*	NA	308.0	67.2	c	13	9.0	44.4	f	7
	Largemouth bass	*M. salmoides*	NA	323.0	72.8	c	13	40.0	65.0	g	11
	Largemouth bass	*M. salmoides*	NA	400.0	47.0	c	13	5.0	20.0	h	7
Exotic											
Cichlidae	Zebra Malawi cichlid	*Pseudotropheus zebra*	Africa	59.7	-	d		12.0	8.3	g	7
	Malawi gold cichlid	*P. auratus*	Africa	105.0	-	a		15.0	-	h	
	Marbled cichlid	*Haplochromis venustus*	Africa	81.5	-	a		19.0	21.1	g	5
	Peacock	*Aulanacara stuartgranti*	Africa	65.0	-	a		11.0	27.3	h	5
	Angelfish	*Pterophyllum scalare*	SA	49.0	-	a		11.5	65.2	f	7
Cyprinidae	Giant danio	*Danio malabaricus*	Asia	271.3	-	d		8.0	62.5	f	7
	Goldfish	*Carassius auratus*	Asia	17.5	-	d		8.7	15.4	f	5
	Blue danio	*Brachydanio kerri*	Asia	5.0	-	a		22.0	22.7	f	7
	Red-tailed shark	*Labeo bicolor*	Asia	170.0	-	a		6.0	-	h	
	Golden barb	*Barbus semifasciolatus*	Asia	1.5	-	a		7.0	28.6	h	5
	Tiger barb	*B. tetrazona*	Asia	167.5	-	a		71.5	-	i	
	Black ruby barb	*B. nigrofasciatus*	Asia	581.5	-	c		3.0	-	i	
	Scissortail rasbora	*Rasbora trilineata*	Asia	223.5	-	a		8.0	-	f	
	Brilliant rasbora	*R. einthoveni*	Asia	482.5	-	c		8.0	62.5	f	5

Pangasiidae	Pangasius catfish	*Pangasius sutchii*	Africa	164.5	- a		14.0	21.4 g	9
Gyrinocheilidae	Chinese algae-eater	*Gyrinocheilus aymonieri*	Asia	209.5	- a		31.0	41.9 g	5
Gymnotidae	Glass knifefish	*Eigenmannia virescens*	SA	20.0	- b		10.0	30.0 g	11
Amphibia									
Ranidae	Northern leopard frog	*Rana pipiens*	NA	93.0	- a		57.0	8.8 g	7
	Bullfrog	*R. catesbeiana*	NA	57.5	- b		55.0	29.1 g	5
Xenopidae	African clawed frog	*Xenopus laevis*	Africa	103.0	- a		4.0	75.0 g	13
Ambystomidae	Tiger salamander larva *	*Ambystoma tigrinum ssp.*	NA	204.0	11.0 e	9	274.0	6.6 g	11
	Tiger salamander larva *	*A. tigrinum ssp.*	NA	83.0	16.0 e	19			
	Tiger salamander larva *	*A. tigrinum ssp.*	NA	132.0	2.3 e	9			
	Tiger salamander larva *	*A. tigrinum ssp.*	NA	1096.0	0.3 e	11			
Decapoda									
Palaemonidae	Ghost shrimp	*Palaemonetes sp.*	NA	26.0	- a		0.7	- g	
Cambaridae	Rusty crayfish	*Orconectes rusticus*	NA	37.0	- a		30.0	- i	
	Virile crayfish	*Cambarus virilis*	NA	95.0	- a				

a = 3500; b = 2250; c = 7000; d = 3200; e = unknown; f = 1416; g = 291; h = 375; i = 551

* see Watters (in press, b)

