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淡水産真珠貝 *Hyriopsis* (*Limnoscapha*) *myersiana* のグロキヂウムの感染実験

ソムサク・パナ

Infection Experiment of the Glochidium of a Freshwater Pearl Mussel, *Hyriopsis* (*Limnoscapha*) *myersiana* (Lea, 1856)

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Abstract: Infection experiment of the glochidium of a freshwater pearl mussel, *Hyriopsis* (*Limnoscapha*) *myersiana* in 11 species of fish was carried out, using 4 levels of glochidia concentration. The mortality of the fish after infection was plotted against these exposure levels. After 30-days, the interpolated LE_{50} values (exposure concentration of glochidia that killed 50% of the fry) for Iridescent mystus (*Mystus vittatus*), Striped catfish (*Pangasius sutchi*), and Yellow mystus (*M. nemurus*), were 15,000, 20,000, and 50,000, respectively. The mortalities were very low in Striped tiger nandid (*Pristolepis fasciatus*) and Temminck's kissing gourami (*Helostoma temminckii*). The most resistant were Sand goby (*Oxyeleotris marmoratus*) and Striped tiger nandid (*Pristolepis fasciatus*), which also gave the highest production of juveniles. Species of *Rasbora* were not resistant at all, and all specimens died soon after infection.

Introduction

Freshwater pearl mussels have a very interesting life-cycle. In the larval stage (glochidium), it needs to parasitise (glochidiosis) with fish or some amphibians (Lefevre and Curtis, 1910; Seshaiya, 1941; Howard, 1951; Walker, 1981; Kraemer and Swanson, 1985). Panha (1990) reported 14 species of fish that are host to glochidia. Due to inadequate knowledge of its life-cycle, the pearl mussels are being directly and indirectly obliterated, e.g. by over fishing, fishing in the reproductive season and water pollution (Bauer et al., 1980; Bauer, 1988).

Thailand has great potential for producing freshwater pearl on a large scale. This is because so many of Thai species of freshwater pearl mussels are capable of making pearls (Nakjinda et al., 1989; Panha, 1990). Apart from studies by Panha (1989, 1990) there has not been any work done on the relationship between glochidia and host in Thailand. Glochidia are very host specific. Studying their relationship is an essential for

conservation and management of both fish and pearl mussels. This will also benefit the pearl industry in the long run.

The studies on relationship of mussels and hosts in other countries can be summarized as follow:

Murphey (1942) studied the relationship of freshwater pearl mussels and trouts in U.S.A. and found that trouts are more preferred as host than other fish. Tedla and Fernando (1969) studied the attachment of glochidia of *Lamsiline radiata* on *Perca flavescens* in Canada and the favorite sites were found to be around gills. Meyers et al. (1977) investigated glochidiosis of salmon and learned that 40–50 mm long salmon are suitable host for *Margaritifera margaritifera*. Heard (1975) found that glochidia of the genus *Anodonta* would encyst on carp. Zale and Neves (1982) found 4 families of fish which can host glochidia. Kondo (1983) found that the parasitic stage of glochidia to *Anodonta woodiana* takes about 12–15 days. Morton and Dudgeon (1984) showed that glochidia of *Anodonta woodiana* would encyst around gills, abdomina and fins. Bauer and Vogel (1987) found that encystment of glochidia induced in the fish an immune system and a protective mechanism. Previously encysted fish are capable to resist new encystment. Bauer (1987b) discovered 4 species of fish which host *Margaritifera margaritifera*. They are *Salmo trutta, S. salar, Hucho hucho* and *Salvelinus fontinalis*.

The present study deals with laboratory and field investigations of glochidiosis by glochidia of the mussel *Hyriopsis* (*Limnoscapha*) *myersiana* on eleven species of fish hosts, namely, Silver barb, *Puntius gonionotus*; Julien's carp, *Cirrhina jullieni*; Rasbora, *Rasbora* sp.; Yellow mystus, *Mystus nemurus*; Iridescent mystus, *M. vittatus*; Sand goby, *Oxyeleotris marmoratus*; Striped tiger nandid, *Pristolepis fasciatus*; Temminck's kissing gourami, *Helostoma temmincki*; Java tilapia, *Tilapia mossambica*; Common climbing perch, *Anabas testudineus*; Striped catfish, *Pangasius sutchi*.

