

Glochidial metamorphosis of the freshwater mussel *Lampsilis cardium* (Bivalvia: Unionidae) on larval tiger salamanders, *Ambystoma tigrinum* ssp. (Amphibia: Ambystomidae)

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Abstract: Larval tiger salamanders (*Ambystoma tigrinum* ssp.) were infected with glochidia of the freshwater mussel *Lampsilis cardium* in laboratory experiments. At 20–21°C, metamorphosis occurred from 9 to 39 days, primarily between 9 and 17 days. The percentage of attached glochidia that metamorphosed varied from 0.27 to 15.7%. Metamorphosis on the salamanders occurred more quickly than on a known piscine host, largemouth bass (*Micropterus salmoides*), but a smaller percentage of the total attached glochidia metamorphosed. The role of amphibians as hosts of freshwater mussels in North America has not been addressed. Recognizing such a relationship could have important consequences for our understanding of mussel zoogeography.

Résumé : Des larves de la salamandre *Ambystoma tigrinum* ssp. ont été infectées de glochidies de la moule d'eau douce *Lampsilis cardium* dans des expériences de laboratoire. À 20–21°C, la métamorphose s'est produite entre les jours 9 et 39, principalement entre les jours 9 et 17. La métamorphose des moules s'est produite plus tôt chez la salamandre que chez un poisson hôte, l'Achigan à grande bouche (*Micropterus salmoides*), mais un pourcentage moins élevé des glochidies fixées sont parvenues à la métamorphose. Le rôle des amphibiens comme hôtes de la moule d'eau douce en Amérique du Nord n'a jamais été étudiée. L'approfondissement de cette relation pourrait contribuer à augmenter notre compréhension de la zoogéographie des moules.
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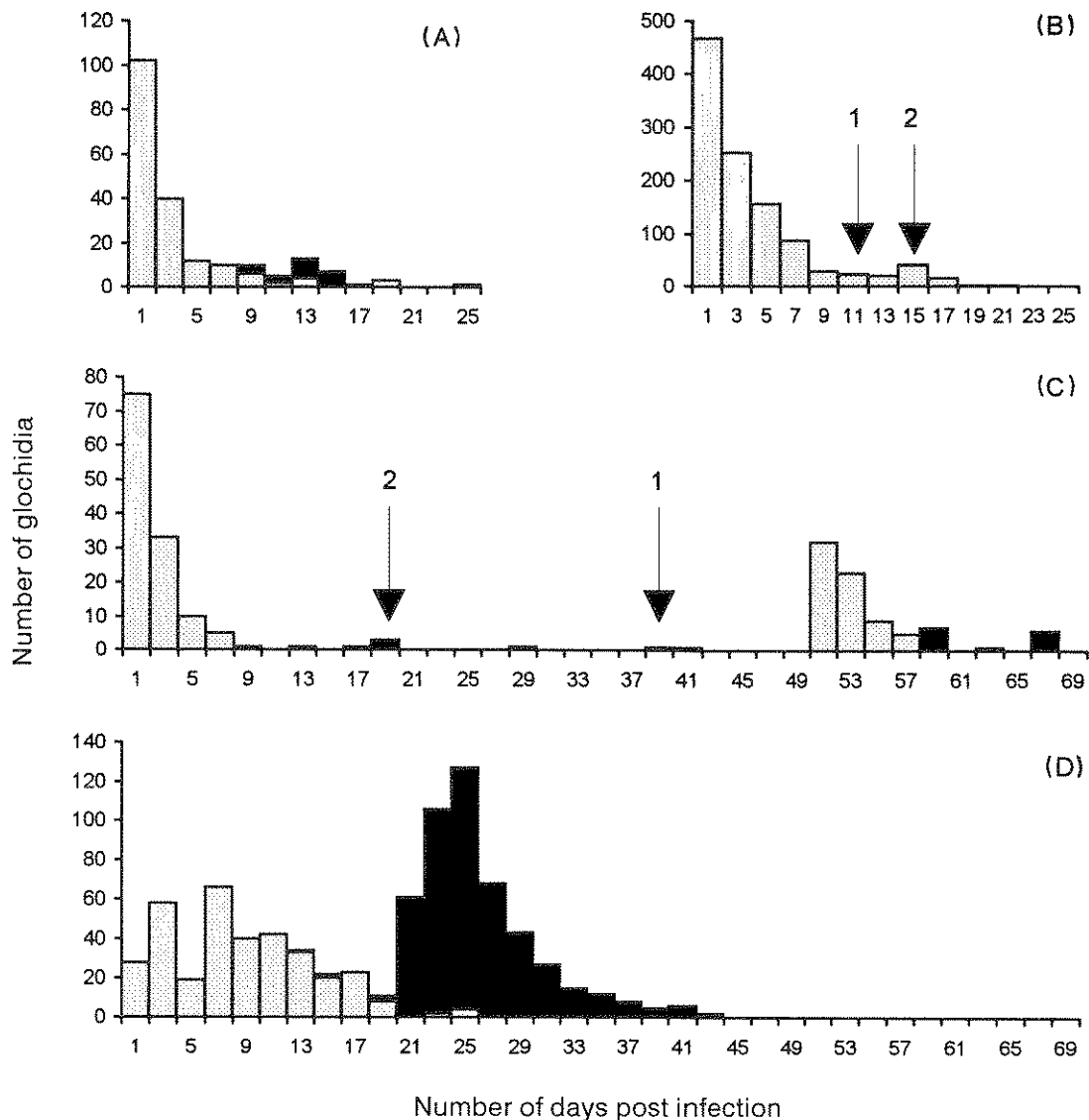
Received April 14, 1996. Accepted September 13, 1996.