NA = North America; SA = South America

Characidae	Red-eye tetra	*Moenkhausia oligolepis*	SA	525.5	-	d		69.0	82.6	f	5
	Silver tip tetra	*Hemigrammus nanus*	SA	3.5	-	a		13.5	18.5	h	3
	Von Rio tetra	*Hyphessobrycon flammeus*	SA	4.0	-	a		20.0	70.0	g	11
	Glowlight tetra	*H. erythrozonus*	SA	961.0	-	c		2.7	12.5	g	7
	Black neon	*H. herbertaxelrodi*	SA	174.0	-	c		34.0	-	i	
	Darter characin	*Characidium fasciatum*	SA	292.0	-	b		-	-	g	
	Silver dollar	*Metynnis argenteus*	SA	306.0	-	a		32.0	68.8	f	5
Gasteropelecidae	Silver hatchetfish	*Gasteropelecus levis*	SA	169.0	-	a		900.0	-	f	
Callichthyidae	Leopard catfish	*Corydoras julii*	SA	234.5	-	d		2.0	-	h	
	Peppered catfish	*C. paleatus*	SA	25.0	-	a		6.0	-	h	
Fundulidae	Panchax killifish	*Aplocheilus lineatus*	Asia	156.0	0.3	a	11	20.0	35.0	g	11
Poecilidae	Painted sword	*Xiphophorus helleri*	SA	73.0	5.5	d	19	3.0	33.3	g	11
	Guppy	*Poecilia reticulata*	SA	422.0	2.1	d	15	23.0	21.7	h	7
	Golden wagtail platy	*Xiphophorus maculatus*	SA	-	-	a		15.0	0.0	h	
Belontiidae	Lavender gourami	*Trichogaster trichopterus*	Asia	41.0	11.0	d	11	31.0	22.6	f	9
	Pearl gourami	*T. leeri*	Asia	9.0	-	a		33.0	9.1	f	9
	Chocolate gourami	*Sphaerichthys osphromenoides*	Asia	13.0	-	a		3.0	-	g	
	Flame gourami	*Colisa lalia*	Asia	52.0	13.6	b	17	39.0	41.0	f	5
	Siamese fighting fish	*Betta splendens*	Asia	40.0	41.3	b	17	5.0	80.0	f	9
Atherinidae	Australian rainbow fish	*Melanotaenia macculloch*	Australia	6.5	-	a		18.0	16.7	f	7
Cobitididae	Clown loach	*Botia macracantha*	Asia	58.5	-	a		6.0	-	f	
	Black loach	*Pangio kuhlii myersi*	Asia	381.0	-	c		15.0	26.7	g	9
Loracariidae	Common plecostomus	*Hypostomus plecostomus*	SA	99.0	-	a		14.0	-	g	
Siluridae	Glass catfish	*Kryptopterus bicirrhis*	Asia	402.0	-	a		9.0	-	g	
Pimelodidae	Pictus catfish	*Pimelodus pictus*	SA	232.0	-	a		19.0	-	g	

Watters MS

This revision is much better than the initial manuscript, and is certainly suitable for publication. I'm pleased to see that the wild speculation on phylogeny has been deleted. With no statistical tests in this MS, it is imperative that speculation from results of inadequate sample size be curtailed. I have included a few editorial comments on the paper, and really have only 3 substantive comments on contents.

Comment 1 - The Management Implications section is lengthy and extrapolates far beyond the research results presented. U. imbecillis uses a wide variety of common native host fishes (~15 species), as do most species of the Anodontinae. Thus the value of exotics to produce juveniles is a moot point. The results of host testing with L. cardium, which has seemingly few natural hosts, show that the use of exotic fishes could be helpful. However, the % metamorphosis of glochidia on largemouth bass (62%) was significantly greater than that on exotics (3-22%). Thus the greater host size (more juveniles/fish) and transformation success on largemouth bass, which can be obtained year-round from various hatcheries, makes it a good, readily available host for the production of juvenile L. cardium. My point is that the management implications described do not apply to these two mussel species. The one page narrative is more speculative than substantive, based on the results reported in the paper. Page 11 should be reduced to 2 paragraphs, maximum.

Comment 2 - The author references his own work in press, when there are good, published papers already in the literature to support statements made (e.g., p. 5, 10). It almost appears that the author is promoting his own work and "taking credit" for concepts and facts shown by earlier publications.

Comment 3 - There are too many short, choppy paragraphs (2-3 sentences). The paper doesn't read smoothy because of this. Some of these can easily be combined to improve the flow of information.

DEPARTMENT OF BIOLOGY

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January 9, 1997

Dick Neves
Department of Fisheries & Wildlife
Virginia Tech
Blacksburg, VA 24061

Dear Dr. Dick,

Earlier you agreed to review the manuscript "Metamorphosis of freshwater mussel glochidia (Bivalvia: Unionidae) on exotic fishes and nonpiscine species: the search for the universal host" by G.T. Watters. Both you and a second reviewer suggested a complete revision of the manuscript followed by a new review.

Dr. Watters has revised his manuscript and I would appreciate your candid opinions on this revision. I have enclosed the comments that you and the other reviewer made on the first submission of this manuscript as well as Dr. Watters specific responses to these comments. Would you recommend this revision for publication?

It would be helpful if your review could be returned within the next three-four weeks. Please return the manuscript and your comments to me.

Again, thank you for your willingness to review this manuscript once again. Your help is greatly appreciated.