Materials and Methods

In December 1990, 40 new hatched specimens of each of the following species Silver barb (*Puntius gonionotus*), Julien's carp (*Cirrhina jullieni*), Rasbora (*Rasbora sp.*), Yellow mystus (*Mystus nemurus*), Iridescent mystus (*M. vittatus*), Striped tiger nandid (*Pristolepis fasciatus*), Temminck's kissing gourami (*Helostoma temmincki*), Java tilapia (*Tilapia mossambica*), Common climbing perch (*Anabas testudineus*), Striped catfish (*Pangasius sutchi*) of 12–17 mm in total length were exposed individually to 7,700, 15,400, 30,800, and 61,600 glochidia for 3 hours in small aquaria containing 3,750 ml of aerated, dechlorinated tap water. The juvenile fishes had never been infected by glochidia before. Of the Sand goby (*Oxyeleotris marmoratus*) only seven juveniles were collected and exposed only to 7,700 glochidia. The fry of all species was obtained from commercial suppliers. The aeration and fish movement kept the glochidia in suspension. Ten fishes were used for each exposure level and 10 for unexposed control specimens all of which were subjected to the same conditions as the test fish. After exposure, the group of infected

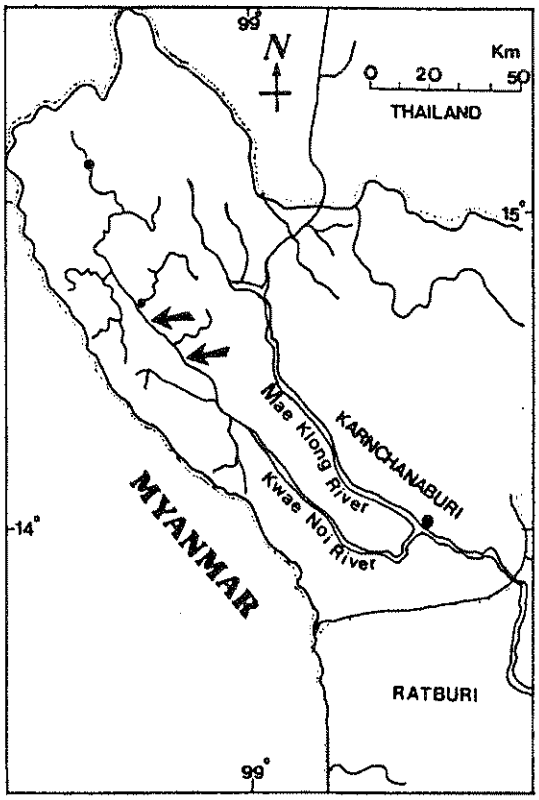

Fig. 1. Map showing main study area, Kwae Noi River, Karnchanaburi Province (arrows).

fish and the controls were kept in separate compartments of big aquaria. The fish were fed once daily with artificial fish pellets.

Gravid mussels from the Kwae Noi River were the major source of glochidia (Fig. 1). During the spawning period from 21 December 1990 to 26 January 1991, the mussels were collected by local people and by dredging from the river bottom and then transported to the laboratory and held to let them spawn.

In the laboratory, each gravid female mussel was held in 1,000 m*l* of dechlorinated tap water. Glochidia were usually released within 1–2 hrs after the water warmed to 28–29°C. (4–5 hrs at room temperature, 24–25°C). The larvae were examined for viability using movement of the valves as criterion. Glochidia from different mussels were pooled and the average number per m*l* of suspension was determined by serial dilution to make the volume of the suspension desired concentration. Only glochidia spawned on the days of fish exposure were used.

The 48-hr mortalities were recorded and plotted against the exposure levels on semi-logarithmic paper. The 48-hr LE_{50} values (the exposure levels lethal to 50 % of the fish

in 48 hr) were interpolated from the graphs for each fish species.

Dead fishes were preserved in 10% buffered formalin for the count of attached glochidia.

The average number of glochidia on fish from each exposure level that died between the first hour of exposure and the time of juvenile encystment, approximately 11–27 days after exposure, was determined.

The cumulative mortalities for all groups of fish were determined 30 days postexposure when parasite encystment was complete for all fish species. Dead fish were preserved for later parasite examination.

Results

1. *Encystment in fish from natural habitat*:

Fishes from the same area as *Hyriopsis* (*Limnoscapha*) *myersiana* were collected by nets of different size and by a trawl. They were fixed with 10% neutral formalin before being examined for the encystment of glochidia. All the eight species of fish collected were found to have glochidia encysted at various parts. The principal sites were fins and gills (Table 1). Smaller fishes had more glochidia than bigger ones (Fig. 2).

