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1996

# Shedding of Untransformed Glochidia by Fishes Parasitized by *Lampsilis fasciola* Rafinesque, 1820 (Mollusca: Bivalvia: Unionidae): Evidence of Acquired Immunity in the Field?

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ABSTRACT

Fifteen fish species were exposed to glochidia from the freshwater mussel *Lampsilis fasciola.* Mussels and fishes were collected in the Big Darby Creek system in Ohio. All fishes began shedding untransformed glochidia within 24 hrs of exposure and continued for approximately eight days. On Day 19, after two weeks of inactivity, rosefin shiner began to shed numerous untransformed glochidia again. This release lasted 24 days. Rainbow darter began shedding untransformed glochidia on Day 25 and continued to do so for 14 days. No transformation occurred on either fish species. We believe this reaction was due to an acquired immunity and may represent the first report of this phenomenon in wild-caught fishes.

## Introduction

Host specificity may be the result of multiple causes, including ecological, behavioral, and physiological factors. If potential hosts are present in the right place at the right time, physiological responses, such as immunity to infestation, become an important aspect of freshwater mussel biology. Fishes are believed to demonstrate two types of immunity to glochidial infestation: natural and acquired. Natural immunity occurs in unsuitable hosts and is believed to be a tissue response (Bauer and Vogel 1987). This response is temperature dependent (Hruska 1992) and sloughing of glochidia occurs within 11 days, usually commencing within 24 hours (Fustish and Millemann 1978, Yeager and Neves 1986, Bauer 1987a, Waller and Mitchell 1989). Acquired immunity is the result of multiple previous infestations such that subsequent exposures are rejected by otherwise suitable hosts. Details of this response are sketchy and often contradictory. Bauer and Vogel (1987) documented acquired immunity between brown trout and *Margaritifera margaritifera*, but Young et al. (1987) found no evidence for this in another study of the same species. Acquired immunity is believed to be a humoral (antibody) reaction that takes longer to manifest itself than natural immunity (Bauer and Vogel 1987). Arey (1932) reported that sloughing of glochidia due to acquired immunity occurred within two days, but recent studies suggest a longer reaction time. The number of exposures needed to elicit acquired immunity depends on both the degree and frequency of infestation. Acquired immunity has been reported after two and three exposures (Bauer 1987a), four exposures (Arey 1932), and seven exposures (Seshaiya 1969), all using different mussels and hosts. Reuling (1919) believed that acquired immunity to one mussel species granted immunity against all species, but this has not been substantiated. How long acquired immunity lasts is unknown.

Acquired immunity has only been demonstrated in induced laboratory situations. Its existence in the wild is predicted but unproven. This study reports several unexpected observations found in conjunction with another experiment. We believe it offers strong, indirect evidence for acquired immunity in the wild. The original experiment was not designed to test any hypothesis concerning acquired immunity and therefore lacks controls and statistical support for that aspect.

## Methods and Materials

Two female *Lampsilis fasciola* were collected from Little Darby Creek, Ohio, on 7 August, 1995, and placed in a flow-through 38 liter aquarium at 20° C supplied with 10 cm of pea gravel. Flow-through was approximately 300 ml/hr, insufficient to wash glochidia from the bottom into the standpipe. Females expelled glochidia when mantle flaps were touched. These glochidia were placed in a liter beaker. The initial concentration of glochidia was unknown. Subsequent preparations taken in the same manner have yielded between 3000 and 4000 glochidia/liter. Fifteen fish species were collected from Little Darby Creek and Big Darby Creek by seining (Table 1). These were placed unanesthetized, by species, in 500 ml of water containing the glochidia. An airstone was introduced to facilitate suspension of glochidia. Fishes were exposed to glochidia for five minutes and were then segregated by species in 38 or 57 liter flow-through tanks kept at 20° C on a 12 hour daylight cycle. Our subsequent studies have shown no relationship between numbers of attached glochidia and sequence of infestation. Fishes exposed last were not necessarily less parasitized than fishes exposed at the beginning of the experiment. Fishes were fed every other day. A liter of water was siphoned from the bottom of each tank every other day and passed through a 125μ sieve; the debris was examined for glochidia. Samples were taken for a total of 53 days.