Sincerely,

Daniel J. Hornbach
Associate Editor, American Midland Naturalist

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17 December 1996

Dr. Robert P. McIntosh
Box 369 / Room 285 GLSC
University of Notre Dame
Notre Dame, IN 46556-5645

Dear Dr. McIntosh:

Enclosed please find a revised MS of "Metamorphosis of freshwater mussel glochidia on exotic fishes and amphibians." I have made substantial changes in the MS along the suggestions of the two reviewers. I greatly appreciate the time and effort they made in bettering this paper. I do not, however, agree with all of their comments and cannot make some suggested changes. I have indicated on the returned comment sheets with a check mark or marginalia what concerns I feel I have addressed. Several issues remain, and these are identified with a red number, discussed below.

Two general comments. First, both reviewers do not seem to appreciate that this was the first study of its kind. It introduces a new way of doing things. It is supposed to raise questions for other workers to ponder and tackle. It is not the last word on the subject. Second, it was conducted in the same manner as all other host identifications - for better or worse. Perhaps I patterned the study after the reviewers' work? Yet the reviewers seem to want me to jump through hoops that no one else has attempted - with apparently unlimited money, time, and personnel.

Reviewer 2.

1) I am aware of this, and it plagues all studies of this kind. And it does so for a very good reason - we have X dollars and Y time. I would loved to have used hundreds of each exotic on dozens of mussels, but it just isn't going to happen. This is just the initial report - now everyone can jump in and test these theories to their heart's content.

2) Of course it's difficult to control, this isn't a physics experiment. The results clearly show that fishes exposed at the same time with the same glochidial concentration had very different glochidial loads. There is no apparent cause for this. Knowing this, how could I control infection numbers and initial attachments? Don't get me wrong I agree that this is desirable. I just don't think it can be done. Even if we pipetted glochidia directly on the gills or fins, the 'susceptibility' problem would still be there.

Reviewer 1.

3) The reviewer has missed the point. I specifically chose *Utterbackia* because it was a weed - as I stated, I wanted the "extremes." In this context, *Utterbackia* was an optimal choice. The fact that it can transform in artificial media is irrelevant - other species can as well, and presumably all can once we perfect the technique. *Utterbackia* metamorphosing without host? Except for Howard's original observation, parroted by several later authors, there is no evidence this happens. Indeed, we know it metamorphoses on quite a few hosts (as other anodontines do), and Dr. Heard has stated that it cannot metamorphose without a host. I don't know what Howard saw, but no one else has seen it.

4) Good question. I've preferred 'infest,' but other reviewers have insisted on 'infect,' which I used here. Either is OK with me.

5) The reviewer is asking me to abandon experimental design. It was important, as a control, that bass and exotics were exposed at the same time with the same mussel. Only then do we get comparable results. If the control was 'weak,' then so be it. It was comparable. It was what really happened. Is the reviewer suggesting I replicate the test until I get a predetermined result?

6) Again, a hoop. Show me a host identification study that evaluated juveniles for 'health, survival, etc.' Even Neves has identified 'good' hosts based on the fact that the fish died but still had glochidia attached! This evaluation simply isn't done, and the reviewer gives no suggestions as to how to do it. What do I look for? How long do they have to live once transformed? A day? A week? What if they die? Was it because of a 'bad' host, or bad conditions after they transformed? The transformed juveniles in this study were alive, moving, and indistinguishable from those that came off the control.

Of course I would argue that I did not make 'exaggerated claims' or wax 'far too speculative.' I have grudgingly removed most of this from the paper (but you know it will turn up somewhere else). Perhaps the reviewers need to practice synthesizing data a little (smile).

I really do appreciate the effort and comments of the reviewers. Life would be dull if everyone agreed with everyone else.

Although I have shortened the Table, I am uncomfortable about shortening it further. I think eliminating the common fish names inserts a new level of difficulty for the reader. Most people recognize the name Black Neon, and know it as a common aquarium fish. But how many would recognize *Hyphessobrycon herbertaxelrodi*? I have included a version with and without common names.

Sincerely,

G Thomas Watters

Rev #1

Manuscript by Watters

This manuscript contains interesting data on the suitability of some exotic fishes and amphibians to two species of native unionids. While I heartedly support publication of results, I also feel that the exaggerated statements of research applicability to ancillary topics need to be toned down. The following comments are provided for consideration by the author, in addition to those included directly on the Ms.

✓ **Title** - revise as stated on Ms.

Abstract - See Ms. Broad statements such as relationships between Anodontinae and Lampsilinae, based on testing of 2 species, are unsupported by this research and should be deleted. — OK, but wrong. This research, plus others given, point to this conclusion.