2. *Infection experiment*:

Individual fry from 7 families and 11 species of fish were exposed to a glochidium suspension of *H.* (*L.*) *myersiana* at four concentration levels: 7,700, 15,400, 30,800 and

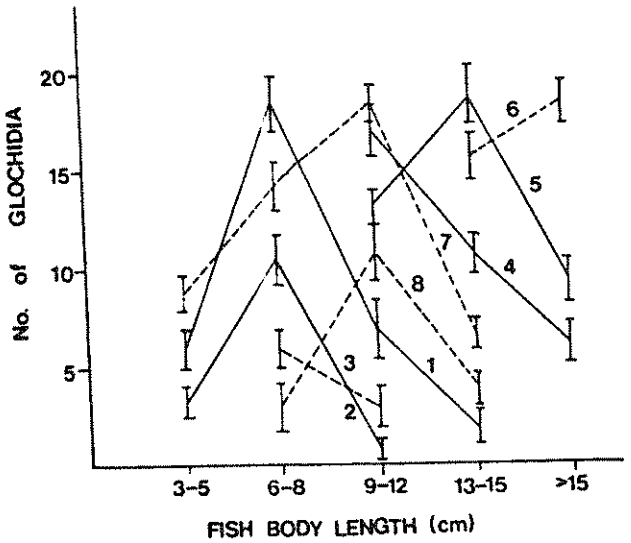

Fig. 2. Number of encysted glochidia of *Hyriopsis (Limnoscapha) myersiana* on 5 size classes of 8 species of fish collected from irrigation canal, Chainat, Province. (1–8 species of fish are as Table 1).

Table 1. Position and quantities of encysted glochidia on 8 species of fish collected from natural habitat. 1. *Puntius gonionotus* 2. *Cirrhina jullieni* 3. *Rasbora* sp. 4. *Mystus nemurus* 5. *M. vittatus* 6. *Oxyeleotris marmoratus* 7. *Pristolepis fasciatus* 8. *Puntioplites proctozysron*

Position / fish species	Fin				Mouth	Gill	Abdomen	n*
	Tail	Dorsal	Pectoral	Anal				
1.	2.01±5.9	0.42±0.62	0.7±1.06	–	–	2.3±0.85	1.6±2.1	14
2.	0.57±0.85	0.55±0.78	0.78±0.63	–	–	–	–	26
3.	0.75±0.5	0.61±0.96	–	–	0.63±0.76	–	–	31
4.	1.9±1.4	0.87±0.8	2.7±3.8	1.9±3.6	0.84±1.2	4.5±4.3	2.09±1.3	12
5.	4.3±3.5	2.9±1.5	2.1±1.3	2.1±1.1	0.4±0.7	4.8±5.1	2.0±1.9	41
6.	5.2±6.4	–	2.1±3.8	–	2.7±2.3	8.3±2.1	2.5±2.0	8
7.	4.4±6.4	3.9±3.1	2.3±0.9	3.6±1.3	3.0±1.9	8.0±3.5	1.6±0.5	27
8.	0.8±0.83	–	1.5±1.4	1.8±2.4	–	1.3±2.3	–	11

*n = Number of fries examined

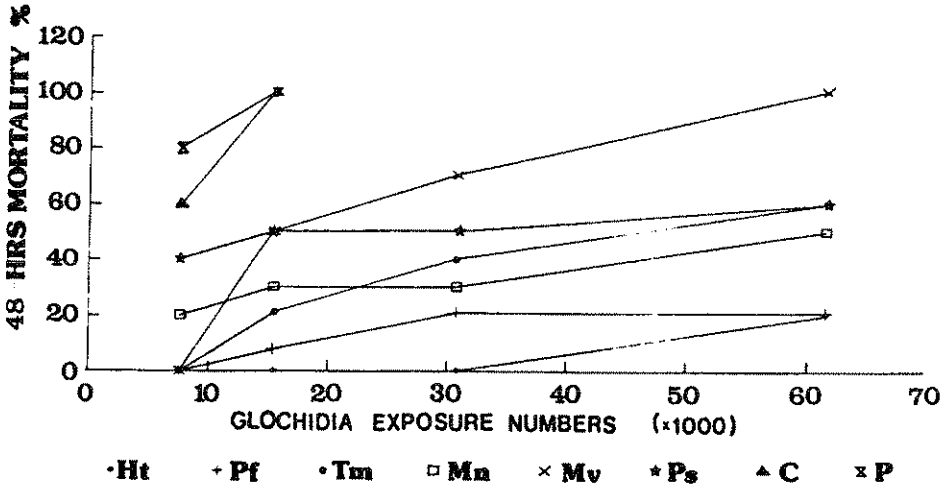

Fig. 3. 48 hours mortality (%) of fry exposed to known numbers of glochidia of *Hyriopsis (Limnoscapha) myersiana* (Ht, *Helostoma temmincki*; Pf, *Pristolepis fasciatus*; Tm, *Tilapia mossambica*; Mn, *Mystus nemurus*; Mv, *M. vittatus*; Ps, *Pangasius sutchi*; C, *Cirrhina jullieni*; P, *Puntius gonionotus*)