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Introduction

With rare exceptions (Kondo 1990), freshwater mussels are obligate vertebrate parasites as larvae. Of the approximately 300 species in North America, only one, the salamander mussel, *Simpsonaias ambigua*, was known to use a non-

Fig. 1. Numbers of metamorphosed (solid bars) and nonmetamorphosed (shaded bars) glochidia versus number of days post infection. Numbers above the arrows indicate the number of metamorphosed glochidia. (A) Larval salamander (host 1). (B) Salamander (host 2). (C) Salamander (host 3), second infection at day 50. (D) Control (largemouth bass).



piscine host, the mud puppy *Necturus maculosus* (Howard 1915, 1951). It was assumed that all other species used exclusively fishes, and that use of *S. ambigua* was an anomaly. In reality, there was little evidence to support this generalization.

The discovery that fishes act as hosts for freshwater mussels dates from Leydig (1866) and Forel (1866). The idea that fishes were the sole hosts quickly became entrenched in the minds of many malacologists. To this day, research has focused on identifying new fish hosts. Virtually no effort was made to determine if non-piscine vertebrates also acted as hosts, despite obvious indications that this was the case. Seshaiya (1941, 1969) and Walker (1981) demonstrated that some unionaceans successfully metamorphosed on tadpoles. Howard (1951) found glochidia of the washboard *Megaloniais nervosa* on mud puppies. But the earliest report was by Faussek (1901), who reported that *Anodonta* spp. glochidia metamorphosed on larval tiger salamanders

(*Ambystoma tigrinum* ssp.), the Austrian cave salamander (*Proteus* sp.), and tadpoles of *Rana* spp. and *Pelobates* spp. These reports have largely been ignored.

This study attempted to duplicate the metamorphosis of glochidia on larval tiger salamanders reported by Faussek (1901). Unfortunately, he did not document how he performed his experiment or give details of the results. His conclusions were strictly qualitative. Nor was he sure which *Anodonta* species he used. Thus, it is impossible to recreate Faussek's experiment exactly as he performed it. Instead, I have taken his hypothesis that glochidia will transform on larval salamanders and tested it with contemporary and replicable methods.

I chose to use a lampsiline mussel rather than an anodontine. Many anodontines are considered host generalists, able to parasitize a wide range of fishes, including exotics (see the review of Watters 1994). One species, the giant floater, *Pyganodon grandis*, parasitizes more kinds of hosts than any

other known mussel (Trdan and Hoeh 1982). Lampsilines are considered more host specific, although the species used here, the plain pocketbook, *Lampsilis cardium* (= *ventricosa*), is less specific than most of its congeners. In theory, if lampsilines will transform on an amphibian, then other mussel groups may do so as well.

Methods and materials

Individuals of *L. cardium* with marsupia distended with glochidia were collected from Conneaut Creek in northeastern Ohio, U.S.A., in September 1995. They were held in a flow-through 38-L aquarium at 13–16°C with 10 cm of pea gravel as substrate. Three larval tiger salamanders were obtained commercially from an unspecified locale in Texas, U.S.A. The salamanders were 13–15 cm in length. They were held in 38-L aquaria at 20–21°C with no substrate. They were fed daily with aquatic oligochaetes.

Mussels were acclimated from 13–16 to 20–21°C for 24 h prior to their use. Glochidia were removed by inserting a water-filled insulin syringe into the distal portion of the marsupium. Injecting water caused glochidia to be flushed from the gills. Glochidia were tested for maturity by subjecting a sample of them to salt, which causes their valves to close if they are infective. Glochidia were suspended in 1 L of 20–21°C water by gentle agitation with an airstone. Glochidial densities are unknown. The three larval salamanders were exposed according to the following schedule. Hosts 1 and 2 were subjected to glochidia for 1 h. Host 3 was subjected to glochidia for 15 min on day 1 and for 1 h on day 50, 11 days after the last metamorphosed glochidium was found from the prior exposure. After exposure, salamanders were rinsed and returned to individual aquaria.