Introduction -

✓ 1. Move the second paragraph to the end.

2. Describe the pertinent reproductive biology and host information for L. cardium and U. imbecillis (long-term vs. short-term, number of known hosts, difference in glochidial attachment, etc.). Rather than vague comments in the first paragraph of M&M, give the reader what he/she should know about their differences up front.

3. In my opinion, the selection of U. imbecillis for this testing was less than optimal. The paper pondshell is a unionid "weed species" that can transform on most fishes tested, can readily transform (unlike many other species) in an artificial medium, and has been reported to metamorphose from glochidium to juvenile in the female marsupium (no host necessary). Given these atypical traits, when compared to other unionids, can test results with exotic fish and amphibians be assumed to apply to other unionid species? Does it typify the Anodontinae? A sample size of one tells me that inferences are unjustified at this time.

4. State why natural hosts should be identified in addition to testing the potential value of unusual hosts. — ? not clear what is wanted here.

Materials and Methods

✓ 1. Revise first paragraph and remove speculation.

✓ 2. Is it transformed glochidia or transformed juveniles? If they've transformed, then they are no longer glochidia, no?

✓ 3. Where did glochidia attach? Hooked glochidia can attach to gills, epidermis, etc. as well as fins.

4. Infest vs. infect - don't parasites infest their hosts?

Results

Lampsilis cardium - As shown in Table 1, exotic fishes and the tiger salamander were suitable but poor hosts for glochidia. The number of juveniles produced and the % metamorphosis was low (x = 9%) relative to the natural host LMB (x = 62%). These results indicate that they are poor surrogates, yet I see no elaboration of this in Discussion, only their value as surrogates.

Utterbackia inbecillis - In my opinion, the reference test with LMB was weak; 2 of the 3 fish

had < 10 glochidia and only 1 and 4 juveniles were obtained from these 2 fish. The low numbers and disparity among fish should have signalled the need for a replication of this test. How can experimental tests be compared to such a disparate reference test?

Discussion

1. The length (4.5 pages) relative to Results (1 page) is excessive, and touches on issues unrelated to this research (highly speculative). Without knowing the immunological mechanism that controls host recognition, the familial claims (first 2 pages) are speculative. Because other host testing has shown that specificity can occur within but not necessarily between genera of the same family of fishes, the claim (" host specificity has a fundamental immunological component operating at the host family level") is incorrect in many cases. I sense that the author is neither an immunologist nor parasitologist, and may not have a good grasp of the physiological mechanisms that can regulate specificity and confer natural immunity. Unless the author can present an hypothesis to support his contention of familial-level immunology at work, these 2 pages should be reduced in length, eliminating much of the speculation on exotic families. — a RONG.

2. "It is suggested that belontiids are suitable hosts for North American unionids" - based on the test of 2 mussel species, how can the author possibly make such a generalization?

3. I would like to see explanations for "anodontines have the physiological capability to parasitize successfully numerous types of hosts" and "this study shows that this difference in host specificity is not only behavioral but immunological as well." There was no physiological or immunological testing done, and yet statements such as this appear in the Discussion without reference to credible studies that can support such notions.

4. Statements of phylogeny between subfamilies (p.10) cannot be derived from the testing of only 2 mussel species. — again, this is supported by a suite of studies

5. Management implications - The author should reiterate that most of the non-traditional hosts were poor hosts and not comparable to natural hosts. As written, my concern is that some administrator will use this section as an alternative for true recovery of rare species (habitat restoration, natural reproduction with native hosts, etc.); i.e., why not use exotic fish to produce juveniles and not worry about the real aquatic world?

6. Ambystomids typically reside in temporary pools and ponds as larvae, even neotenic populations. Because of heavy predation by fish, they are uncommon to rare in even sluggish streams. I don't see how this family, even if suitable as host to more mussel species, will change our views of zoogeographic patterns. It provides an avenue, perhaps, for dispersal among ponds, but few mussel species would be affected.

7. One last point that bears mentioning. None of these non-traditionally produced juveniles were evaluated for health, survival, etc. Before the author claims (p. 11) that "surrogate hosts may be a valuable alternative to natural hosts" or that "surrogate hosts may be more cost-effective and practical", trials with juveniles produced from natural vs. exotic hosts must be run. Overall, I feel that the author has been far too speculative, with statements and claims based on feelings rather than facts. He has gone far beyond what 2 mussel species can tell us about phylogeny, immunology, physiology, and zoogeography of North American unionids.

No. I said amphibians in general, not just ambystomids

I don't think so!