61,600. None of the control fish, but most of the fish exposed to large numbers of glochidia died during the first 48 hr after infection. In *Rasbora* sp. all fish were dead after exposure of every concentration of glochidia. However, no mortality at all occurred in *Anabas testudineus* (Fig. 3). Low resistance was shown by Cyprinidae, e.g. *Puntius gonionotus, Cirrhina jullieni*. *A. testudineus* had the highest resistance followed by *Helostoma temmincki, Pristolepis fasciatus* and *Mystus nemurus*, respectively. In between were *Tilapia mossambica,*

Table 2. Average number of encysted glochidia in dead fry 48 hours after experiment (15,400 concentration in *Puntius gonionotus* and *Cirrhina jullieni*, 61,600 in the other 6 species). Glochidia were counted after fixed in 10% neutral formalin for 1–2 months. (n = Number of fry examined)

Fry Species	No. of glochidia found (mean ± S.D.)	n
1. *Puntius gonionotus*	60.3 ± 17.9	10
2. *Cirrhina jullieni*	25.4 ± 14.0	10
3. *Mystus vittatus*	53.2 ± 28.8	10
4. *Mystus nemurus*	58.6 ± 8.7	5
5. *Pangasius sutchi*	34.5 ± 16.2	6
6. *Tilapia mossambica*	37.8 ± 13.6	6
7. *Pristolepis fasciatus*	76.5 ± 10.6	2
8. *Helostoma temmincki*	63.5 ± 6.4	2

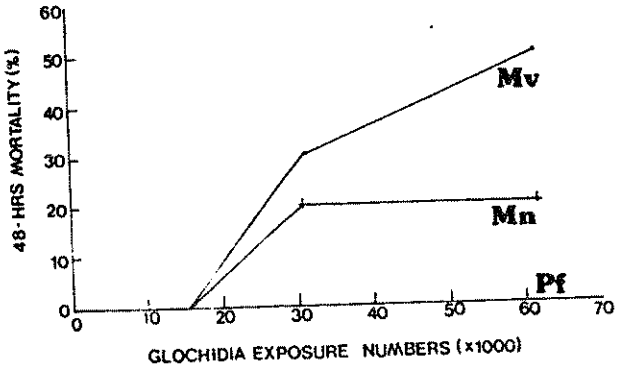

Fig. 4. 48 hours mortality (%) of 3 species of fry observed at Kwae Noi River. See Fig. 3 for abbreviations of fish species

Pangasius sutchi and *Mystus vittatus* which all died within 48 hrs at a concentration of 61,600 glochidia.

The average number of encysted glochidia in dead fish at 48 hrs after infection are shown in Table 2. The largest number of encysted glochidia were found on *Pristolepis fasciatus, Helostoma temmincki, Puntius gonionotus, Mystus nemurus* and *M. vittatus* in decreasing order.

Only 7 *Oxyeleotris marmoratus* juveniles were exposed to glochidia at the lowest concentration and observed at River Kwae Noi. The same concentration was used in natural environment with *Pristolepis fasciatus, Mystus nemurus* and *M. vittatus*, of which fry was abundantly available there. In all 3 species the mortality rate here was lower than in the lab, especially with *Pristolepis fasciatus* where no death occurred at all with this glochidia concentration (Fig. 4).

Thirty days later, at concentration 61,600 there were 100% mortality in *Mystus vittatus*,

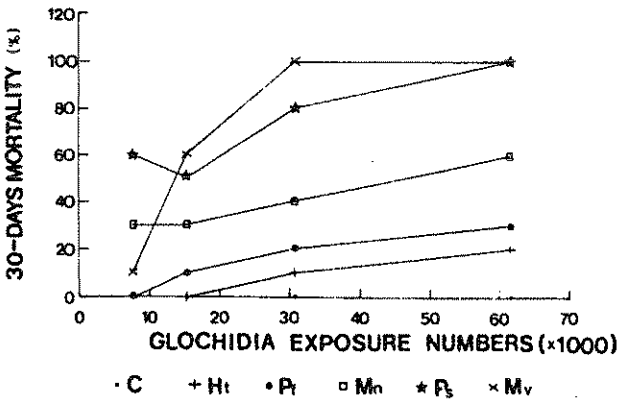

Fig. 5. 30 days mortality (%) of fry of 6 species after infection. See Fig. 3 for abbreviation of fish species.