As a control, a hatchery-raised largemouth bass (*Micropterus salmoides*) was exposed for 1 h at 20–21°C at the same time as the amphibians. Largemouth bass are known hosts of this mussel (Waller et al. 1985). Animals were maintained according to the Ohio State University Animal Care and Use protocols.

On alternating days, beginning the day after exposure, 1 L of water was siphoned from the aquarium bottom and passed through a 145- μ m sieve. The debris was examined for glochidia using the polarized-light method devised for detecting zebra mussel veligers (Johnson 1995). Metamorphosed glochidia were identified by the presence of two adductor muscles and a foot, a darker shell, and the ability to crawl. Nonmetamorphosed individuals retain a single adductor muscle, lack a foot, are generally lighter colored, and are immobile. Lampsiline glochidia do not increase in size while encysted or during metamorphosis. The total number of attached glochidia was the sum of metamorphosed and sloughed nonmetamorphosed glochidia recovered from a single host.

Results

Salamanders and bass were examined under a dissecting microscope for encysted glochidia. Glochidia attached to the gills and tail fins of salamanders and to the gills of the bass. All hosts shed nonmetamorphosed glochidia for approximately 1 week. Host 1 shed metamorphosed glochidia from days 9 to 17 (Fig. 1A). Based on the total number of glochidia that attached to the host (204), 11.3% metamorphosed. Host 2 shed metamorphosed glochidia from days 11 to 15 (Fig. 1B). Of the 1096 glochidia that attached, only 0.27% metamorphosed. Host 3 shed metamorphosed glochidia from the first infection from days 9 to 39 (Fig. 1C). The 15-min exposure resulted in 132 attached glochidia, of which 2.3% metamorphosed. The second infection, for 1 h, resulted in 83 glochidia attaching, of which 15.7% meta-

morphosed. Metamorphosis occurred from days 9 to 17 of this second infection. There was no indication that the host had an acquired immunity by this second exposure. This host died on day 69 of unknown causes. No remaining glochidia were found on examination with a dissecting microscope.

The control bass shed metamorphosed glochidia from days 13 to 43 (Fig. 1D). Of the 823 glochidia that attached, 58% metamorphosed. This is substantially more metamorphosed glochidia per host than have been reported for this host-parasite association (Waller et al. 1985).

Discussion

Although larval tiger salamanders were able to act as hosts, they reacted differently from the control bass. Most glochidia metamorphosed more quickly on the salamander larvae than on the bass at the same temperature. Excystment from salamanders occurred from days 9 to 39 post infection, primarily from days 9 to 17. Metamorphosis on the bass lasted from days 13 to 43, with most excystment occurring between days 21 and 29. Metamorphosis on salamanders was not distributed over time in a single, well-defined event as seen in the control bass, but appeared more erratic. Determining whether these differences between amphibian and fishes are inherent to the hosts requires more data and additional controls.

The percentage of attached glochidia that metamorphosed on larval salamanders varied from 0.27 to 15.7%, much less than on the control bass (58%). Nevertheless, the number of metamorphosed glochidia per salamander (3–23) was within the range reported for fishes considered to be hosts for mussels in general (0.8–3.07, mean = 15; Neves et al. 1985; Yeager and Neves 1986; Zales and Neves 1982). However, comparisons are difficult to make because of differences in host species, glochidial densities, and durations of exposure.

Both tiger salamanders and *L. cardium* are distributed over much of North America, and undoubtedly occur together in the wild. How frequently they encounter one another, and how long the salamander would be exposed, are unknown. Faussek (1901) observed mild infections on amphibians, but we do not know how he conducted his experiments. He believed that glochidia attaching to the tail of tadpoles would be knocked off by the movements of the host, reducing the overall number of attached glochidia. Glochidia encysted on gills may be more likely to remain attached.

Salamanders and other amphibians may be attracted to the mantle displays of lampsiline mussels and to expelled glochidia encased in specialized matrices (conglutinates) of amblemines. Free individual glochidia broadcast by anodontines lying on the substrate could also encounter amphibians. Thus, mussel reproductive strategies involving glochidial release could be as effective for parasitizing amphibians as for parasitizing fishes.

Freshwater mussel distribution, at least on the large scale, is a function of the host's range. Previous work has focused on piscine hosts as the sole agent for dispersal. As described by Viktor Faussek nearly a century ago, amphibians also can be hosts for mussels. This study shows that mussels may use amphibians as efficiently as some reported piscine hosts. However, further studies are needed to demonstrate a symbi-

otic relationship between mussels and amphibians in the wild. We do not know which mussels are able to use amphibians, or which amphibians best serve as hosts. Amphibians may be a second, though perhaps less important, factor in the distribution of freshwater mussels.

Acknowledgements

The Ohio State University Aquatic Ecology Laboratory provided laboratory and office space. Funding was provided by the Division of Wildlife, Ohio Department of Natural Resources, through the Do Something Wild! state income tax checkoff program.

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