Reviewer Comments

The manuscript provides some very important information for people interested in the production of juvenile mussels for research and conservation activities. It clearly indicates that we may be able to increase populations of threatened species without grinding out numerous host experiments with native fish species. I commend the author for his effort in this area.

However, the paper suffers in areas from weak writing. The methods section is too sketchy and needs further explanation. The author also tends to make broad statements that are not well supported or explained adequately. The discussion needs to be re-organized or rewritten in several places to make the paper better flow.

I have made specific comments on the text of the manuscript and also include further comment and explanation, by section, below.

Title	Shorten the title, perhaps deleting the section after the colon.
Introduction	
p. 3 par.1	Include references on culture work in artificial media.
P. 3. par.2	Move to last paragraph of the section as your objectives statement.
p.3. par.3	What is meant by "systematic" study of exotic hosts? (Testing all possible families?)
p. 3 par.4. line 1	"Reports of ....., but largely ignored." This is one of those general statements that I mentioned above that needs clarification if it is left in the text.
p. 4. par 1	Howard 1951 is not in lit. cited list.
p.4 par 2	Be consistent in the use of "metamorphosis" or "transformation."
Methods p.4. par 3	Explain what subfamilies are represented by each of these species. How do they represent extremes in reproduction? Some of this material may be more appropriate in the Introduction. Do you think that all amblemines will respond somewhere between these two species? This is a large extrapolation from just one representative of each subfamily and none from the amblemines. How many female mussels did you collect glochidia from for these tests? What time of the year were they collected? ~~How long were they held in the lab and under~~ what conditions before testing?
P. 5. Par.1	N=1-3 for each test host is a very low sample. We have seen that a fish species isn't necessarily an "all-or-nothing" host. In lab-reared fish from the same lot, glochidia may metamorphose on some and not on others. With a small sample size you risk misinterpreting the suitability of a species.

p. 5. par.2 Please provide additional information in the methods, specifically to address the following questions:

- ✓ Did you check glochidia for viability? – ~~they were alive~~ see method
- ✓ What concentration of glochidia was used for infection? – given in table
- ✓ Did you add fresh glochidia to the container for each infection? – simultaneous
- ✓ Did you check for initial attachment and levels of infection – No
- ✓ Were all fish of a given species placed in a single aquaria? – yes
- ✓ What size are glochidia of these two species? ? – irrelevant
- ✓ Did you check for viability of metamorphosed juveniles (e.g., movement)? yes

p.5 par 4. You assume that you recovered most of the glochidia that attached to the host, but fish may eat them off of the bottom. Moreover, the original number attached to each fish was not determined and the number of glochidia each fish received was highly variable. In heavily infected hosts, more glochidia may slough relative to a lighter infection, because of gill tissue reaction (even in suitable hosts). I think its difficult to make good quantitative comparisons of %metamorphosis without better control of infection numbers and initial attachments.

Results P. 6. Par.1 Delayed sloughing also occurs in marginal hosts that have not been previously infected. – REF?

P. 6. Par.3 ✓ What is the differences between producing a high percentage of metamorphosis and numerous juveniles?

P. 7.par 1 Would glochidia be sloughed from the gills during molting? – of course

Host mortality should be reported in the Results. – it already is

Discussion p. 9. Par.1 ✓ This paragraph belongs in the Results.

P. 9. Par.2 Variation in numbers of attached glochidia probably reflect the concentration of glochidia and time of exposure more than any thing. How could one species be more susceptible than another when the glochidia will initially close on anything when stimulated? Please explain. – I can't. I just report the result.

P. 9. Par.3 Mortality was also likely related more to the degree of infection (did the author compare number attached with mortality to fish?). The last two sentences in the paragraph should be explained or deleted.

P. 9. Par.4 ✓ Are all of the exotics that were suitable hosts for L. cardium attracted to their lures? unknown

I think the conclusions drawn make sense and are not far afield, but there is no discussion in this section about the relationship of amblemines in this scheme. The author also needs to be cautious about using data from 1 species in a subfamily as conclusive evidence. The whole section needs to be more closely organized.

P. 11 par.3 Stay away from one sentence paragraphs. ? 2 sentence!

Table the #meta and %mf aren't both necessary since they really convey the same information. I would report %mf only. –OK

Superscript IN so the reader knows where to find the levels of infection density.

Should the data on larval tiger salamander for L. Cardium be reported as original data here, if it is also in press in another manuscript? – see bottom of table and elsewhere

Can any of this data be compared statistically? N's too low

Good luck with your manuscript. I look forward to seeing it in print.