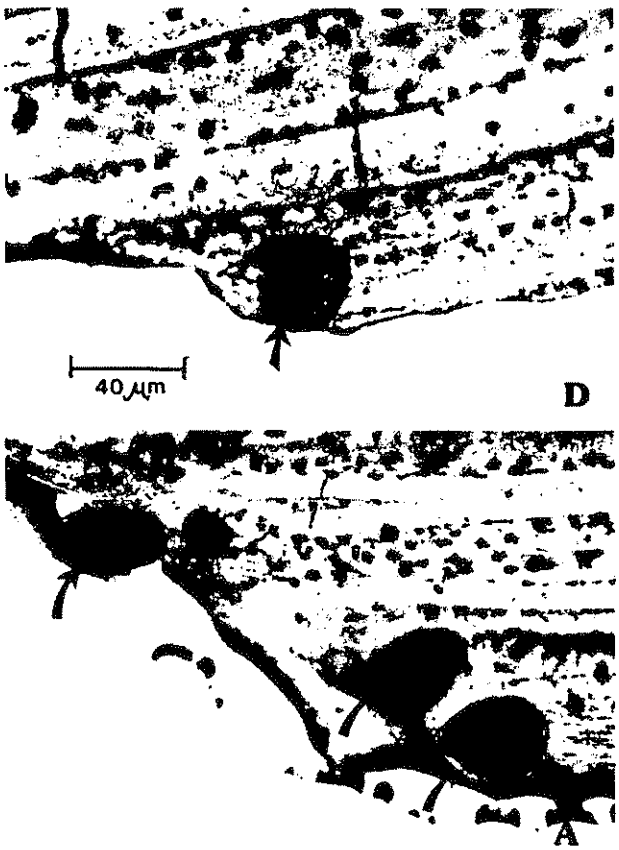

Fig. 6. Photomicrographs of encysted glochidia of *Hyriopsis (Limnoscapha) myersiana* on the dorsal fin (D) anal fin (A) of a Temminck's kissing gourami (*Helostoma temmincki*) (arrows indicate glochidia).

Pangasius sutchi and *Tilapia mossambica* (Fig. 5). The LE_{50} of *M. vittatus* at 30 days after infectiion was about 15,000. This means that at glochidial concentration of 15,000, there will be a 50% death in *M. vittatus*, while LE_{50} of 20,000 and 50,000 were observed for *Pangasius sutchi* and *M. nemurus*, respectively. Both *Pristolepis fasciatus* and *Helostoma temmincki* have a very low death rate. The most encysted areas in all species were fins and gills (Table 3, Fig. 6).

Survived fish were kept on at a glochidium concentration of 7,700 to observe juvenile development both in actual environment and in the laboratory (Fig. 7). Mussel juveniles were found only from five species of fish: *Mystus nemurus, M. vittatus, Helostoma temmincki, Pristolepis fasciatus* and *Oxyeleotris marmoratus*. These juveniles were found with two peaks: on the 11th and 12th days, in *Mystus vittatus*; 13th–15th, in *M. nemurus*; 18th–23rd in *Helostoma temmincki* and *Pristolepis fasciatus*. The latter can be found up till the 27th day. In the natural environment observation on the 18th–24th days of *Helostoma temmincki* had at least three to four times more juvenile mussels than those kept under laboratory conditions. In *Oxyeleotris marmoratus* the mussel juveniles were found on the 22nd–26th day in the same quantities as those of *Helostoma temmincki*. The juvenile mussels were continued to be cultured to observe their development onto adult mussels.

Table 3. Number of glochidia (Mean ± S.D.) of *Hyriopsis* (*Limnoscapha*) *myeriana* in 6 fry species.