A female *Lampsilis cardium* was collected from Coneaut Creek, Ohio, on 31 August. The two rosefin shiners used in the previous experiment were exposed to glochidia of this mussel on 27 November, 80 days after the first exposure (34 days after the last untransformed *Lampsilis fasciola* glochidium was shed). Exposure was conducted as above.

## Results

No glochidia in any state of development were found associated with banded darter. Apparently no glochidia attached during the exposure. Untransformed glochidia were shed by all other species for approximately one week post exposure (Figure 1, Table 1). Several species continued to shed glochidia into the second and third week.

Untransformed glochidia again appeared from 19-43 days post infestation. Rosefin shiner and rainbow darter released numerous glochidia (>30/fish) during this time, eight other species released many fewer (<4), and six did not release additional glochidia after 19 days. No transformed glochidia were recovered from any fish. No glochidia other than those of *Lampsilis fasciola* or *L. cardium* were ever found in any tanks.

Subsequent exposure of the rosefin shiners to *Lampsilis cardium* resulted in an initial shedding of glochidia for 13 days. No secondary shedding was observed. No transformed glochidia were recovered.

Table 1. Fish species exposed to glochidia and average number of glochidia released/fish. Day 19 represented the first occurrence of secondary glochidia release in any species.

| Family | Species | # exposed | Glochidia released before Day 19 | Glochidia released on/after Day 19 |
|---|---|---|---|---|
| Cyprinidae | Rosefin Shiner | 2 | 48 | 264.5 |
| | Hornyhead Chub | 1 | 21 | 3 |
| | Central Stoneroller | 4 | 8 | 1.25 |
| | Creek Chub | 5 | 11.6 | 0.2 |
| | Striped Shiner | 17 | 4.2 | 0.12 |
| | River Chub | 4 | 16.5 | 0 |
| | Bluntnose Minnow | 4 | 0.5 | 0 |
| | Rosyface Shiner | 2 | 9 | 0 |
| Centrarchidae | Bluegill | 1 | 8 | 1 |
| | White Crappie | 2 | 30 | 1.5 |
| | Longear Sunfish | 1 | 59 | 0 |
| Percidae | Rainbow Darter | 12 | 9 | 39 |
| | Tippecanoe Darter | 1 | 12 | 1 |
| | Greenside Darter | 1 | 11 | a |
| | Yellow Perch | 1 | 83 | 0 |
| | Banded Darter | 1 | 0 | 0 |

a - died on Day 13

## Discussion

Zale and Neves (1982a) reported that *Lampsilis fasciola* spawned in late August and released glochidia the following May through August. Based on observations in the field and laboratory, apparently this species also may release in autumn of the same year they spawn. In the laboratory at 20°C, female *Lampsilis fasciola* burrowed beneath the surface for two to three days, surfaced and displayed for two days, reburrowed, and emerged in a different part of the aquarium. This cycle was repeated for over two months. By October, both females had stopped displaying and were barren of glochidia. Displays included rhythmic undulations of spotted fish-like mantle flaps. The marsupial regions of the gills protruded between the shells and the mantle flaps. When touched, the flaps were withdrawn, and simultaneously the shells closed on the marsupia, causing them to burst and release a cloud of glochidia.

Except banded darter, all exposed fish species exhibited an immediate sloughing of untransformed glochidia. This generally lasted for eight days, although white crappie continued to shed glochidia almost continuously for at least three weeks. This is similar to the observation of Zale and Neves (1982b) that some centrarchids initially shed glochidia over prolonged periods. This initial release corresponds, in part, to a natural immunity reaction.

Rosefin shiner and rainbow darter began shedding untransformed glochidia again after about a two week period of inactivity. This secondary release in rosefin