Position / Fish numbers and species	Fin				Mouth	Gill	Head	Abdomen
	Tail	Dorsal	Pectoral	Anal				
(10) *Puntius gonionotus*	10.7±3.7	5.3±3.5	9.2±4.1	1.3±1.2	0.5±1.3	15±1.3	1.2±1.4	1.0±1.5
(10) *Cirrhina jullieni*	7.3±5.7	2.6±3.4	2.3±2.6	0.3±0.7	0.5±0.5	11.1±3.7	2.3±2.0	1.4±1.5
(5) *Mystus vittatus*	15.6±4.7	8.8±4.3	6.4±7.4	1.0±1.2	1.4±1.1	22.4±7.5	1.6±1.5	3.2±1.9
(5) *Mystus nemurus*	9.4±3.4	5.0±2.7	4.6±2.9	5.6±2.5	3.2±1.9	11.6±7.8	2.8±1.6	2.8±1.3
(7) *Pristolepis fasciatus*	23.6±3.9	6.0±4.4	7.7±4.4	20.4±6.0	7.3±3.6	17.1±6.2	1.6±1.5	0.9±0.9
(5) *Helostoma temmincki*	8.6±3.5	2.4±1.1	1.8±0.8	1.2±2.3	1.0±1.4	7.8±4.2	3.4±2.9	2.0±1.0

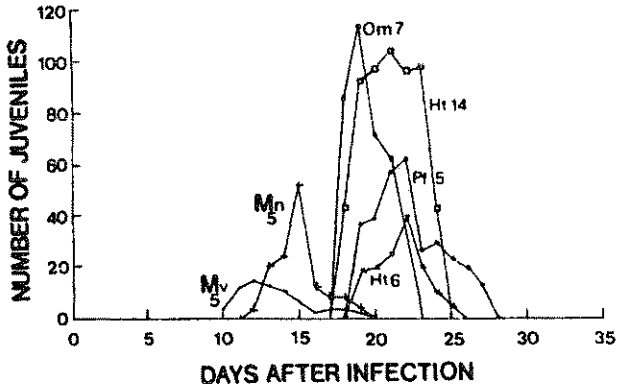

Fig. 7. Number of mussel juveniles found in each fry species after infection with the glochidia of *H. (L.) myersiana* at concentration 7,700 (number after fish names is number of fry observed, abbreviation is the same as Fig. 3).

Discussion

The infection experiment of glochidia of *Hyriopsis (Limnoscapha) myersiana* in 11 species of 7 families of fishes showed that *Pristolepis fasciatus* (Nandidae) and *Oxyeleotris marmoratus* (Eleotridae), gave the highest number of juvenile mussels and glochidial encystment. Experiments on *O. marmoratus* were limited to 7 juvenile fishes and done in the River Kwae Noi only. All data of *P. fasciatus* were from the laboratory. This species may prove to be a suitable host of *H. (L.) myerisiana*, thus it could be of great potential in producing small mussels in large quantity under laboratory condition.

The mortality of the small fishes at 48 hours after infection may be due to physiological stress from encysted glochidia. Mortality after 30 days may be caused by chronic physiological stress, due to growing glochidia as well as infection by fungi and bacteria. Glochidial infection may cause fish epidemics. Black (1981) found that glochidia hinder movement of Brook charr (*Salvelinus fontinalis*). Some charrs undergoing smoltification do not go into the sea whilst others do. There are many studies on the quantity, and specific identity of parasitic glochidia found on many species of commercial fish as well as their pathology. (For example, Molnar et al., 1974; Hare and Frantsi, 1974; Hare and Burt, 1976; Cone and Anderson, 1977; Hanex and Fernando, 1978; Watson and Dick, 1980).

Host specificity proves very important in the present experiments. Bagrid fish have low to moderate resistance to glochidia. The production of juveniles with the mussels parasitic on Bagridae is low to moderate. Still the peak was high in *Mystus nemurus*, but juvenile mortality was also rather high. In the parasites of Bagridae, the transition from glochidia to juveniles is not very successful. This may be due to host resistance.

Table 4. A verified diagram of resistance of 11 species of fries versus production of juveniles mussel, *Hyriopsis* (*Limnoscapha*) *myersiana* is shown below. (At, *Anabas testudineus*; C, *Cirrhina jullieni;* Ht, *Helostoma temmincki*; Mn, *Mystus nemurus*; Mv, *M. vittatus;* Om, *Oxyeleotris marmoratus*; P, *Puntius gonionotus*; Pf, *Pristolepis fasciatus*; Ps, *Pangasius sutchi*; R, *Rasbora* sp.; Tm, *Tilapia mossambica*).

Resistance	Juvenile Production		
	High	Low	No
High	Om, Pf	Ht, Mn	At
Low		Mv	Ps, C, P, Tm
None			R

Glochidia may be expelled from the host during encystment resulting in poorly developed or even diseased. Futish and Millemann (1978) showed that the Coho salmon (*Oncorhynchus kisutch*) has a better immunity to glochidia of *Margaritifera margaritifera* than the Chinook salmon (*Oncorhyncus tshawytscha*) has. The glochidia may be ejected from the gills by 4.5 days after infection. Bauer (1987a) concluded that the pattern of glochidial mortality suggests that less susceptible hosts respond to infection with a rapid tissue response whereas the susceptible host shows a delayed response presumably due to a humoral factor.

In our experiments we found that the numbers of encysted glochidia depend on the size of the host and the positions of the parasite on the host body. On fish which are the specific host to the glochidia will mostly develop to juveniles. Fig. 7 shows that *Pristolepis fasciatus* gives the highest juvenile yield of the species studied in the laboratory. While in the natural environment, *Helostoma temmincki* and *Oxyeleotris mormoratus* are capable of producing more juveniles. Furthermore, the appearance of these juveniles occurs in two intervals: The Bagridae (*Mystus vittatus* and *M. nemurus*) appear on the 11th–19th days post-infection, with the peak at the 12th and 15th day, respectively. Unfortunately, these juveniles, according to our observations, have a high mortality after further cultivation. The juveniles of the Anabantidae (*Helostoma temmincki*) and the Nandidae (*Pristolepis fasciatus*), occur on the 18th–27th days. Both in the field and in the laboratory experiments yield the same results for *Helostoma temmincki* even though field juvenile yield is many times larger than the laboratory population.

A verified diagram of resistance of 11 species of fry versus production of young mussel, *Hyriopsis* (*Limnoscapha*) *myersiana* is shown in Table 4.

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要　約

淡水真珠貝の*Hyriopsis myersiana*のグロキヂウムの感染実験を11種の魚類について行った。グロキヂウムの密度4階級に分けた。30日後のLE_{50}(50%の稚魚が感染によって死滅する密度)は*Mystus vittatus*では15,000, *Pangasius sutchi*では20,000, *Mystus nemurus*では50,000であった。しかし*Pristolepis fasciatus*と*Helostona temminckii*の死亡率は低く，*Oxyeleotnis marmoratus*と*Pristolepis fasciatus*は最も抵抗力が強かった。*Rasbora*の魚類は最も弱く，感染と同時に死滅した。

References

Adam, W. 1960. *Faune de Belgique. Mollusques.* Vol. 1. Institut Royal des Sciences Naturelles de Belgique, Bruxelles, 402 pp.

Bauer, G., Schrimpf, E., Thomas, W. and Herrmann, R. 1980. Relation between the decline of the freshwater pearl mussel (*Margaritifere margaritifera*) in the Fichtelgebirge and the water quality. *Arch. Hydrobiol.* 88 (4): 505–513.

Bauer, G. 1987a. The parasitic stage of the freshwater pearl mussel (*Margaitifera margaritifera*). III. Host relationship. *Arch. Hydrobiol. Suppl.* 76(4): 413–423.

Bauer, G. 1987b. The parasitic stage of the freshwater pearl mussel (*Margaritifera margaritifera*). II. Susceptibility of brown trout. *Arch. Hydrobiol. Suppl.* 76(4): 403–412.

Bauer, G. and Vogel, C. 1987. The parasitic stage of freshwater pearl mussel, *Margaritifera margaritifera* L. I. Host response to glochidiosis. *Arch. Hydrobiol. Suppl.* 76(4): 393–402.

Bauer, G. 1988. Threats to the freshwater pearl mussel (*Margaritifera margaritifera*) in Central Europe. *Biol. Conserv.* 45: 239–253.

Black, G. A. 1981. Metazoan parasite as indicators of movements of anadromous brook charr (*Salvelinus fontinalis*) to Sea. *Can. J. Zool.* 59: 1892–1896.

Brodniewicz, I. 1968. On glochidia of the genera *Unio* and *Anodonta* from the quaternaires d'eau douce. *Mitt. int. Verein. theor. angrew. Limnol.* 17: 315–318.

Cocker, R., Shira, A. F., Clark, H. W. and Howard, A. D. 1921. The natural history and propagation of freshwater mussels. *Bull. Bur. Fish. Wash.* 37: 75–181.

Cone, D. K. and Anderson, R. C. 1977. Parasites of pumpkinseed (*Lepomis gibbosus* L.) from Ryan Lake, Algoquin Park, Ontario. *Can. J. Zool.* 55: 1410–1423.

Futish, C. A. and Millemann, R. E. 1978. Glochidiosis of Salmonid fishes. II. Comparison of tissue response of coho and chinook salmon to experimental infection with *Margaritifera margaritifera* (L.). *J. Parasitol.* 64(1): 155–157.