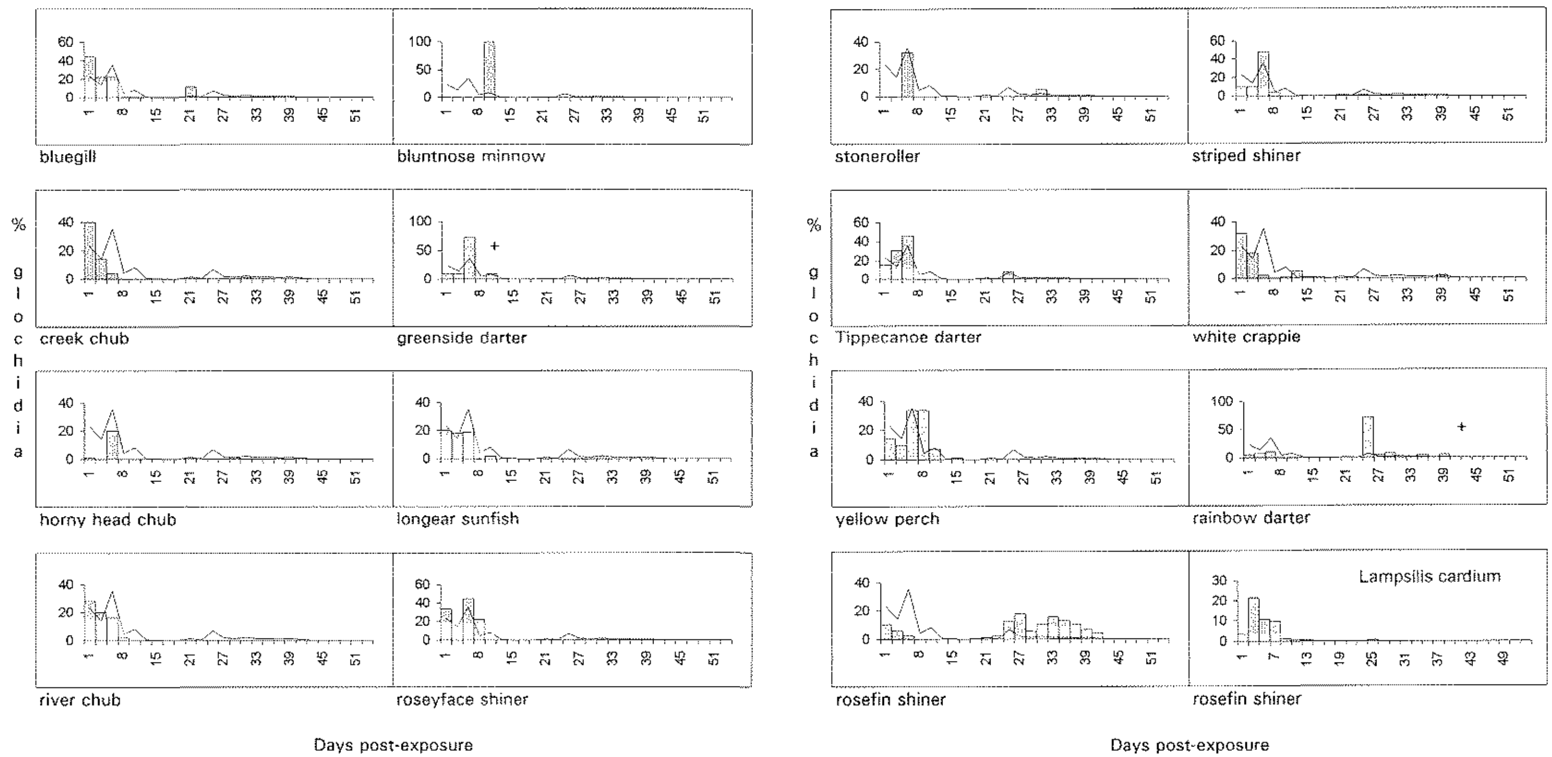


Figure 1. Percent of total glochidia released (bars) by days post-exposure. Line indicates average release of all species. + - all individuals of this fish species dead by this day. All glochidia are *Lampsilis fasciola* except where noted.

shiner began on day 19, lasted 24 days, and consisted of two peak releases that may correspond to the two individual shiners. The secondary release in rainbow darter began on day 25, lasted 14 days, and consisted of a single peak, consistent with a single individual. Eight other species shed glochidia at this time as well. However, based on the very low numbers released by these eight species, we believe that these were incidental glochidia that may have been previously missed, or took an unnaturally long time to slough off. Rainbow darter shed 81% of its glochidia in the second release, and rosefin shiner shed 87% at that time. In contrast, the greatest amount shed by any other species during this time was 8%. We believe that this secondary release corresponds to an acquired immunity. It is of interest to note that both rosefin shiner and rainbow darter initially shed glochidia in the same fashion as the other fish tested. This may be due to weakened or damaged glochidia, or it may represent glochidia that did not attach properly, and fell off the fishes. Bauer (1987b) found glochidial mortality from acquired immunity remained constant for the 43 days of his experiment. Our results suggest a much more episodic mortality.

Reinfestation of the rosefin shiners with glochidia of *Lampsilis cardium* resulted in a shedding of glochidia that peaked on the third day, and lasted for an additional ten days. This is a much shorter duration than the 27 day peak seen for *Lampsilis fasciola* but longer than the immediate sloughing of glochidia that resulted from natural immunity. It may be that the acquired immunity generated from the infestation 80 days previous was still active and resulted in quick glochidial mortality because antibodies were already present. This would support Reuling's (1919) finding that immunity to one mussel species confers immunity to all. However, both mussel species used here are members of *Lampsilis*. It may not be surprising then to find that both react in an immunologically similar fashion. Unfortunately, the timing of the shedding does not permit an unequivocal identification as either a natural or acquired immunity reaction.

Bauer (1987b) stated that highly susceptible hosts have long term serum responses, such as acquired immunity. If so, then rosefin shiner and rainbow darter may be suitable hosts under other conditions. Zale and Neves (1982b) exposed 14 fish species to *Lampsilis fasciola* glochidia. Only smallmouth bass was believed to be a host. Neither rosefin shiner nor rainbow darter was used in that study. Rainbow darter was proposed as a host for *Pyganodon grandis* (Trdan and Hoeh 1982) and *Epioblasma florentina curtisi* (Buchanan 1987). Rosefin shiner was proposed as a host for *Pleurobema collina* (Hove and Neves 1991) and *P. cordatum* (Yokley 1972). Rosefin shiner has not been identified as a host for *Lampsilis cardium* in other studies (Watters 1994b).

No transformed glochidia were recovered from either rosefin shiner or rainbow darter. Other tested fish species shed all glochidia as the result of the primary reaction (natural immunity). It was the secondary reaction that resulted in the complete expulsion of all glochidia from rosefin shiner and rainbow darter. Delayed, or acquired immunity also has resulted in complete immunity in other studies (Bauer 1987b).

We do not believe that any extrinsic factor, such as water chemistry, was responsible for the secondary shedding of glochidia. Fishes were not stressed and were fed regularly. Rosefin shiner developed breeding color during the experiment. Water temperatures did not vary more than 1-2° C. Continuous flow-through water maintained essentially constant conditions. Glochidia have successfully transformed in the laboratory setup used here before (Watters 1995) and since.

There was no evidence that any fishes already bore glochidia when caught. However, *Lampsilis fasciola* is widespread in the Big Darby Creek system (Watters 1994a) and occurs at or near the fish collection sites. In addition, Big Darby Creek supports a highly diverse unionid fauna; 41 species have been recorded. Thus it is not unlikely that some fish used were infested previously with the glochidia of some mussel species. If the conclusions of Reuling (1919) are correct, then acquired immunity may be granted by any mussel species. We have no way of knowing if the rosefin shiner and rainbow darter were previously exposed, or to what mussel species, but clearly they exhibited a long-term immune response. This response was of the magnitude and timing reported for acquired immunity in laboratory experiments.

Several circumstances may have affected the outcome. It has been suggested that for bradytictic mussels such as *Lampsilis fasciola*, glochidia that overwinter till spring in the marsupia experience less mortality than those that are released in the autumn or winter (Corwin 1920, Higgins 1930, Tedla and Fernando 1969, Zale and Neves 1982b). It may be that the glochidia used here, obtained early in the autumn, might successfully have transformed if left to overwinter. It also is questionable whether rosefin shiner and rainbow darter would be attracted to the fish lure displayed by *Lampsilis fasciola,* and therefore ever serve as natural hosts. Clearly, the nature of immunity to glochidia in wild fishes is complex and deserves more rigorous study.

Acknowledgments

We thank Dan Rice (Ohio Department of Natural Resources) for collecting and identifying the fishes and Dave Ross (ODNR) for assisting in fish collections. This project was a cooperative study supported by the Ohio Division of Wildlife and the Aquatic Ecology Laboratory of the Ohio State University.