Giusti, F., Castagnolo, L. Moretti, L. and Renzoni, A. 1975. The reproductive cycle and the glochidium of *Anodonta cygnea* L. from Lago Trasimeno (Central Italy). *Monitore Zool. Ital.* 9: 99–118.

Hanex, G. and Fernando, C. H. 1978. Spatial distribution of gill parasites of *Lepomis gibbosus* (L.) and *Ambloplites rupestris* (Raf.). *Can. J. Zool.* 55: 1235–1240.

Hare, G. M. and Fransti, C. 1974. Abundance and potential pathology of parasites infecting salmonids in Canadian Maritime Hatcheries. *J. Fish. Res. Board Can.* 31: 1031–1036.

Hare, G. M. and Burt, M. D. B. 1976. Parasites as potential biological tags of atlantic salmon (*Salmo salar*) smolt in the Miramichi River system, New Brunswick. *J. Fish. Res. Board. Can.* 33: 1139–1143.

Harms, W. 1909. Postembronale Entwicklungsgeschichte der Unioniden. *Zool. Jb.* (Anat.) 28: 325–386.

Heard, W. H. 1975. Sexuality and other aspects of reproduction in *Anodonta* (Pelecypoda: Unionidae). *Malacologia.* 15: 81–103.

Howard, A. D. 1951. A river mussel parasitic on a salamander. *Nat. Hist. Misc. Chicago.* 77: 1–6.

Kondo, T. 1983. The parasitic period of *Anodonta cygnea*. *Venus.* 42(3): 187–189.

Kraemer, L. R. and Swanson, C. M. 1985. Functional morphology of eyespots of mantle flaps of *Lamsilis*: evidence for their role as effectors, and basis for hypothesis regarding pigment distribution in bivalve mantle tissues. *Malacologia.* 26(1–2): 241–251.

Lefevre, G. and Curtis, W. C. 1910. Reproduction and parasitism in the Unionidae. *J. Exp. Zool.* 9: 79–115.

Meyers, T. R. and Millemann, R. E. 1977. Glochidiosis of salmonid fishes. 1. Comparative susceptibility to experimental infection with *Margaritifera margaritifera* (L.). *J. Parasitol.* 63: 728–732.

Molnar, K., Hanex, G. and Fernando, C. H. 1974. Parasites of fishes from Laurel Creek, Ontario. *J. Fish. Biol.* 6: 717–728.

Morton, B. and Dudgeon, D. 1984. Site selection and attachment duration of *Anodonta woodiana* glochidia on fish hosts. *J. Zool.* (*London*). 204: 355–362.

Murphey, G. 1942. Relationship of the freshwater mussel to trout in the Truckee River. *Calif. Fish and Game.* 28: 89–102.

Nakjinda, O., Sahassanonta, K. and Tispijit, N. 1989. Preliminary study on pearl formation in 2 species freshwater mussel. *Abstr. Ann. Meet. Kasetsart Univ. Fish Section.* Bangkok. p.177.

Panha, S. 1989. Observation on the parasitic glochidia of *Physunio superbus* infesting yellow mystus, *Mystus nemurus* in an irrigation canal, Central Thailand. *Abstr. 5th International Congress in Invertebrate Reproduction* (July 23–28). Nagoya, p. 82.

Panha, S. 1990. The site survey and the study on reproductive cycles of freshwater pearl mussels in the central part of Thailand. Venus, 49(3): 240–257.

Seshaiya, R. V. 1941. Tadpoles as hosts for the glochidia of the freshwater mussel. *Current Science*, 10: 535–536.

Tedla, S. and Fernando, C. H. 1969. Observation on the glochidia of *Lampsilis radiata* (Gmelin) infesting yellow perch *Perca flavescens* in the bay of Quinte. *Can. J. Zool.*, 47: 705–712.

Walker, K. F. 1981. The ecology of freshwater mussels in the River Murray. *Aust. Water. Res. Cou. Tech.*, (63).

Watson, R. A. and Dick, T. A. 1980. Metazoan parasites of pike, *Esox lucius* Linnaeus, from Southern Indian Lake, Manitoba, *Canada. J. Fish. biol.* 17: 255–261.

Zale, A. V. and Neves, R. J. 1982. Fish hosts of four species of lamsiline mussels in Big Moccasin Creek, Virginia. *Can. J. Zool.*, 60: 2535–2543.

Zhadin, V. 1952. Mollijuski presnychi solonovatych vod SSSR. Opred. *Faune SSSR* 46: 1–376.

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