## Literature Cited

Arey, L. B. 1932. A microscopical study of glochidial immunity. J. Morph. 53:367-379.

Bauer, G. 1987a. The parasitic stage of the freshwater pearl mussel (*Margaritifera margaritifera* L.). II. Susceptibility of brown trout. Arch. Hydrob., Suppl. 76:403-412.

Bauer, G. 1987b. The parasitic stage of the freshwater pearl mussel (*Margaritifera margaritifera* L.). III. Host relationships. Arch. Hydrob., Suppl. 76:413-423.

Bauer, G. and C. Vogel. 1987. The parasitic stage of the freshwater pearl mussel (*Margaritifera margaritifera* L.). I. Host response to glochidiosis. Arch. Hydrob., Suppl. 76:393-402.

Buchanan, A. C. 1987. Aspects of the life history of the Curtis' pearly mussel, *Epioblasma florentina curtisi* (Utterback 1915). Final Rep., End. Sp. Proj. SE-3-2, Mo. Depart. Cons. 21 pp.

Corwin, R. S. 1920. Raising freshwater mussels in enclosures. Trans. Am. Fish. Soc. 49:81-84

Fustish, C. A. and R. E. Millemann. 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. Parasit. 64:155-157.

Higgins, E. 1930. Fresh-water mussel investigations. Rep. Comm. Fish. for 1929, App. 10:670-673.

Hove, M. and R. Neves. 1991. Distribution and life history of the James River spinymussel. End. Sp. Tech. Bull. 16(3): 9.

Hruska, J. 1992. The freshwater pearl mussel in South Bohemia: Evaluation of the effect of temperature on reproduction, growth and age structure of the population. Arch. Hydrob. 126:181-191.

Reuling, F. H. 1919. Acquired immunity to an animal parasite. J. Infect. Dis. 24:337-346.

Seshaiya, R. V. 1969. Some observations on the life-histories of south Indian freshwater mussels. Malacologia 9:286-287.

Tedla, S. and C. H. Fernando. 1969. Observations on the glochidia of *Lampsilis radiata* (Gmelin) infesting yellow perch, *Perca flavescens* (Mitchell) in the Bay of Quinte, Lake Ontario. Can. J. Zool. 47:705-712.

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.

Waller, D. L. and L. G. Mitchell. 1989. Gill tissue reactions in walleye *Stizostedion vitreum vitreum* and common carp *Cyprinus carpio* to glochidia of the freshwater mussel *Lampsilis radiata siliquoidea*. Dis. Aqua. Org. 6:81-87.

Watters, G. T. 1994a. Unionidae of the Big Darby Creek system in central Ohio, USA. Malac. Rev. 27:99-107.

Watters, G. T. 1994b. An annotated bibliography of the reproduction and propagation of the Unionoidea (primarily of North America). Ohio Biol. Surv. Misc. Contr. (1): 1-158.

Watters, G.T. 1995. New hosts for *Anodontoides ferussacianus* (Lea, 1834). Trian. Unionid Rep. (7): 7-8.

Yeager, B. L. and R. J. Neves. 1986. Reproductive cycle and fish hosts of the rabbit's foot mussel, *Quadrula cylindrica strigillata* (Mollusca: Unionidae) in the upper Tennessee River drainage. Am. Midl. Nat. 116:329-340.

Yokley, P. 1972. Life history of *Pleurobema cordatum* (Rafinesque, 1820) (Bivalvia: Unionacea). Malacologia 11:351-364.

Young, M., G. J. Purser, and B. al-Mousawi. 1987. Infection and successful reinfection of brown trout [*Salmo trutta* (L.)] with glochidia of *Margaritifera margaritifera* (L.). Am. Malac. Bull. 5:125-128.

Zale, A. V. and R. J. Neves. 1982a. Reproductive biology of four freshwater mussel species (Mollusca: Unionidae) in Virginia. Freshw. Invert. Biol. 1:17-28.

Zale, A. V. and R. J. Neves. 1982b. Identification of a host fish for *Alasmidonta minor* (Mollusca: Unionidae). Am. Midl. Nat. 107:386-388.



Received: 8 January 1996

Accepted: 29 April 1996